ABSTRACT

Joseph T. Brozinick, Jr. FUEL SELECTION IN MUSCLE: THE REGULATION OF
PYRUVATE DEHYDROGENASE ACTIVITY DURING EXERCISE AND ITS INFLUENCE UPON
GLYCOGEN UTILIZATION AND ENDURANCE. Department of Biology, May, 1986.

    Increases in fatty acid mobilization and utilization result in sig-
nificant glycogen sparing during exercise. Regulation of pyruvate dehy-
drogenase (PDH) activity was investigated to determine if PDH regulates
glycogen utilization. Muscle glycogen is utilized at a rate that is
lower in red than in white muscle during exercise. Measurement of
muscle glycogen and PDH in the red and white portions of the vastus
lateralis of exercised rats showed less muscle glycogen depletion in red
vastus, but no difference in PDH activity between the two muscle types.
Training rats for one week resulted in an increase in the amount of PDH
in the active form. Exercise following training resulted in no signifi-
cant increase in PDH activation. Elevation of plasma free fatty acids
by oil ingestion did not result in either glycogen sparing or a decrease
in PDH activation in anesthetized rats. Fasted groups of exercised rats
showed significant glycogen sparing and a decrease in PDH activation due
to elevated plasma free fatty acids as compared to fed rats. Inhibition
of beta-oxidation by sodium 2-tetradecylglycidic acid resulted in a sig-
nificant increase in glycogen utilization during exercise, but no
increase in PDH activation. These data show that PDH does not play a
major regulatory role in glycogen utilization during exercise as origi-
nally hypothesized.

FUEL SELECTION IN MUSCLE: THE REGULATION OF PYRUVATE DEHYDROGENASE

ACTIVITY DURING EXERCISE AND ITS INFLUENCE UPON GLYCOGEN UTILIZATION

AND ENDURANCE

A Thesis

Presented to

the Faculty of the Department of Biology

East Carolina University

Submitted in partial fufillment

of the requirements for the degree of

Master of Science

by

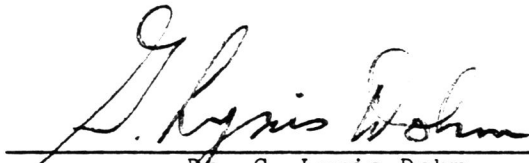Joseph T. Brozinick, Jr.

April, 1986

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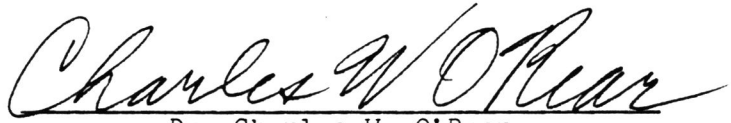
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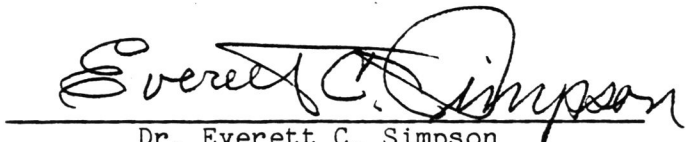
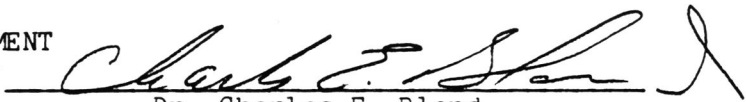APPROVED BY:

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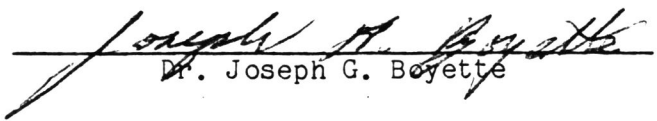THESIS COMMITTEE

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INTRODUCTION

The mechanisms of biochemical control which are responsible for preferential selection of fuels during exercise are important in metabolism. For example, during fasting, carbohydrate stores are conserved and lipid utilization and mobilization are increased. Although hormones mediate part of this response by increasing fatty acid mobilization, there is also a cellular regulatory mechanism in the tissues that utilizes fatty acids, resulting in reduction of carbohydrate metabolism. Randle et al. (1963, 1981) demonstrated this by perfusing rat hearts with fatty acids and finding that glucose uptake and glycolysis were inhibited. Perfusion with fatty acids also inhibited pyruvate and glucose oxidation and these effects were similar to those induced by starvation and alloxan diabetes. From these data Randle postulated the glucose fatty acid oxidation cycle which, briefly stated, says that fatty acid oxidation imposes restrictions upon glucose uptake and utilization in muscle (Figure 1). (For an excellent review and description, see Newsholme and Leech, 1983).

Although the regulation of glucose oxidation by cardiac muscle is of biochemical importance, the overall contribution to glucose conservation during exercise is small in relation to that of skeletal muscle. Rennie et al. (1976) found that dosing rats with oil to increase plasma free fatty acids decreased the degree of glycogen depletion in slow red and fast red muscle during exercise. In fast white muscle, glycogen sparing did not occur and this may be due to white muscle having a lower capacity to oxidize fatty acids compared to red muscle.

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Figure 1. An overview of the glucose fatty acid oxidation cycle. Dia-
        gram shows the mechanisms by which increased fatty acid oxida-
        tion inhibits glucose utilization and oxidation.
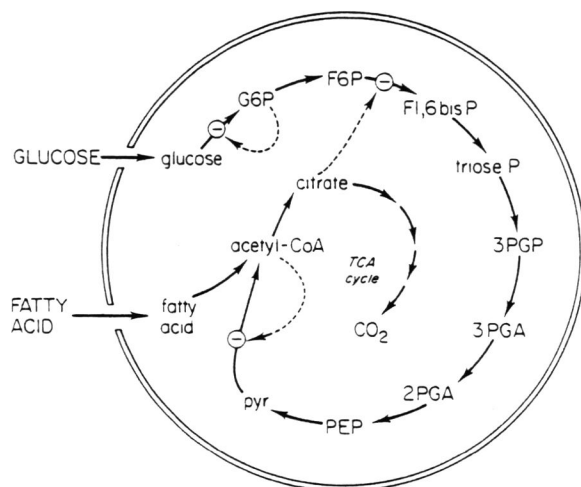
A follow-up study by Rennie and Holloszy (1977) investigated the effect of elevating plasma free fatty acids in well oxygenated perfused skeletal muscle and found that glucose oxidation and uptake were decreased. Also, in agreement with their previous results, glycogen depletion was reduced in fast and slow red, but not in fast white muscle. Concentrations of citrate and glucose-6-phosphate were elevated in red muscle of rats dosed with oil and it was postulated that glucose utilization could be regulated by inhibition of hexokinase by glucose-6-phosphate and by inhibition of phosphofructokinase with citrate.

Sparing of muscle glycogen is of vital importance to athletes since glycogen levels and depletion of glycogen are important determinants of endurance (Bergstrom et al., 1967; Karlsson and Saltin, 1971; Pernow and Saltin, 1971). Hickson et al. (1977) demonstrated that dosing rats with oil to elevate plasma free fatty acids resulted in increased endurance time to exhaustion and, in addition, muscle glycogen was spared. Costill et al. (1977) found that elevation of plasma free fatty acids by injection of heparin decreased muscle glycogen depletion in humans by as much as 40%, compared to controls, during 30 min of treadmill running at 70% VO_2. Ivy et al. (1979) reported that stimulation of fatty acid mobilization by ingestion of caffeine resulted in a 7.4% increase in work production during bicycle ergometer exercise. Jansson (1980) reported that elevation of plasma free fatty acids by a pre-exercise fatty meal resulted in an increase in fat utilization and a decrease in carbohydrate metabolism in humans during 25 min of submaximal treadmill exercise. Recently, Ravussin et al. (1986) showed that elevation of plasma free fatty acids by injection of heparin did not significantly

alter the rate of carbohydrate utilization in humans during prolonged (2.5 hr) exercise. However, the subjects exercised at only 44% VO_2max as compared to 70% in the other studies previously mentioned (Jansson, 1980; Ivy et al., 1979).

Dohm et al. (1983) recently reported that fasting increased endurance time to exhaustion in rats. This was a surprising finding since fasted rats had lower muscle glycogen before exercise than fed controls. The rate of glycogen utilization of fasted rats was decreased, however, and at exhaustion glycogen levels were higher in fasted than in fed rats. Plasma free fatty acids were elevated during exercise in fasted rats, a finding in agreement with Rennie et al. (1976). It was noted that the increased endurance and decreased glycogen depletion were the result of increased mobilization and utilization of fatty acids in fasted rats (Dohm et al., 1983). Recently, Zorzano et al. (1985) in a similar follow-up study found that exercising of fasted rats caused sparing of glycogen in soleus muscle, a red slow twitch muscle.

Physical training is another physiological condition which decreases muscle glycogen depletion rates and increases endurance (Karlsson, et al., 1974; Fitts et al., 1975). The mechanism of glycogen sparing in training is as yet unknown, but it may be related to the increased capacity of trained animals to oxidize fatty acids (Askew et al., 1972; Dohm et al., 1972; Newsholme and Leech, 1983).

Although conservation of carbohydrate during exercise may involve multiple regulatory sites, it was postulated that pyruvate dehydrogenase (PDH) might play an important regulatory role (Newsholme and Leech,

1983; Denton and Halestrap, 1979; Jansson, 1980). PDH is at the interface between carbohydrate and lipid metabolism because its substrate, pyruvate, can be converted to glucose whereas its product, acetyl CoA, can be used for fatty acid or ketone body synthesis, but can't be converted to glucose. Therefore, any substance which inhibits PDH activity ultimately leads to carbohydrate conservation since the pyruvate can be reconverted to glycogen.

The structure and regulation of PDH have been described in several excellent reviews (Randle, 1981; Wieland, 1983). In relation to this discussion, the most important regulatory mechanism is the phosphorylation-dephosphorylation interconversion cycle of PDH by specific kinase and phosphatase enzymes. The kinase and phosphatase are allosterically modulated by intracellular metabolite levels; the kinase is activated by acetyl-CoA, ATP, and NADH and is inhibited by ADP, CoA, NAD^+, pyruvate and Ca^{+2}, whereas the phosphatase is activated by Ca^{+2} and inhibited by acetyl-CoA and NADH. The phosphorylated form of PDH is inactive and the nonphosphorylated form is active so that an increase in the ATP/ADP, acetyl-CoA/CoA, and $NADH/NAD^+$ mitochondrial ratios would activate the kinase and lead to PDH deactivation, whereas an increase in amounts of mitochondrial Ca^{+2} would activate the phosphatase and lead to PDH activation (Figure 2) (Wieland et al., 1971, Randle, 1981; Wieland, 1981; Newsholme and Leech, 1983).

The majority of the regulatory mechanisms of PDH have been worked out for liver, cardiac muscle, and adipose tissue. Recently, however, Fuller and Randle (1984) and Ashour and Hansford (1983) have demonstrated that phosphorylation-dephosphorylation was also a major

Figure 2. An overview of regulation of PDH activity. Phosphorylation-
dephosphorylation cycle of mammalian PDH complex. PDH_a,
dephosphorylated, active form; PDH_b, phosphorylated, inactive
form. Physiologically relevant effectors for regulation of the
steady state by activation (+) or inhibition (−) of the kinase
and phosphatase, respectively are indicated by bold print.

Acetyl–CoA
NADH

MgATP

+

PDHa

[KINASE]

–

ADP

PDH$_b$

NAD, ADP
CoA–SH
Pyruvate

NADH

P$_i$

–

[PHOSPHATASE]

+

HOH

Ca^{+2}
Mg^{+2}
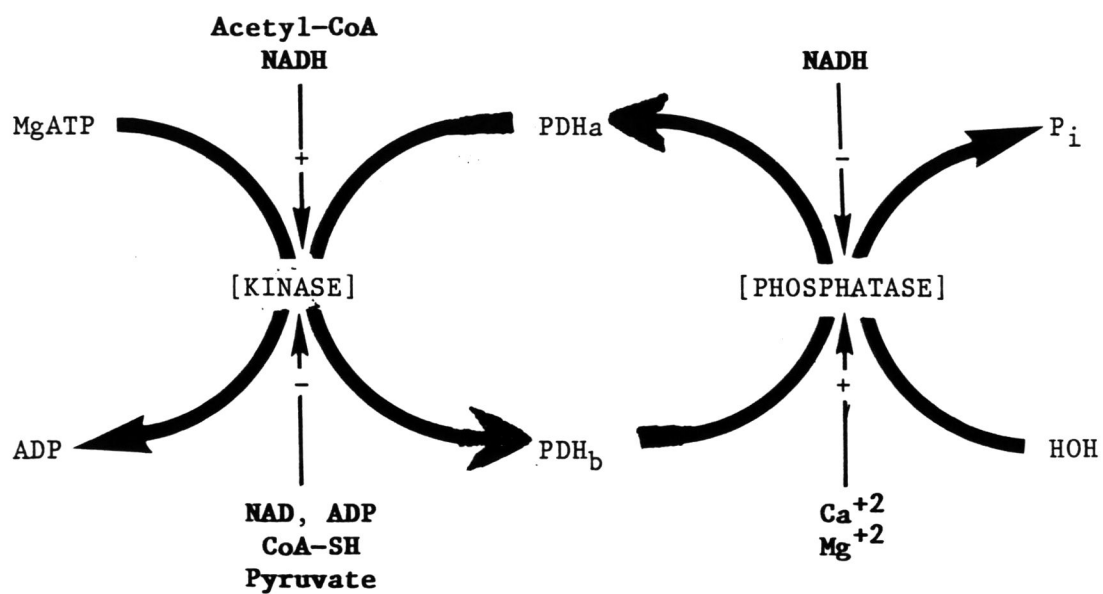
regulatory mechanism in skeletal muscle. The proportion of PDH complex in the active form from freshly isolated skeletal muscle mitochondria was 34%, whereas this activity decreased to 10-14% when the mitochondria were incubated with respiratory substrates (Fuller and Randle, 1984). The activity of PDH complex was increased by pyruvate, Ca^{+2}, and dichloracetate (Whitehouse and Randle, 1973; Fuller and Randle, 1984), while palmitoyl-L-carnitine and acetoacetate both decreased the flux through PDH (Ashour and Hansford, 1983). Palmitoyl-L-carnitine increased the $NADH/NAD^+$ and acetyl-CoA/CoA ratios so that the decreased PDH activity is a result of increased PDH kinase activity.

Alloxan diabetes or starvation (48 hr) had no effect on total activity of PDH complex in skeletal muscle mitochondria, but each reduced the concentration of active complex. This was accompanied by a 2-3 fold increase in activity of PDH kinase and greater concentrations of pyruvate, dichloroacetate, and Ca^{+2} were required to obtain half maximal activation (Fuller and Randle, 1984). Caterson et al. (1982, 1984), using a fatty acid oxidation inhibitor, concluded that the effects of starvation and alloxan diabetes on the proportion of active complex in rat heart muscle are dependent on fatty acid oxidation.

Hennig et al. (1975) were the first to show that skeletal muscle PDH activity was increased as the result of muscle contraction. They electrically stimulated the hindlimb muscles of fed, fasted and diabetic rats. A summary of their data is shown in Table 1. Exercise increased the activity of PDH, while diabetes and fasting decreased the PDH activity. The main conclusion from these data is that although exercise caused an approximate doubling of PDH activity for each group, the mag-

Table 1. The Effect of Fasting, Diabetes, and Muscular Contraction
          on PDH Activity (Hennig et al., 1976)

| | Activity (nmoles/min/mg protein) | |
	PDHa (rested)	PDHa (stimulated)
Fed	2.3	5.6
Fasted	.8	1.7
Diabetic	1.2	2.1

nitude of the increase was greater for fed than for either the fasted or diabetic group (Table 1). In other words, fasting and diabetes damps the stimulation of PDH by muscle contraction so that it can not reach the activity levels seen in fed animals. These results were replicated shortly afterward by Hagg et al. (1976) using a perfused muscle preparation.

The results of Hennig et al. (1975) and Hagg et al. (1976) were very important in relation to the effects of fasting on endurance and glycogen depletion during exercise because they suggested a molecular mechanism for the glycogen sparing previously observed by Dohm et al. (1983). It was felt, however, that these experiments should be performed on intact animals since this is the normal physiological condition in which free fatty acids are being mobilized and hormone levels are changing in response to exercise.

As a preliminary study, Dohm et al. (unpublished) measured PDH activity in rat muscles from fed and fasted rats (Table 2). As can be seen, exercise increased the basal PDH activity approximately two-fold and fasting decreased the basal activity. Exercise increased PDH activity in fasted rats but the degree of activation was less than that for fed rats. These data are qualitatively similar to results of Hennig et al. (1975) and are also in agreement with the results of Hagg et al. (1976) and Ward et al. (1982). Thus, these data supported the hypothesis that increased endurance time to exhaustion and decreased glycogen depletion in fasted rats were due to lower PDH in fasted rested rats and decreased activation of PDH during exercise.

Table 2. The Effect of Fasting and Exercise on Muscle PDH Activity
(Dohm et al., unpublished).

	PDH (nmoles/min/g tissue)		
	Basal Activity	Total Activity	% Active
Fed-Rested	410 ± 30	1200 ± 100	34
Fasted-Rested	130 ± 10[a]	990 ± 60	13
Fed-Exercised	910 ± 30[a,b,c]	1110 ± 60	82
Fasted-Exercised	210 ± 60[a]	1040 ± 60	20

[a]significantly different ($p < 0.05$) than fed-rested.
[b]significantly different ($p < 0.05$) than fasted-rested.
[c]significantly different ($p < 0.05$) than fasted-exercised.

Since endurance training is another physiological condition that spares muscle glycogen (Fitts et al., 1975), the effects of endurance training and exercise on PDH activity was also investigated. Rats were trained for 4 weeks as described by Dohm et al. (1977). Muscle PDH was determined in trained and untrained animals that were rested or exercised for 2 hr on a treadmill. The results of this experiment, shown in Table 3, demonstrated that as in earlier experiments, exercising untrained rats caused an increased activity of PDH. However, exercise did not increase PDH activity in trained rats. This supported the hypothesis that glycogen sparing in trained animals is the result of decreased activation of PDH since the basal activity was not increased by exercise. However, PDH activity in trained rested rats was just as high as in trained exercised rats.

A similar study was performed by Ward et al. (1982) on human subjects. They found that the enzyme was 40% active in the rested condition and this increased to 88% after anaerobic exercise to exhaustion. In addition, they found the PDH in resting muscle of four well trained athletes to be 70% active compared to 40% for untrained subjects. Recently, Ward et al. (1986) also found similar changes in PDH activity following strength training. The adaptation to training described by Dohm et al. (unpublished) in trained rats may then also occur in humans.

A survey of the literature has demonstrated that relatively little is known about regulation of PDH during exercise. Several studies reported that exercise increased muscle PDH activity but most of them used a nonphysiological model. Although these studies suggested a link between carbohydrate conservation and PDH activity, it was desired to

Table 3. The Effect of Training and Exercise on Muscle PDH Activity
(Dohm et al., unpublished).

	PDH (nmoles/min/g tissue)		
	Basal Activity	Total Activity	% Active
Untrained-Rested	220 ± 20	620 ± 50	35
Trained-Rested	370 ± 30[a]	590 ± 100	63
Untrained-Exercised	370 ± 40[a]	620 ± 50	60
Trained-Exercised	340 ± 60[a]	560 ± 60	61

[a]significantly different (p < 0.05) than untrained-rested.

investigate these findings in intact animals to determine if they support the hypothesis that glycogen depletion varies directly with PDH activity (i.e., the higher the PDH activation, the greater the glycogen depletion).

Since the oxidative decarboxylation of pyruvate is an irreversible reaction, the need for a complex regulatory mechanism is understandable since unregulated loss of pyruvate through this step would have serious adverse effects on glucose homeostasis in starvation, diabetes, and exercise. The studies that have been performed have increased our knowledge of this important regulatory enzyme.

METHODS AND MATERIALS

Experimental Animals

Male Sprague-Dawley rats supplied by the East Carolina University
Medical School Animal Resources Center were individually caged and given
water and laboratory chow ad libitum. The lighting schedule was regu-
lated with the lights on from 7 a.m. to 7 p.m. The Medical School ani-
mal facility keeps a colony of Sprague-Dawley rats and they were avail-
able upon request. The rat colony was established from rats obtained
from Charles River Breeding Labs and at regular intervals new breeding
stock is obtained to prevent genetic drift from that line of animals.

Some experiments involved running trained or untrained rats on a
treadmill. We had learned to make untrained rats (225-275 gm) run at 28
m/min, zero grade for 2-5 hr before becoming exhausted. The rats were
first placed on the treadmill and allowed to walk slowly until they
became accustomed to the treadmill. The treadmill speed was then gradu-
ally increased until the rats were running at 28 m/min. The warm up
period was usually completed within 15 min. Use of a shock grid was
kept to a minimum and limited to very short intervals. Training of rats
was performed as previously described by Dohm et al. (1977). Rats
weighing approximately 100 gm at the start training were run for 2 hr
daily on the treadmill for one week. On the first day, the rats started
running at 15 m/min and the time and speed were increased until the rats
were running at 28 m/min for two hours daily.

Rested (control) and acutely exercised rats were sacrificed by cer-
vical dislocation and a gastrocnemius muscle was removed and frozen
immediately between aluminum tongs cooled in liquid nitrogen. Total

elapsed time between removal of the rat from the treadmill and freezing of the muscle was no longer than 20 sec. Preliminary studies performed by Dohm et al. (unpublished) have shown that the metabolite levels present within 20 sec of the termination of exercise are representative of the state of the muscle while it is exercising.

In addition to exercising the rats on a treadmill, electrically stimulated contraction of one of the hindlimbs was alternatively used as a model for exercise. Preliminary studies (Table 8) performed by Dohm et al. (unpublished) showed that electrical stimulation of one hindlimb produced a two fold increase in PDH activity in the gastrocnemius. This approximated the increase seen in rats exercised on a treadmill. This procedure was performed as follows: rats were anesthetized by a subcutaneous injection of 0.25 ml of Inovarvet. The skin on both hindlimbs was carefully removed to avoid severing major blood vessels and a weight was attached to the achilles tendon. The achilles tendon was then severed and the sciatic nerve was exposed and electrically stimulated to cause muscle contraction. Following stimulation, the muscle was frozen in situ as it contracted and was then cut free from the body. The unstimulated gastrocnemius muscle was frozen and removed in the same manner and served as a rested control. This method allowed the muscle to be frozen while it contracted, which insured that the metabolite levels to be measured were adequate representations of the muscle in an exercised state.

PDH Assay

PDH was assayed radiochemically by measuring release of $^{14}CO_2$ from

$1-[^{14}C]$pyruvate by a modification of the procedure of Hagg et al. (1976). PDH exists in either a nonphosphorylated-active form or a phosphorylated-inactive form (Figure 2) (Wieland, 1971, 1983). Since these two forms are readily interconverted by the associated kinase and phosphatase enzymes, it was important to prevent the conversion of one form to the other for measurement of basal PDH activity. Basal activity is defined as that fraction of the enzyme which is in the active form in vivo. This was determined by rapidly freezing the tissue, by adding fluoride and triton X-100 to the homogenization buffer, and by starting the enzyme assay immediately after the frozen muscle was homogenized. To measure the total PDH activity (sum of the active and inactive forms), the homogenate was preincubated under conditions that favored the rapid conversion of the inactive form to the active (nonphosphorylated) form.

To assay basal PDH activity, frozen muscle was homogenized in a buffer containing 200 mM sucrose, 50 mM KCl, 5.0 mM $MgCl_2$, 5.0 mM EGTA, 50 mM tris-HCl (pH 7.8), 50 mM NaF, and .1% triton X-100. The assay was performed in a buffer containing 220 mM mannitol, 70 mM sucrose, 2.0 mM $MgCl_2$, 2.0 mM HEPES (pH 7.4), .45 mM NAD^+, .45 mM thiamine pyrophosphate, .8 mM CoA, and 2.0 mM $1-[^{14}C]$pyruvate (.05 µCi/ml). $^{14}CO_2$ was trapped in a center well containing ethanolamine: methylcellosolve (1:2) and counted by liquid scintillation.

To measure total PDH activity frozen muscle was homogenized in a buffer like that for basal activity except omitting NaF and triton X-100. The assay was performed as for basal activity except that 5 mM

$CaCl_2$ was added to the assay buffer and the homogenate was preincubated at 30°C for 5 min before the radioactive pyruvate, fluoride, and triton X-100 were added to initiate the reaction.

In all cases PDH activity was expressed in terms of nmol/min/gm tissue.

Glycogen Assay

Glycogen was assayed by the method of Lo et al. (1970). Frozen muscle samples weighing 50-100 mg were placed in test tubes and 1.0 ml of 30% KOH saturated with Na_2SO_4 was added. The samples were homogenized using a polytron for approximately 15 sec. The tubes were then placed in a boiling water bath for 20-30 min until a homogeneous solution was obtained. At the end of this period, the samples were removed from the water bath and refrigerated overnight. Five ml of 95% ethanol was added to precipitate the glycogen from the alkaline digest. The samples were then cooled in ice for 30 min and centrifuged at 840 x g for 20-30 min. The supernatants were carefully aspirated and the glycogen pellet was dissolved in 3 ml of distilled water. A 1 ml aliquot of the above solution was pipeted into a test tube and 0.5 ml of 5% phenol solution was added, followed by rapid addition of 2.5 ml of 95% H_2SO_4. The tubes were allowed to stand for 10 min and placed in a 25-30°C water bath for 20-30 min before readings were taken. The absorbance was read on a spectrophotometer at 490 nm and recorded. All tests were performed in duplicate to minimize errors. A standard curve was obtained using solutions containing 0-100 µg glycogen which were treated as above. The curve was plotted as a straight line using least square analysis. The

amount of glycogen in the samples was determined by reference to the standard curve and was expressed in terms of μmol glycosyl units/mg tissue.

Experiment 1

The purpose of experiment 1 was to determine whether the degree of activation of muscle PDH is greater in white than in red muscle. Since red muscle possesses a higher capacity to oxidize fatty acids it was postulated that PDH would be activated to a lesser extent in red than in white muscle during exercise. In addition, it was thought that this could be the reason that glycogen depletion is decreased in red, but not in white muscle during exercise when fatty acid availability is increased (Baldwin et al., 1972; Gollnick, et al., 1975; Rennie et al., 1976; Rennie and Holloszy, 1977).

To test this hypothesis, two groups of rats were used, rested-controls and treadmill exercised (10 animals/group). Rats were sacrificed in either the rested or exercised condition by cervical dislocation and the red and white portions of the vastus lateralis were removed and frozen between aluminum tongs cooled in liquid nitrogen. The frozen muscles were then assayed for basal and total PDH and glycogen levels.

Experiment 2

Experiment 2 was designed to investigate if high intensity exercise would cause PDH activation in trained rats. Previous work performed by Dohm et al. (unpublished) (Table 3) and the data of Ward et al. (1982) had shown that training increases the percentage of PDH in the active form in skeletal muscle and that exercise did not activate PDH. It was

postulated that a higher intensity of exercise might be required in trained rats. To investigate this, rats were trained for 1 week and then exercised at three different intensities.

Rats were trained 2 hr daily for 1 week as described in a previous section. The animals were then divided into 4 groups: group 1 was exercised at 20 m/min for 2.5 hr, group 2 at 20 m/min for 5.0 hr, group 3 at 40 m/min for 2.5 hr. Group 4 was not exercised and served as rested-controls. Following exercise, the rats were sacrificed by cervical dislocation and a gastrocnemius muscle was removed and quickly frozen. Active (basal) and total PDH were then measured.

Experiment 3

The purpose of experiment 3 was to investigate whether increasing the availability of free fatty acids reduced the activation of muscle PDH during exercise. Rats were dosed with oil and given 200 units of sodium heparin subcutaneously to stimulate lipoprotein lipase activity (Hickson et al., 1977). Control animals were given methylcellulose (which is not absorbed or digested) in place of the oil and 0.9% saline in place of the heparin. If this hypothesis were correct, a decrease in activation of muscle PDH in the oil treated group would have been expected.

Rats were divided into two groups (10 animals/group): oil dosed and control (methylcellulose dosed). Both groups were given 5 ml of either oil or methyl cellulose three hr before the exercise bout, and injected with heparin or 0.9% saline 10 min before exercise. The exercise was performed by electrical stimulation of one of the hindlimbs as previ-

ously described. The rested and stimulated gastrocnemius muscles were removed and quickly frozen. Active and total PDH and muscle glycogen levels were measured.

Experiment 4

The purpose of this experiment was to investigate whether inhibition of fatty acid oxidation accentuates the activation of PDH during exercise. Rats were treated with the fatty acid oxidation inhibitor methyl 2-tetradecylglycidic acid (TDGA) (McN-3716) which has been shown to be an effective inhibitor of beta-oxidation by blocking activity of carnitine palmitoyl transferase I (Tutwiler et al., 1978, Tutwiler and Mohrbacher, 1981). The effects of exercise on PDH activation were then determined. If this hypothesis were correct, then it would have been expected to find that inhibition of fatty acid oxidation would increase muscle glycogen utilization and PDH activation during exercise as compared to untreated rats.

Rats were divided into two groups (10 animals/group), control and TDGA treated. Rats were dosed with TDGA (25 mg/kg body weight) suspended in 0.5% methyl cellulose by stomach tube for three days. Control animals were given a similar volume of methyl cellulose on the same schedule. Two hr after the last dose was administered, the gastrocnemius muscle was exercised by electrical stimulation. The rested and stimulated gastrocnemius muscles were frozen and removed. Basal and total PDH activity and muscle glycogen levels were then measured.

Experiment 5

In light of problems experienced with the anesthetic used during electrical stimulation in preliminary experiments and in experiments 3 and 4 (see Results and Discussion for details), a fifth experiment was performed on treadmill exercised animals to eliminate the effect of the anesthetic.

Control (fed) and fasted rats were treated by oral ingestion with the fatty acid oxidation inhibitor TDGA (50 mg/kg body weight) (dissolved in mineral oil) and the effects of exercise on PDH activation and glycogen depletion were determined. Fasting increases fatty acid oxidation and it was proposed that this adaptation is responsible for decreasing the activation of PDH during exercise. If this hypothesis were correct, then inhibition of fatty acid oxidation would reverse the effects of fasting on PDH activation and an equal amount of activation of PDH in control (fed) and fasted groups would be observed. In addition, it was also expected that inhibition of beta-oxidation would increase muscle glycogen depletion during exercise compared to untreated groups.

Rats were divided into two groups: drug treated and mineral oil treated (placebo dosed) (32 animals/group). Half of each group was subdivided into fed (control) and fasted groups (16 animals/group). Half of each fed (control) and fasted group (8 animals/group) were exercised by treadmill running as previously described (see Methods and Materials) and the other half served as rested controls. The animals were run for 60 min or exhaustion (whichever came first), and were sacrificed by cervical dislocation. Rested controls were sacrificed at the same time. A

gastrocnemius muscle was removed and quickly frozen. Basal and total
PDH activities and muscle glycogen levels were measured.

Data Analysis

Data from experiments 1 and 2 were analyzed by a Newman-Keuls post
hoc analysis (Zar, 1984) to determine the effects of exercise and muscle
type (experiment 1) and exercise and training (experiment 2). The elec-
trical stimulation preliminary studies were also analyzed using a
Newman-Keuls analysis within groups (exercised or control) and a paired
t-test to determine the significance between exercise and control
groups. Experiments 3 and 4 were analyzed using a non-paired t-test
when comparing treated and control groups and a paired t-test when com-
paring muscles from the same rat (exercise effect). Experiment 5 was
analyzed by a 3-way ANOVA to explore the main effects of fasting, TDGA
administration, and exercise as well as the interactions between these
main effects.

All statistics were performed on a NWA StatPak program graciously
supplied by Dr. Richard Marks of the E.C.U. School of Medicine's Depart-
ment of Biochemistry.

RESULTS

Experiment 1: As expected, and in agreement with the results of Baldwin et al. (1972), Gollnick et al. (1975), Rennie et al. (1976), and Rennie and Holloszy (1977), there was significantly less glycogen utilization in red muscle than in white muscle (Figure 3, Table 4). Initially, white muscle possessed higher muscle glycogen levels, but as exercise progressed, white muscle glycogen was depleted more rapidly than in red, and a significant amount of sparing of glycogen was observed in red muscle. Contrary to the hypothesis, however, there was no significant difference in PDH activation during exercise (Figure 4, Table 5). As can be seen, exercise of a duration longer than 30 min resulted in a decrease in PDH activity. The decreased basal activity after 60 min of exercise was contrary to the observation in gastrocnemius and thus it was decided to repeat the experiment. A supplemental experiment was performed in which basal and total PDH activities were measured in control-rested and 2 hr exercised rats. Results from this experiment are shown in Table 6. These data duplicated results of the initial experiment as they also showed a decrease in the % active (basal/total) PDH with exercise. Both experiments led to the same conclusion, that contrary to the hypothesis, there does not appear to be a difference in PDH activation between white and red muscle.

Experiment 2: In agreement with previous studies (Table 3) (Ward et al., 1982; Dohm et al., unpublished), one week of training increased the % active PDH in muscle (Table 7). However, exercise at any of the three intensities used did not result in any significant amount of PDH activation.

Table 5. The Effect of Muscle Type and Exercise on Glycogen Depletion
in Muscle

Muscle Glycogen (μmole/mg muscle)

Muscle Type	Rested	30 min Exer	75 min Exer	Exhausted	Exhausted + 1 hr
Red	36.7 ± 3.7	21.0 ± 2.0*	20.2 ± 0.9*	19.9 ± 2.4*	34.3 ± 4.1^{*+}
White	47.6 ± 3.0	21.8 ± 1.1*	14.7 ± 1.9*	13.8 ± 2.4*	20.4 ± 2.5*

*Statistically different ($p < 0.05$) than rested.
$^+$Statistically significant difference ($p < 0.05$) between Exhausted and Exhausted + 1 hr
rest.

Figure 3. The effects of muscle type and exercise on muscle glycogen
depletion in rat quadriceps. Rats were divided into 5 groups
(10 animals/ group) and were exercised for 30 min, 75 min,
exhaustion (~3 hr), and 3 hr + 1 hr rest. The fifth group
served as rested controls. Animals were sacrificed by cervi-
cal dislocation and the red and white portions of the quadri-
ceps were removed and frozen between aluminum tongs cooled in
liquid nitrogen. Muscle glycogen was assayed as described in
the Materials and Methods section.

RED
MUSCLE GLY
─────────

WHITE
MUSCLE GLY
───── ─ ▪ ─

Muscle Glycogen (umol/mg muscle)

* statistically different (P<.05) than rested.

+ statistically significant difference (P<.05) between exhausted and exhausted + 1 h.

RESTED   30 MIN. EX.   75 MIN. EX.   EXH. (3 h)   EXH.+1 h

Exercise Time (Min.)

28
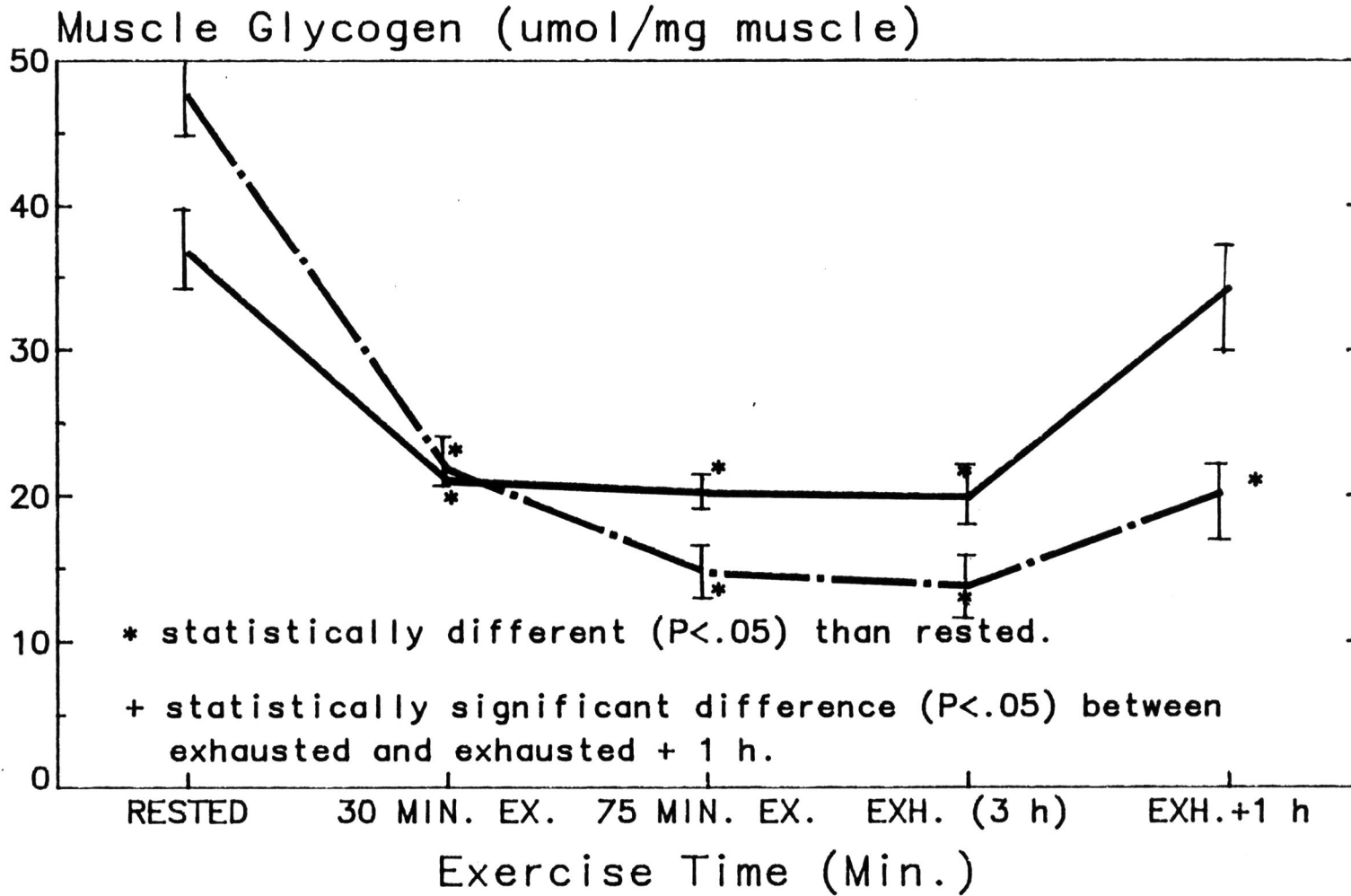
Table 4. The Effects of Muscle Type and Exercise on Basal PDH
Activity in Muscle.

Active Muscle PDH (nmol/min/gm)

Muscle Type	Rested	30 min Exer	75 min Exer	Exhausted	Exhausted + 1 hr
Red	944.2 ± 115.8	1345.5 ± 198.7	697.4 ± 70.4	1113.4 ± 182.1	393.5 ± 57.7[*+]
White	1048.2 ± 77.8	1424.5 ± 60.8[*]	955.9 ± 95.6	937.4 ± 64.9	450.8 ± 53.1[*+]

[*]Statistically different (p < 0.05) than rested.
[+]Statistically significant difference (p < 0.05) between Exhausted and Exhausted + 1 hr rest.

Figure 4. The effects of muscle type and exercise on muscle PDH activity
in rat quadriceps. Rats were divided into 5 groups (10 ani-
mals/group) and were exercised for 30 min, 75 min, exhaustion
(3 hr), and 3 hr + 1 hr rest. The fifth group served as res-
ted controls. Animals were sacrificed by cervical dislocation
and the red and white portions of the quadriceps were removed
and frozen between aluminum tongs cooled in liquid nitrogen.
Muscle PDH was assayed as described in the Materials and
Methods section.

RED
MUSCLE PDH

WHITE
MUSCLE PDH

Active Muscle PDH (nmol/min/gm)

* statistically different (P<.05) than rested.
+ statisically significant difference (P<.05) between
  exhausted and exhausted + 1 h

RESTED   30 MIN. EX.   75 MIN. EX.   EXH. (3 h)   EXH.+1 h

Exercise Time (Min.)

32
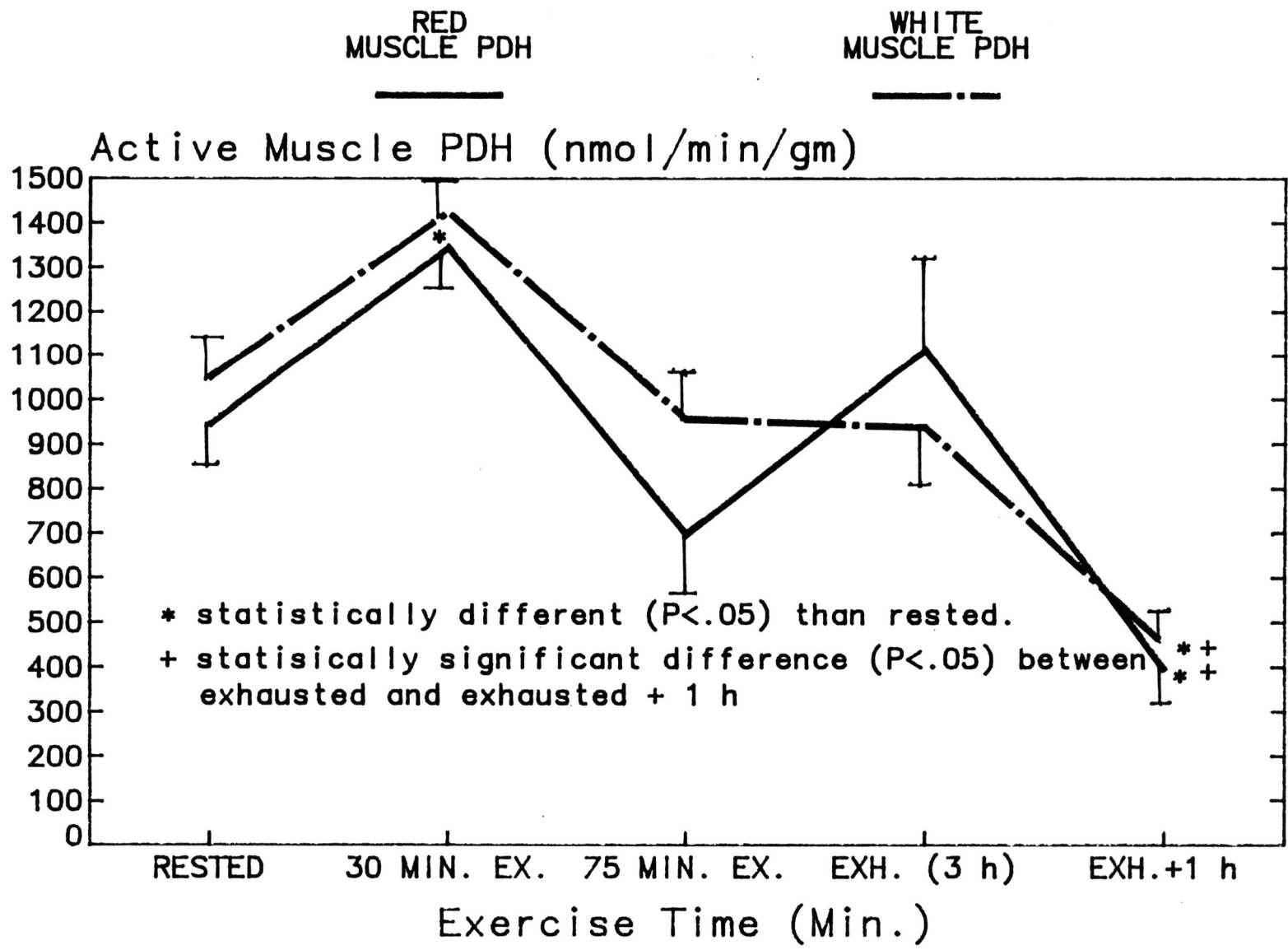
Table 6. The Effects of Muscle Type and Exercise
on PDH Activation in Muscle.

Pyruvate Dehydrogenase (nmol/min/gm)

	Basal Activity	Total Activity	% Active
Red			
Control	1549.5 ± 160.6	2361.4 ± 270.8	66
2 hr Exer	759.5 ± 52.7^a	2326.8 ± 330.6	33
White			
Control	707.8 ± 60.1^b	1574.0 ± 242.8^b	45
2 hr Exer	515.2 ± 17.1	1854.3 ± 208.8^c	28

[a]Statistically different ($p < 0.05$) than control.
[b]Statistically different ($p < 0.05$) than red control.
[c]Statistically different ($p < 0.05$) than red exercised.

Table 7. The Effects of Training and Exercise on PDH Activity.

Pyruvate Dehydrogenase (nmol/min/gm)

	Basal Activity	Total Activity	% Active
Rested	385.1 ± 63.7	624.2 ± 57.6	62
E1	426.5 ± 45.2	674.9 ± 80.7	63
E2	341.8 ± 45.8	664.7 ± 51.8	51
E3	502.8 ± 39.9	720.2 ± 114.5	70

E1 = 20 m/min for 2.5 hr.
E2 = 20 m/min for 5.0 hr.
E3 = 40 m/min for 2.5 hr.

Experiment 3: Preceding the use of electrical stimulation in experiments 3 and 4, several preliminary studies were performed investigating the rate and duration of the stimulus, and the amount of weight to be used. Table 8 shows results from the first study in which the muscle was stimulated for 15 min at a rate of 12 pulses/sec. As can be seen from the data, electrical stimulation caused a two-fold increase in PDH activation. This increase approximated that seen with treadmill exercise. Following this study, the time and weight which gave maximum stimulation was investigated. Rats were stimulated at a rate of 12 pulses/sec for either 15 min, 30 min, 1 hr, or 2 hr with a 25 gm weight, and 2 hr with a 50 gm weight (6 animals/time point). PDH activity and muscle glycogen were then measured. As can be seen in Table 9 and Figure 5, stimulation for 15 min, 30 min, or 1 hr with the 25 gm weight produced a doubling in PDH activity, which is consistent with previous results. Stimulation for 1 hr with a 50 gm weight or 2 hr with a 25 gm weight increased PDH activity, but the magnitude of the increase was not as great for the 15 min, 30 min, or 1 hr (25 gm) time points. Additionally, there was no significant difference between the first three time points. The increase in PDH activity was accompanied by a rapid decrease in muscle glycogen, as can be seen in Table 10 and Figure 6. However, the control glycogen values were much lower than observed previously in unexercised rats. This may be due to the fact that the anesthetic (Inovarvet) caused catecholamine secretion which accelerated glycogen depletion. Additionally, animals were anesthetized up to 10 min before use allowing for an additional effect of the anesthetic.

Table 8. The Effect of Electrical Stimulation on Basal PDH
Activity in Muscle.

	Pyruvate Dehydrogenase (nmol/min/gm)
Control	151.2 ± 10.6
Stimulated	356.0 ± 27.8*

*Statistically different ($p < 0.05$) than control.

Table 9. The Effect of Electrical Stimulation on Basal PDH
Activity in Muscle.

Pyruvate Dehydrogenase (nmol/min/gm)

	15 min	30 min	(25 gm) 1 hr	(50 gm) 1 hr	2 hr
Stim.	$494.0 \pm 130.0^{*}$	$594.0 \pm 80.0^{*}$	$596.0 \pm 70.0^{*}$	$4340 \pm 70.0^{*}$	214.1 ± 40.0
Control	144.0 ± 40.0	172.6 ± 60.0	231.1 ± 70.0	158.4 ± 50.0	214.1 ± 40.0

*Statistically different ($p < 0.05$) than control.

Figure 5. The effect of duration of stimuli and the amount of weight
attached to the achilles tendon on muscle PDH activation
during electrical stimulation. Rats were divided into 5
groups (6 animals/group) and were anesthetized with Inovarvet.
The gastrocnemius of one of the hindlimbs was electrically
stimulated (12 pulses/sec) to contract for 15 min, 30 min, 1
hr, or 2 hr with a 25 gm weight, and 1 hr with a 50 gm weight.
The stimulated gastrocnemius was quick frozen as it contracted
in situ. The contralateral hindlimbs gastrocnemius was also
quick frozen and removed and served as a rested control.
Basal muscle PDH was assayed as described in the Materials and
Methods section.

STIMULATED

CONTROL

Pyruvate Dehydrogenase (nmol/min/gm)

Exercise Time (Min.)

* statistically different (P<.05) than control.

Table 10. The Effect of Electrical Stimulation on Glycogen Depletion in Muscle.

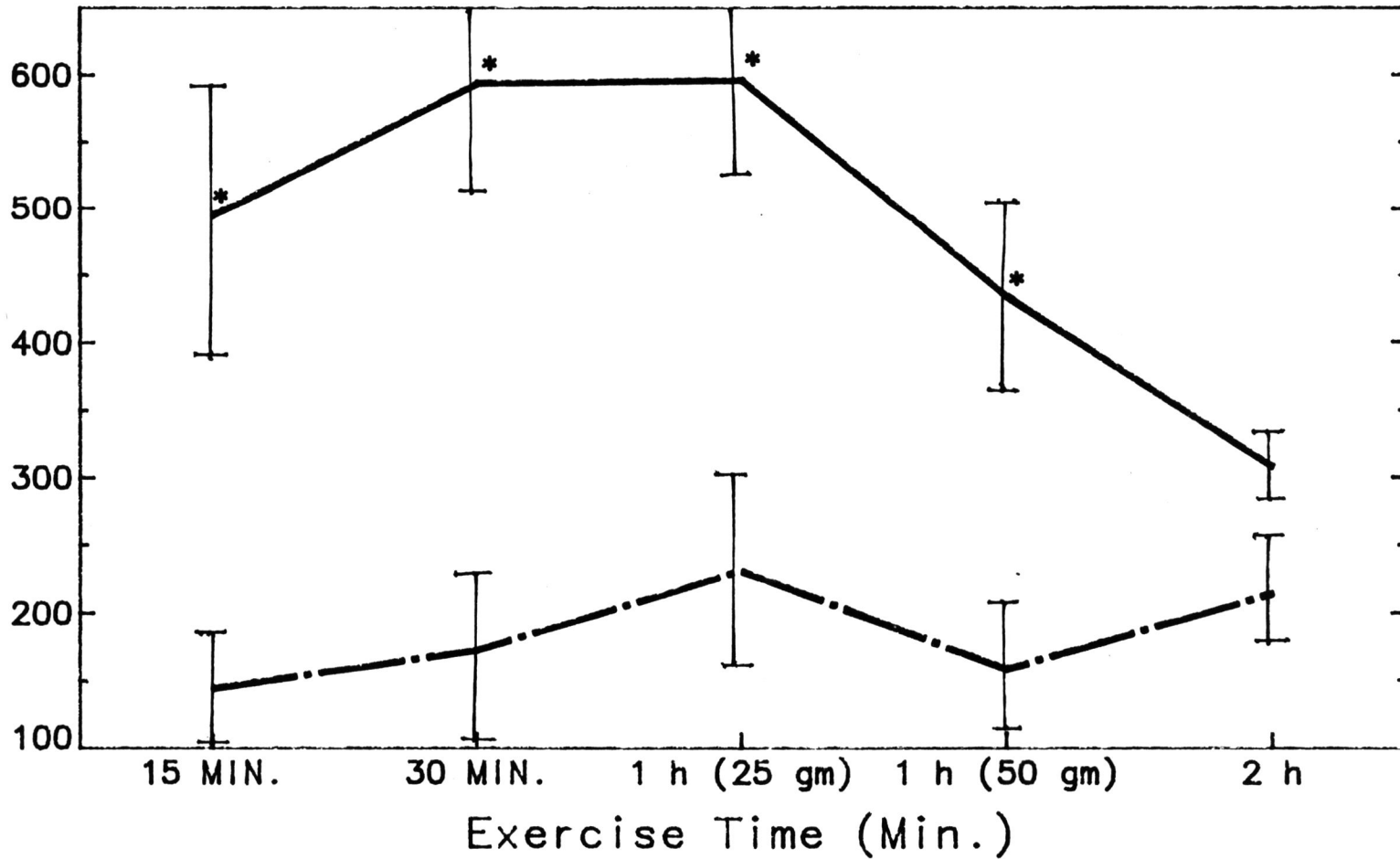| | Muscle Glycogen (μmol/mg muscle) | | | | |
	15 min	30 min	(25 gm) 1 hr	(50 gm) 1 hr	2 hr
Stimulated	10.0 ± 1.2	7.7 ± 1.2	9.0 ± 0.9	8.7 ± 2.0	7.7 ± 2.3[*]
Control	14.6 ± 4.2	13.4 ± 4.1	13.0 ± 0.9	11.7 ± 3.4	13.6 ± 3.1

[*]Statistically different ($p < 0.05$) than control.

40

Figure 6. The effect of duration of stimuli and the amount of weight
attached to the achilles tendon on muscle glycogen depletion
during electrical stimulation. Rats were divided into 5
groups (6 animals/group) and were anesthetized with Inovarvet.
The gastrocnemius of one of the hindlimbs was electrically
stimulated (12 pulses/sec) to contract for 15 min, 30 min, 1
hr, or 2 hr with a 25 gm weight, and 1 hr with a 50 gm weight.
The stimulated gastrocnemius was quick frozen as it contracted
in situ. The contralateral hindlimbs gastrocnemius was also
quick frozen and removed and served as a rested control.
Muscle glycogen was assayed as described in the Materials and
Methods section.

STIMULATED ————— CONTROL —‥—‥—

Muscle Glycogen (umol/mg muscle)

Exercise Time (Min.)

15 MIN. 30 MIN. 1 h (25 gm) 1 h (50 gm) 2 h

* statistically different (P<.05) than control.

The rate of the stimuli may have also contributed to the very rapid rate of glycogen depletion. Therefore a third preliminary study was performed in which muscles were stimulated for 5, 10, or 15 min with a 25 gm weight (8 animals/group). Rats in this experiment were used immediately after being anesthetized and were stimulated at a rate of 6 pulses/sec. As can be seen in Table 11 and Figure 7, stimulation once again resulted in a doubling in PDH activity, except for the 10 min point for which I have no explanation. The 5 min stimulation time point showed the highest PDH activation although it was not significantly different from the 15 min stimulation time point. Muscle glycogen in the stimulated leg again showed a rapid decrease with time, but the control values were as high as previous results (Table 12, Figure 8) (Dohm et al., 1983). However, the control muscle glycogen levels still decreased with time, which showed that the influence of the anesthetic could not be completely overcome. Due the results of this experiment, 5 min was selected as the stimulation time for experiments 3 and 4 since 5 min produced the greatest PDH activation and the least amount of anesthetic (catecholamine) induced glycogen depletion.

Contrary to the hypothesis, and the results of Rennie et al. (1976), Hickson et al. (1977), Rennie and Holloszy (1977), and others, there was no significant glycogen sparing observed in oil treated animals, as can be seen in Table 13. In fact, there was no significant difference between the oil (+) and oil (-) stimulated muscle glycogen values. Also, contrary to the hypothesis, and the results of Hennig et al. (1975), Ashour and Hansford (1983), and Fuller and Randle (1984), the oil (+) group did not show a decrease in PDH activation, either as

Table 11. The Effect of Electrical Stimulation on Basal PDH
Activity in Muscle.

| | Pyruvate Dehydrogenase (nmol/min/gm) | | |
	5 min	10 min	15 min
Stimulated	587.2 ± 81.4*	385.9 ± 56.7	472.6 ± 63.5*
Control	238.9 ± 17.8	276.8 ± 37.5	275.6 ± 4.5

*Statistically different ($p < 0.05$) than control.

44

Figure 7. The effect of duration of stimuli on muscle PDH activation

during electrical stimulation. Rats were divided into 3

groups (8 animals/group) and were anesthetized with Inovarvet.

The gastrocnemius of one of the hindlimbs was electrically

stimulated (6 pulses/sec) to contract for 5, 10, or 15 min

with a 25 gm weight. The stimulated gastrocnemius was quickly

frozen as it contracted in situ. The contralateral hindlimbs

gastrocnemius was also frozen and removed and served as a res-

ted control. Basal muscle PDH was assayed as described in the

Materials and Methods section.

STIMULATED   CONTROL

Pyruvate Dehydrogenase (nmol/min/gm)

Exercise Time (Min.)

* statisticaly different (P<.05) than control.

Table 12. The Effect of Electrical Stimulation on Glycogen
Depletion in Muscle.

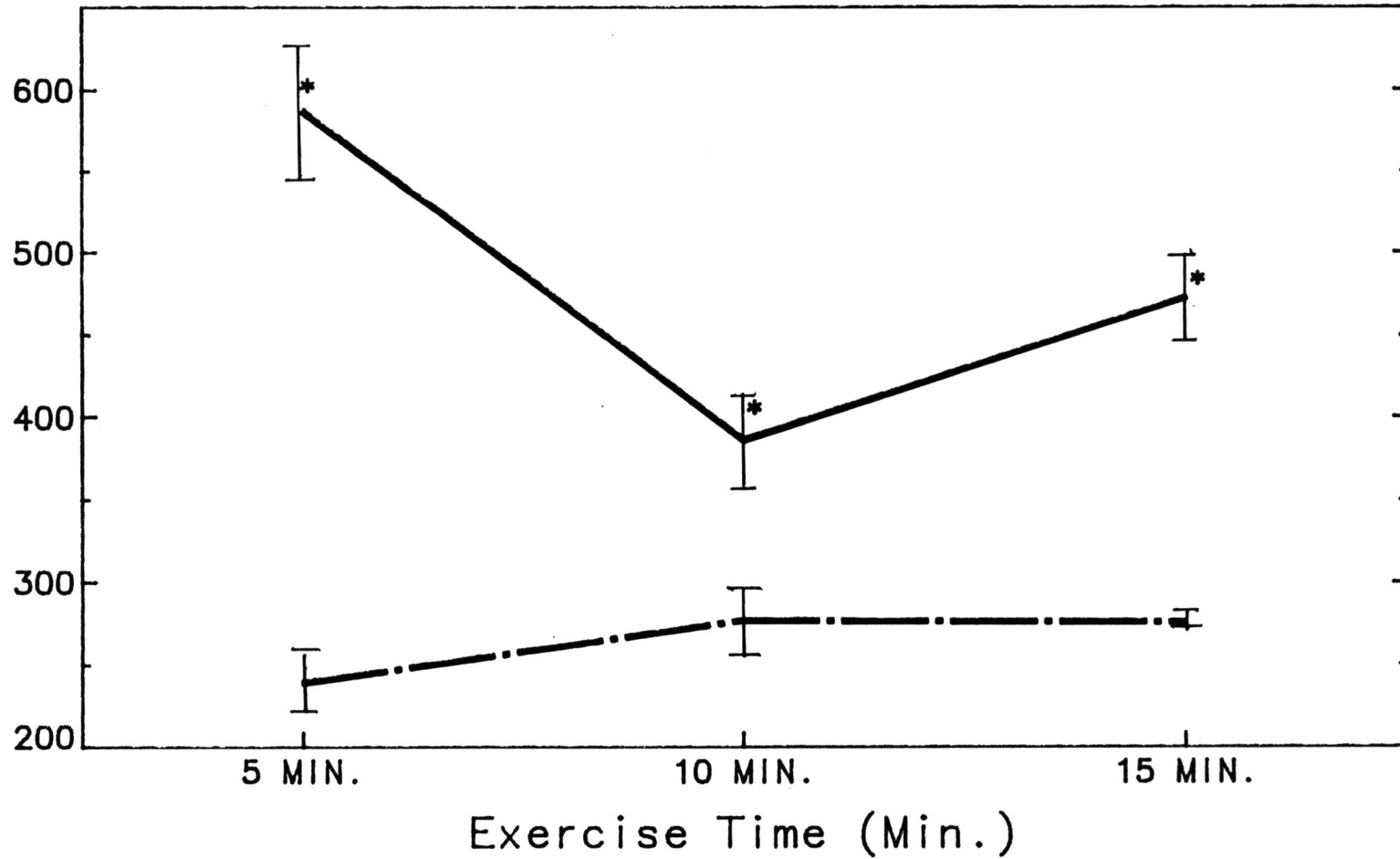| | Glycogen (μmol/mg muscle) | | |
	5 min	10 min	15 min
Stimulated	18.8 ± 2.8*	15.5 ± 2.6*	12.9 ± 1.7*
Control	36.9 ± 2.4	31.8 ± 3.4	30.6 ± 1.9

*Statistically different (p·< 0.05) than control.

Figure 8. The effect of duration of stimuli on muscle glycogen depletion

during electrical stimulation. Rats were divided into 3

groups (8 animals/group) and were anesthetized with Inovarvet.

The gastrocnemius of one of the hindlimbs was electrically

stimulated (6 pulses/sec) to contract for 5, 10, or 15 min

with a 25 gm weight. The stimulated gastrocnemius was quickly

frozen as it contracted in situ. The contralateral gastrocne-

mius was also frozen and removed and served as a rested con-

trol. Muscle glycogen was assayed as described in the

Materials and Methods section.

STIMULATED   CONTROL

Muscle Glycogen (umol/mg muscle)

Exercise Time (Min.)

* statisticaly different (P<.05) than control.

48
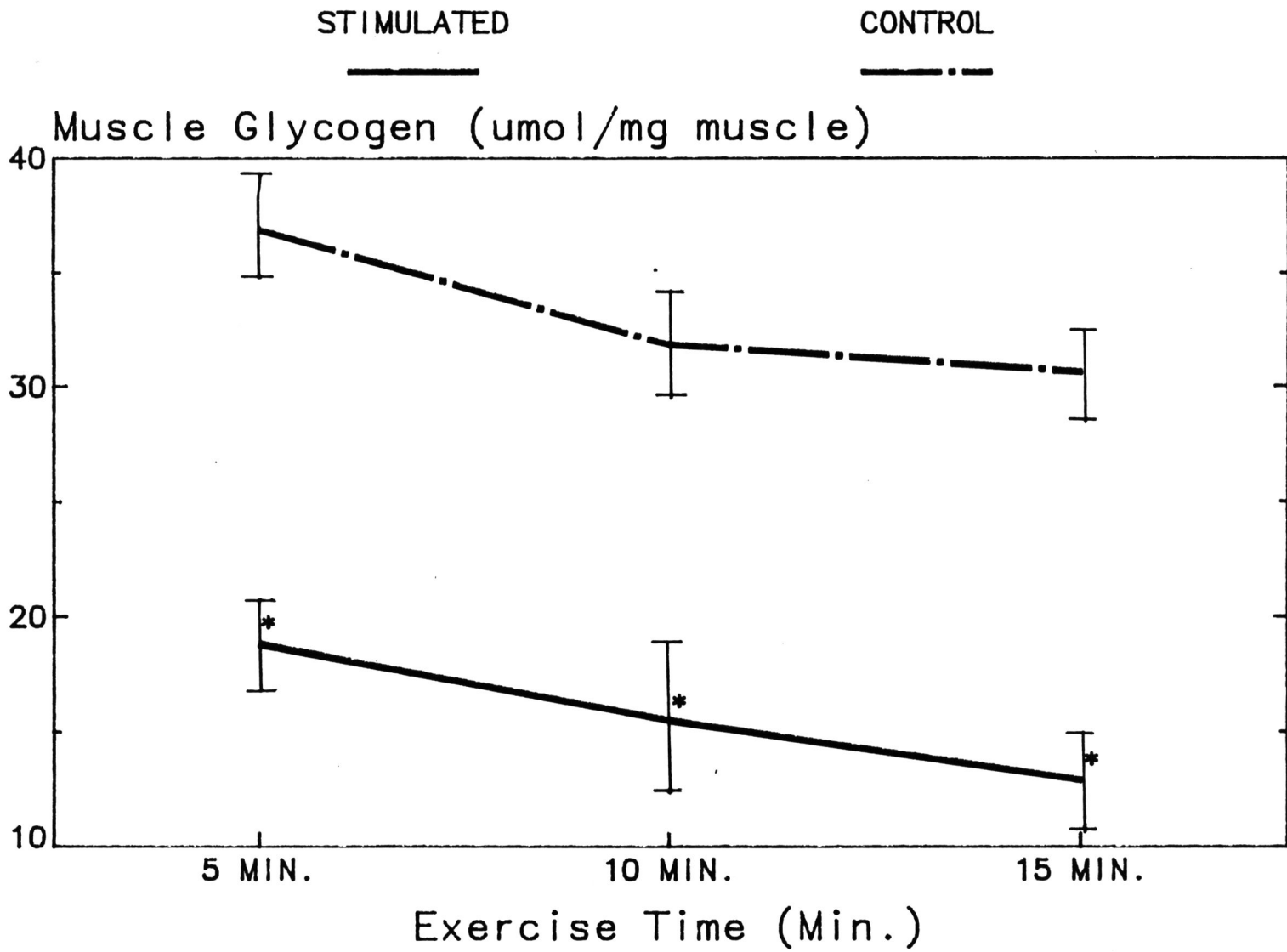
Table 13. The Effects of Oil Ingestion on Glycogen Levels
in Electrically Stimulated Muscle.

	Glycogen (μmole/mg muscle)	
Oil	(-)	(+)
Stimulated	12.6 $\pm$ 1.3*	13.3 $\pm$ 1.3*
Control	28.1 $\pm$ 1.0	26.8 $\pm$ 1.2

*Statistically different (p < 0.05) than control.

can be seen in Table 14. In fact the PDH activity was higher, although not significantly, in the oil (+) group than the oil (−) group. A summary of these data is shown in Table 15 and illustrates the lack of correlation between PDH activity and glycogen depletion. These results may have been due in part to the fact that the animals were anesthetized with ether during the stomach tubing, followed by administration of the anesthetic prior to stimulation 3 hr later. This double anesthesia could have increased the glycogen depletion before stimulation occurred by increasing catecholamine secretion. Since the control glycogen values were slightly low, this would tend to support this contention. However, this lends no explanation as to why the PDH activity in the oil (+) group was not decreased.

Experiment 4: Contrary to the hypothesis and in agreement with the results of Young et al. (1983), TDGA resulted in no increase in muscle glycogen depletion and, in fact, as can be seen in Table 16, the TDGA (−) group showed a greater amount of glycogen depletion than the TDGA (+) group. Administration of TDGA also failed to significantly increase PDH activity when compared to controls, in agreement with the results of Catterson et al. (1982, 1984), as can be seen in Table 17. Table 18 shows a summary of these data. As can be seen, not only did the TDGA (+) group fail to show a greater increase in glycogen depletion and PDH activity, but the TDGA (−) group showed a slightly larger change of both PDH activity and glycogen depletion. As in experiment 3, these data may partially reflect the effect of doubly anesthetizing the animals before use.

Table 14. Effect of Oil Ingestion and Electrical Stimulation of Basal PDH Activity in Muscle.

	Pyruvate Dehydrogenase (nmol/min/gm)			
Oil	(-)	% Active	(+)	% Active
Stimulated	$401.2 \pm 30.6^{*+}$	31	$518.1 \pm 50.9^{*}$	43
Control	146.7 ± 14.2	11	186.9 ± 18.3	15
Total PDH	1298.1 ± 174.8		1216.8 ± 99.3	

[*]Statistically different ($p < 0.05$) than control.
[+]Statistically significant difference ($p < 0.05$) between oil (-) and oil (+) groups.

Table 15. Summary: Effect of Oil Ingestion on PDH Activation and
Glycogen Depletion during Muscle Contraction

| | Oil Dosed | |
	Oil (-)	Oil (+)
Δ PDH Activity	20%	27%
Δ Glycogen Depletion (μmoles/mg muscle)	16	14

Table 16. Effect of TDGA and Electrical Stimulation on Glycogen
Levels in Muscle.

TDGA	Glycogen (μmole/mg muscle)	
	(+)	(-)
Stimulated	16.9 $\pm$ 1.3*	15.5 $\pm$ 1.1*
Control	35.4 $\pm$ 2.0	36.6 $\pm$ 2.1

*Statistically different ($p < 0.05$) than control.

Table 17. Effect of TDGA and Electrical Stimulation on PDH
Activity in Muscle.

	Pyruvate Dehydrogenase (nmol/min/gm)			
TDGA	(+)	% Active	(−)	% Active
Stimulated	854.3 ± 37.2*	72	827.95 ± 71.3*	68
Control	537.9 ± 23.7	46	483.4 ± 23.6	40
Total PDH	1179.9 ± 116.3		1224.4 ± 172.1	

*Statistically different ($p < 0.05$) than control.

Table 18. Summary: Effect of TDGA Administration on PDH Activation and Glycogen Depletion during Muscle Contraction

	TDGA Dosed	
	TDGA (+)	TDGA (−)
Δ PDH Activation	27%	28%
Δ Glycogen Depletion	19	21

Experiment 5: In agreement with the hypothesis, and contrary to the results of Young et al. (1983), administration of TDGA to treadmill exercised fed and fasted rats negated the effect of fasting, and approximately equal amounts of muscle glycogen were found in the fed (+) and fasted (+) exercised groups (Table 19). In addition, administration of TDGA caused a significant increase in muscle glycogen depletion during exercise as compared to untreated animals. Also, the fasted (-) group showed a small, but significant amount of muscle glycogen sparing compared to the fed (-) group, in agreement with previous results (Dohm, et al., 1983) and the results of Zorzano et al. (1985).

Confirming previous work (Table 2), and the results of Hennig et al. (1975) (Table 1), the fasted (-) group showed a decreased control PDH activity, when compared to the fed (-) group, and the magnitude of increase with exercise was not as great when compared with the fed (-) exercised group (Table 20). However, contrary to the hypothesis and in agreement with the results of Caterson et al. (1982), TDGA did not negate the effects of fasting on PDH activation in skeletal muscle. As can be seen, activation of PDH in the fed (+) and fasted (+) groups was not equal, and in fact, the fasted (+) group showed no significant activation at all. Additionally, PDH activity was not increased in the fed (+) and fasted (+) exercised groups when compared to untreated exercised groups.

Table 19. Effects of TDGA, Fasting and Exercise on Muscle Glycogen

| | Muscle Glycogen (μmole/mg muscle) | | | |
Drug	Fed (+)	Fed (−)	Fasted (+)	Fasted (−)
Control	25.8 ± 2.5	28.7 ± 1.2	17.9 ± 0.9	25.9 ± 4.4
Exercised	4.9 ± 1.8	10.8 ± 0.9	3.9 ± 0.7	14.4 ± 1.9

Data was analyzed by 3-way ANOVA. Significant main effects were the drug and exercise. The significant interaction was between the drug and fasting.

Table 20. Effects of TDGA, Fasting and Exercise on Muscle PDH Activities.

Pyruvate Dehydrogenase (nmol/min/gm)

Drug	Fed (+)	% Active	Fed (-)	% Active	Fasted (+)	% Active	Fasted (-)	% Active
Control	248.0 ± 15.4	29	278.5 ± 34.4	34	145.2 ± 14.1	19	143.3 ± 20.6	15
Exercised	526.2 ± 51.1	64	476.5 ± 68.8	70	147.2 ± 12.8	19	202.5 ± 19.2	30

Total Pyruvate Dehydrogenase (nmol/min/gm)

Drug	Fed (+)	Fed (-)	Fasted (+)	Fasted (-)
Control	856.2 ± 139.8	818.4 ± 78.0	767.4 ± 64.7	920.8 ± 127.1
Exercised	822.9 ± 115.6	750.4 ± 50.5	758.4 ± 62.7	664.3 ± 86.3

The data was analyzed by a 3-way ANOVA. Significant main effects were fasting and exercise. The significant interaction was between fasting and exercise.

DISCUSSION

In experiment 1, perhaps more important than there being no dif-
ference in PDH activation between the two muscle types, is the fact that
after 30 min of exercise, PDH activation decreased in both experiment 1
and the supplemental experiment. This was very perplexing, and the pos-
sibility does exist that the added amount of time necessary to remove
the red and white portions of the quadriceps allowed the kinase to inac-
tivate the PDH complex. Since PDH has not been measured in red and
white muscle before, the results observed may have been the normal
situation seen with exercise in the quadriceps. In this light, it is
important to note that Ward et al. (1982) measured PDH in various
species and in tissues from the same animal and reported that there was
not only a significant variation in PDH content between species, but
also between tissues of the same animal.

The results of experiment 2 are very similar to earlier results
obtained by Dohm et al. (unpublished) (Table 3) in that training
increased the % active PDH to approximately 60%, while exercise resulted
in an increase of only 1%. Interestingly, Ward et al. (1982) found that
the PDH in resting muscle of four well trained athletes to be 70% active
compared to 40% for untrained subjects. Since endurance training is
another physiological condition that spares muscle glycogen (Fitts et
al., 1975; Dohm et al., 1977), these data support the hypothesis that
the glycogen sparing observed in trained animals is the result of
decreased activation of PDH since basal activity was not increased by
exercise. However, basal PDH activity in trained rested-control rats was
just as high in trained exercised rats. This presents an intriguing

problem in that the question is not so much why exercise did not increase PDH activity, but why basal PDH was elevated in the trained animals.

The fact that experiments 3 and 4 did not support the hypothesis may have been entirely due to the anesthetic used in both experiments which caused catecholamine release. Compounding this effect was the fact that the animals had to be etherized in order to stomach tube them. Although catecholamines have been shown to not cause activation of PDH (Wieland et al., 1983), they would have accelerated glycogen depletion through activation of phosphorylase. This accelerated glycogen depletion compared to treadmill running did not allow the intended comparison of the stimulation model and treadmill exercise.

A summary of the data from experiment 5 is presented in Table 21 and shows the lack of correlation between PDH activation and glycogen depletion. The fed (+) and fasted (+) groups showed the greatest amount of glycogen depletion when compared to the fed (-) and fasted (-) groups, respectively. However, the fed (+) and fasted (+) groups showed a smaller change in PDH activation than the fed (-) and fasted (-) groups which disproves the original hypothesis that the increased glycogen depletion observed was the result of increased PDH activity. The fact that there was no increase in PDH activity with exercise in the fasted (+) group was perplexing. Since the animals were exercised for 60 min or exhaustion, there is a possibility that PDH was activated early in the exercise bout, and activity decreased as glycogen was rapidly depleted. This will be investigated further by exercising TDGA

Table 21. Summary of Changes in PDH Activation and Glycogen Depletion during Exercise in Response to Fasting and TDGA

	Fed (+)	Fed (−)	Fasted (+)	Fasted (−)
Δ PDH Activation (Change in % Active)	35%	36%	.5%	15%
Δ Glycogen Depletion (μmol/mg muscle)	21	18	14	12

(+) and TDGA (-) groups of rats for 15 and 30 min to determine if PDH was highly activated early in the exercise bout.

The conclusions obtained from these data can be summarized as follows:

1) There was no difference in PDH activity between red and white muscle, and there was no activation of PDH after 30 min of exercise.

2) Training increased the amount of PDH in the active state and exercise did not increase the amount of activation.

3) TDGA administration did not increase PDH activity but did increase glycogen depletion in both fed and fasted animals, in contrast to the results of Young et al. (1983).

Finally, the overall conclusion obtained from these data is that PDH does not play a major regulatory role in glycogen utilization during exercise, as was originally hypothesized.

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