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A COMPARISON OF THE EXERCISE RESPONSE OF PLASMA DOPAMINE-B-HYDROXYLASE BETWEEN TRAINED AND UNTRAINED MALE SUBJECTS

by

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ABSTRACT

The purpose of this study was to compare the response of plasma dopamine-B-hyorcxylase (DBH) to an acute bout of exercise of the same relative intensity between trained and untrained subjects. The exercise intensities were determined relative to both the anaerobic threshold and the maximal oxygen consumption.

Each group consisted of 6 healthy males between the ages of 19 and 33 years. The mean VO2 max of the untrained and trained subjects were 50.6 and 69.6 ml/kg/min respectively. The subjects performed a treadmill running protocol to exhaustion with speeds increasing from 8.0 to 19.0 km/h in the untrained and 9.5 to 23.0 km/h in the trained group, all at a constant 0.0 % elevation during which expired gases were collected. 5.0 ml blood samples were collected via a catheter inserted in the antecubital vein prior to exercise, at rest, during the last 30 seconds of each 2 minute workload accomplished, and 5 minutes post-exercise. All samples were analysed for DBH, lactate, pH, and hematocrit. The sensitivities of the DBH and lactate assays were previously determined to be within standard deviations of 5.1 % and 6.6 % respectively. The lactate values were used to determine the anaerobic threshold (AT), defined as the first exercise

stage prior to that at which a significant accumulation in blood lactate was apparent, for each subject. The VO2 at this point was defined as threshold oxygen consumption (TVO2).

Because of the reported wide interindividual variations in absolute units of DBH at rest and their effect on subsequent exercise values, the enzyme activities were expressed as % Resting DBH. % Resting DBH was plotted against VO2 for each workload. From this graph, the DBH activity was interpolated at VO2 equivalent to 50, 70, 80, 90, 100, and 105 % TVO2 as well as at rest and at the maximum workload achieved, that is the workload during which the subject was exhausted. The DBH activity was also interpolated at oxygen consumptions equivalent to 50, 60, 70, 80, 90, and 100 %VO2 max.

With regards to comparing the response of DBH to exercise between the trained and untrained subjects, no significant differences were determined between the two groups both when the results were reported relative to the anaerobic threshold (%VO2 max) and the maximal oxygen consumption (%VO2 max). Therefore the data of the two groups were pooled to test the DBH response at each exercise intensity. Plasma DBH values were only significantly elevated above resting levels at the maximum workload achieved and 100 %VO2 max. Relative to the AT (i.e. %TVO2), a significant plasma

elevation was apparent between the values obtained at the maximum workload achieved and those at rest, 50 %, and 70 %TVO2. Similarly, the pooled data at exercise intensities relative to VO2 max (i.e. %VO2 max) demonstrated significant differences between the DBH values obtained at 100 %VO2 max and those at rest 50, 60, and 70 %VO2 max.

Thus the results showed that the plasma DBH increased significantly above resting values with exercise only in the pooled (Trained + Untrained) data but not in the individual group data. The results also indicated that only maximal levels of exercise produced a DBH response significantly greater than resting values and that once a moderate exercise intensity was reached (i.e. 70 %VO2 max) the DBH response was not statistically distinct from any higher intensity.

In conclusion, it appears that the DBH response to continuous progressive exercise was not significantly different between the trained and untrained groups. Also, plasma DBH does not appear to be as sensitive an index of acute sympathetic changes as other previously studied parameters such as heart rate and catecholamines.

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Chapter I

THE PROBLEM

1.1 INTRODUCTION

With the popularity of fitness and mass participation in physical activity, it has become clear that exercise is being increasingly used as a stress by many people. Much of the work attempting to quantify exercise as a stress has involved the measurement of catecholamines either in the urine (Pierce, Kuorat, & Henry, 1976) or in the blood (Howley, 1976). However, other biochemical parameters, such as the response of serum enzymes (Kaman, Gcheen, Patton, & Raven 1977), have also been used in the elucidation of exercise as a stress.

Dopamine-B-hydroxylase is an enzyme closely associated with catecholamines and its release into the circulation during exercise has been suggested to reflect the level of sympathetic activity during physical exertion (Bennett, Kemp, & Wilson, 1973). The advantage of using plasma dopa-

mine-B-hydroxylase rather than catechclamines to determine changes in sympathetic activity in routine studies would be its longer half-life and stability in the blood. As well, the enzyme assays for dopamine-B-hydroxylase are less complex than for catecholamines.

The response of catecholamines to a bout of exercise in a trained state has been documented (Hartley et al, 1972a and 1972b; Winder et al, 1975). However little is known about the exercise response of dopamine-B-hydroxylase in relation to the fitness of the individual. Such information would be important to assess the usefulness of this plasma enzyme as a measure of the changes in sympathetic activity during exertion.

When comparing trained and untrained individuals, not only must the maximal oxygen consumption (VO2 max) be considered, but also the point where there is an increase in blood lactate. The rise in lactate is accompanied by a rise in minute ventilation and volume of expired carbon dioxide to compensate for the impending drop in pH. The lactate response appears to be due to anaerobiosis since the increased energy requirements occur at the same time as a possible reduction of muscle blood flow, an increased recruitment of fast twitch muscle fibres and a decrease in the utilization of free fatty acids (Skinner & McLellan, 1980). This reference point has been termed the anaerobic threshold (Kinder-

mann, 1979). Therefore, when prescribing equivalent relative workloads to subjects of different fitness levels, one should take into account their anaerobic thresholds and suitably adjust the intensities. This procedure is appealing due to the submaximal nature of the anaerobic threshold, which provides a reference point for work intensities other than those relative to the higher intensity associated with the VO2 max.

1.2 RATIONALE

Few studies have been conducted to compare the effects of an acute bout of exercise on plasma dopamine-B-hydroxylase activity between trained and untrained subjects. Planz et al (1974) claimed smaller increases in plasma dopamine-B-hydroxylase activity in trained subjects in response to exercise. Wirth and colleagues (1980) did not demonstrate any change in plasma dopamine-B-hydroxylase in either group. Seifen et al (1975) found that after four months of training, the elevation of serum dopamine-B-hydroxylase with exercise was greater than before training.

From the findings in the above studies, it is apparent that further investigation in this area is required. It should include an exercise protocol which prescribes equiva-

lent workload intensities to both trained and untrained groups. The percent of maximal oxygen consumption has been the traditional measure of relative intensity of exercise and is based on the maximum aerobic performance of each individual. The anaerobic threshold can also be employed as a method of equating exercise intensities as it is related to the stresses imposed on the physiological and metabolic systems. The anaerobic threshold is a submaximal parameter, which is an additional reference point to percent of maximal oxygen consumption. Further investigation prescribing workload intensities relative to the anaerobic threshold as well as the maximal oxygen consumption would make it possible to obtain a clearer understanding of the exercise response of plasma dopamine-B-hydroxylase in trained and untrained subjects.

1.3 STATEMENT OF THE PROBLEM

The present study attempted to compare the response of plasma dopamine-B-hydroxylase to exertion of the same relative intensity between trained and untrained male subjects.

A sub-problem of this study was to determine the sensitivity of the changes in dopamine-B-hydroxylase activity with varying exercise intensity regardless of the fitness of the subject.

1.4 HYPOTHESIS

It was hypothesized that there is no difference in the elevation of plasma dopamine-β-hydroxylase activity between trained and untrained male subjects in response to equivalent relative exercise intensities as determined by their anaerobic thresholds.

1.5 LIMITATIONS

There are several factors that restricted the interpretations of the results obtained in this investigation. Uncontrollable variables such as genetically determined basal plasma dopamine-β-hydroxylase levels and maximal capacities to exercise imposed such limitations. Treadmill running was performed in this study because the trained subjects were selected from a group of experienced runners. This may have limited the comparison of results between the present study and previous work which was performed primarily on the bicycle.

To familiarize the subjects with the experimental procedures, they participated in an orientation session.

Resting levels of DBH in the present study refer to the levels obtained 15 minutes into a resting period following catheterization. These resting levels may not be the same as those obtained in other studies.

With regards to the delimitations, a study where the subjects would have trained over a period of time would have been very useful in determining the effects of training on the plasma dopamine-B-hydroxylase response to exercise. However time constraints rendered this approach impractical as an initial investigation. In the present study, only two relatively small groups of trained and untrained male subjects, whose range in age was rather narrow, were employed. These delimitations on subjects and groups restricted the confident interpolation and extrapolation possible from the data. Therefore it was not possible to conclude that humans beyond the experimental parameters characteristic to this study will respond in the same manner to a bout of exercise. Finally, venous blood samples may not have been totally representative of the changes that occurred at the capillary level in the tissues.

1.6 DEFINITION OF TERMS

AT: anaerobic threshold, determined as the first exercise stage prior to that at which a significant accumulation in blood lactate is apparent.

CA: catecholamines.

DBH: dopamine-B-hydroxylase (E.C.1.14.17.1.).

E: epinephrine.

FT fibres: fast twitch muscle fibres.

FTa fibres: fast twitch oxidative muscle fibres.

FTb fibres: fast twitch glycolytic muscle fibres.

NE: norepinephrine.

% Resting DBH: Dopamine-B-hydroxylase levels expressed as a percent of the resting value.

PNMT: phenylethanolamine N-methyltransferase (E.C.2.2.1).

RNA: ribonucleic acid.

SCG: superior cervical ganglion.

SNS: sympathetic nervous system.

ST fibres: slow twitch muscle fibres.

TH: tyrosine hydroxylase (E.C.1.14.16.2).

TVO2: the oxygen consumption at the anaerobic threshold.

Ve: Minute volume expired.

VeCO2: Volume of carbon dioxide expired.

VO2: oxygen consumption.

VO2 max (l/min): Absolute maximal oxygen consumption.

VO2 max (ml/kg/min): Maximal oxygen consumption relative to body weight.

Chapter II

REVIEW OF THE LITERATURE

To obtain a clearer understanding of the relation of dopamine-B-hydroxylase (DBH) to exercise, it is essential to investigate its properties, synthesis, and appearance into the circulation. DBH is involved in the biosynthesis of catecholamines and associated with their levels in the circulation. Therefore, the characteristics of epinephrine (E) and norepinephrine (NE) will be included in this review. As well, when comparing different individuals with regards to exercise, the relative intensity of exertion should be considered. For this reason, the production of lactic acid and its relation to the anaerobic threshold (AT), which can be used as a method of equating relative workload intensities in different subjects will be examined.

In this respect, the review of literature will be comprised of eight sections:

1. Catecholamine biosynthesis and the function of DBH.
2. Induction and regulation of catecholamine and enzyme synthesis.
3. DBH and neurotransmitter storage and release into the circulation.
4. DBH as an index of sympathetic activity.

5. Production of lactic acid.

6. Anaerobic threshold.

7. The influence of exercise on plasma catecholamines.

8. Dopamine-β-hydroxylase and exercise.

2.1 CATECHOLAMINE BIOSYNTHESIS AND THE FUNCTION OF DBH

The pathway and the sites of catecholamine biosynthesis, along with the properties of dopamine-β-hydroxylase will be discussed in the following paragraphs. This is to provide background information for further review of the effects of stress on catecholamines and the biosynthetic enzymes.

The synthesis and release of catecholamines occurs in adrenergic tissues. These structures can be classified under two main subdivisions (Blaschko,1973): (1) Adrenergic neurons, noradrenergic or dopaminergic; found mainly in postganglionic sympathetic fibres and in the central nervous system. The amines reach only those excitable units that are innervated by them. (2) Chromaffin cells; found mainly in the adrenal medulla or singely outside the adrenal glands, the amines serving as hormones.

Catecholamines are synthesized from tyrosine in a pathway involving four enzymes: (1) tyrosine hydroxylase (TH), (2) DOPA-decarboxylase, (3) dopamine-B-hydroxylase (DBH), (4) phenylethanolamine N-methyltransferase (PNMT) (Axelrod, 1977). In adrenergic neurons, it appears that some amine is found in the cell body and is transported down the axon towards the endings by axonal flow (Blaschko, 1973). However the main site of amine formation is in the periphery, in or near the nerve endings (Blaschko, 1973). Tyrosine hydroxylase is the rate-limiting enzyme of the catecholamine biosynthetic pathway (Axelrod,1977) and changes in enzyme activities, particularly of TH will affect the rate of amine synthesis.

In the catecholamine biosynthetic pathway the function of dopamine-B-hydroxylase (E.C.1.14.17.1.) is to hydroxylate dopamine on the B-carbon to form norepinephrine (Axelrod, 1972). DBH is a copper containing enzyme characterized as a tetrameric glycoprotein with a molecular weight of close to 290,000 (Rosenberg & Lovenberg, 1980).Each subunit has a molecular weight near 75,000 and the carbohydrate content of human DBH is approximately 13 % (Rosenberg & Lovenberg, 1980). Oxygen and ascorbate are required as cofactors of the reaction catalysed by the enzyme (Fush & Geffen, 1960). The enzyme lacks specificity and can B-hydroxylate a wide variety of phenylethylamine derivatives including tyramine and amphetamine (Geffen,1974). Since

dopamine-B-hydroxylase (DBH) catalyses the synthesis of octopamine from tyramine, this product can be used to determine the enzyme activity (Nagatsu & Udenfriend, 1972).

2.2 INDUCTION AND REGULATION OF CATECHOLAMINE AND ENZYME SYNTHESIS

Catecholamine synthesis is induced by stress. This is necessary since with increased release during stress the catechclamine supply in adrenergic tissue must be replenished and maintained. The time lag of catecholamine synthesis following stress and the role of TH and DBH will be examined in this section.

A large dose of reserpine is known to deplete the catecholamine stores in the sympathetic nervous system (Carlsson, 1965). Norberg and Hamberger (1964) found that after total depletion by reserpine in the rat, the first reappearance of fluorescence (i.e. catecholamine) in the cell body occurred at 12 hours and nearly reached resting levels by 48 hours after administration of the drug. The terminals on the other hand showed weak fluorescence at 48-72 hours and recovery to roughly normal intensity required several days. Jarlstedt and Dahlstrom (1972) observed that RNA content in ganglion cells seemed to be maximal 24-48 hours after reserpine and the maximal amount of norepinephrine storage gran-

ules accumulated above a nerve ligation during the third to fourth day. Utilizing continuous cold exposure for 1, 7, 21, or 42 days Kvetnansky (1971) demonstrated a decrease in adrenal epirephrine of rats after one day but returned to control on the subsequent days. Adrenal norepinephrine was unchanged after one day but increased after 7, 21, or 42 days.

The above studies provide evidence that sympathetic nerve stimulation causes an accelerated enzyme and catecholamine synthesis and a time lag is apparent before complete restoration of neurotransmitter occurs. Such a time lag may be expected to occur for the following reasons: (1) if the increased synthesis of granule protein is dependent upon increase in RNA content of the cells, then RNA elevation should precede the increase in granule proteins including DBH (Haymire et al, 1979) (2) some time will be needed for the packeting of proteins into granules (Jarlstedt & Dahlstrom, 1972) and (3) some time is consumed for the transport of the formed granules now synthesizing catecholamines down the axons to the terminals (Jarlstedt & Dahlstrom, 1972).

Increased catecholamine synthesis is dependent on the activities of its synthesizing enzymes. The contribution of tyrosine hydroxylase and dopamine-b-hydroxylase will be discussed.

It has been demonstrated that TH activity increases in adrenergic tissue via two mechanisms: (1) induction (Costa et al,1974; Zigmond & Ben-Ari, 1977; Chalazonitis et al, 1980) and (2) activation (Zivkovic et al, 1974; Masserano & Weiner, 1979; Saltman & Roth, 1980). Induction of TH activity has been shown to occur following brief periods of electrical stimulation of rat superior cervical ganglia (Zigmond & Ben-Ari, 1977) and preganglionic cervical trunk (Chalazonitis et al, 1980). The increase in enzyme activity can be totally accounted for by an increase in the number of TH molecules in the ganglion (Zigmond et al, 1978).

The activity of TH in sympathetic ganglia has also been shown to increase following prolonged exposure to stressful conditions such as exposure to cold for 7 and 21 days (Kvetnansky et al, 1971), administration of reserpine for 4 successive days (Joh et al, 1973) and 3 hours of forced immobilization on 5 consecutive days (Palkovits et al, 1975).

The second mechanism responsible for increased TH activity is activation of the enzyme. It has been shown that increased neuronal activity brings about an increase in the affinity of TH to tyrosine and co-factors (Bustos, Roth, & Morgenroth, 1976) thus increasing its activity and in turn enhancing amine synthesis. Studies have indicated that activation of TH may be mediated by a cyclic AMP-dependent

protein kinase reaction which directly phosphorylates the enzyme (Weiner et al, 1978; Masserano & Weiner,1979).

The rapid activation of TH during nerve stimulation is thought to play a primary role in maintaining the releasable norepinephrine stores in adrenergic neurons during sustained activity (Weiner et al, 1978). Thus the short term regulation of amine formation prevents depletion of neurotransmitter (Blaschko, 1973). On the other hand long term regulation of amine synthesis is most likely brought about by formation of new enzyme molecules (Zigmond & Chalazonctis, 1979). Of interest, exercise is a stress that probably affects the synthesis of catecholamines via the increase in the levels of enzyme activity. Since the amount of catecholamine released during physical exertion increases greatly, these mechanisms are likely responsible for short and long term regulation of the neurotransmitter supply.

Increases in the levels of enzymatic activity are not restricted to the rate limiting enzyme tyrosine hydroxylase. Other enzymes involved in the biosynthesis of catecholamines are also increased (Ross et al,1978; Chalazonitis, Rice, & Zigmond, 1980). DBH is also an inducible enzyme (Molinoff et al, 1970; Kvetnansky et al, 1971; Molinoff et al, 1972; Chalazonitis et al, 1980). DbH induction in sympathetic neurons and adrenal glands occurs under the same stresses as employed in TH induction, that is after reserpine injection

(Molinoff et al, 1970), cold exposure (Kvetnansky et al, 1971) and electrical stimulation (Chalazonitis et al, 1980).

DBH is synthesized in the parikaryon and transported in the synaptic vesicles to the nerve ending of adrenergic neurons (Smith, 1979; Kopin, 1979). The induction of DBH and TH in adrenergic tissues appears to be mediated by a transynaptic process in which acetylcholine exerts its influence on the post-synaptic nicotinic receptors (Molinoff et al, 1972). In the adrenal glands, the increment in TH activity can be prevented by blocking these receptors or by severing the splanchnic nerves (Guidotti & Costa, 1973; Guidotti, Zivkovic, Pfeiffer, & Costa, 1973). Chalazonitis (1980) demonstrated that preganglionic nerve stimulation acts only on the nicotinic receptors of the post-ganglionic membrane and in turn causes a rise in DBH and TH activities in the rat SCG via an increase in their rates of synthesis.

Kvetnansky (1980) has presented a model for stress induction of catecholamine enzyme in the adrenal medulla. The induction is mediated by a transynaptic process where acetylcholine released from the innervating neuron stimulates the nicotinic receptors to activate adenylate cyclase. This induces the increase of cyclic AMP during the first minutes of stress, which in turn activates cytosol cyclic AMP-dependent (or possibly independent) protein kinases. The catalytic subunit of protein kinase may translocate to the

nucleus and initiate mRNA synthesis resulting in elevated catecholamine enzyme synthesis. Waymire (1979) presented evidence of a similar process occurring in the neurons. In this case cyclic AMP elevated the levels of TH and DBH in cultured mouse neuroblastoma and an increased production of mRNA may be one of the steps necessary for these effects.

Neuronal and hormonal regulation of adrenal DBH and PNMT in the stress response has been postulated (Ciaranello, Wooten, & Axelrod, 1976; Ciaranello, 1980). Under stress situations, both splanchnic neuronal firing and pituitary ACTH secretion increase (Ciaranello,1980). The accelerated nerve firing stimulates the adrenal cortical and medullary cholinergic receptors (Ciaranello et al,1976; Ciaranello,1980). ACTH-mediated glucocorticoid production in the cortex may be facilitated by cholinergic stimulation (Ciaranello, 1980) while in the medullary cell, induction of catecholamine enzyme synthesis occurs (Mclinoff et al, 1970; Kvetnansky et al,1971). Increased cortical glucocorticoid production results in synthesis of S-adenosyl methionine (SAM) and ascorbate, stabilizing the medullary enzyme against intracellular breakdown (glucocorticoids inhibit proteolysis of the enzyme) (Ciaranello, 1980). Thus the synthesis of the catecholamine enzymes is increased during stress and simultaneously their breakdown is slowed.

2.3 DBH AND NEUROTRANSMITTER STORAGE AND RELEASE INTO THE CIRCULATION

In this section, the storage of DBH and norepinephrine in adrenergic tissue will be discussed as well as the mechanisms by which they are released and appear in the circulation. Several characteristics of DBH in the blood, including twenty-four hour rhythm, inheritance, and the effects of pathological disorders on its levels will be mentioned.

Sympathetic neurons contain two types of noradrenergic vesicles: (1) large dense-cored vesicles (70-80nm diameter) (2) small dense-cored vesicles (~45nm diameter)(Geffen & Levitt, 1971; Smith, 1973).DBH and norepinephrine have been positively identified in the large vesicles however the definite composition of the small dense-cored vesicles, which comprise a large, often predominant part of the store for norepinephrine in the terminal varicosities is not known (Smith,1979). Although it is not universally agreed that the enzyme is also associated with the smaller vesicles (Weinshilboum, 1978), Geffen (1974) maintains that both large and small granular vesicles contain DBH and norepinephrine. The large vesicles release proteins (i.e. DBH) and the small vesicles release transmitter. Exocytosis appears to be the mode of transmitter and protein release from the vesicles (Axelrod,1972; deRotter et al,1969). Smith (1979) has sum-

marized the views about the events in the terminal varicosities of a sympathetic neuron following the arrival of an action potential from the cell body:

(1) and (2): The large vesicles are assembled from their constituents in the cell body and arrive in the varicosities following fast transport along the axon.

(3) large vesicles secrete proteins and norepinephrine by exocytosis.

(4) Membrane of large vesicles is converted into small coated pits.

(5) Coated pits bud from plasma membrane to give coated vesicles which become electron-luscent small vesicles.

(6) Electron-luscent small vesicles take up or synthesize norepinephrine and become small noradrenergic vesicles.

(7) Alternative possible modes of small vesicle formation; either by budding from smooth endoplasmic reticulum or fission of intact large vesicle.

(8) Small vesicles release their content either by exocytosis or by forming a tight junction with plasma membrane.

(9) Empty small vesicles are recharged with norepinephrine.

(10) Empty small vesicles are ultimately digested in multivesicular bodies.

It should be noted that in nerve organelles about 80 % of the DBH is insoluble (Lagercrantz, Kirskey, & Klein, 1974). Insoluble DBH appears to be a major constituent of the membrane proteins for which the term chromomembrin is

used (Blaschko,1973). DBH is present as a portion of the fraction designated as "chromomembrin A" (Winkler,1976). The complete soluble content of the vesicles are released during exocytosis (Blaschko,1973). This includes the transmitter as well as the "chromogranins" which are the soluble fractions of vesicular proteins (Dahlstrom,1971). DBH is the dominant protein in the soluble phase of the nerve vesicles (Lagercrantz,1976).

Several studies (Foldes, Jeffrey, Preston, et al, 1972; Hortnagl, Winkler, & Lochs, 1972) have found that the molecular size , amino acid composition , co-factors, kinetics or immunological identity are very similar in soluble and bound forms of the enzyme.

DBH is stored in vesicles of the chromaffin cells along with norepinephrine in the adrenal medulla (Winkler,1976). The process of release of protein (DBH) and hormone (NE) from chromaffin cells is similar to that in neurons, that is via exocytosis (Viveros, Arqueros, & Kirshner, 1968). Upon lysis of organelles from the adrenal medulla in hypotonic media, about 50 % of the DBH is membrane-bound and 50 % is soluble and released (Schneider, 1972). This compares to 80 % of the enzyme being insoluble in nerve organelles and may be due to the higher proportion of membrane volume in these smaller particles i.e. neurons (Blaschko,1973). Catecholamines and macromolecules released from

the adrenal medulla appear to be cleared equally well into the circulation (Geffen,1974).

DBH is present in the blood of mammals including rats and humans (Weinshilbaum & Axelrod, 1971b) and is primarily derived from the firing of peripheral sympathetic neurons (Weinshilbaum & Axelrod,1971a). This is supported by Roth and Mulrow (1976) who found that DBH activity was comparable in 8 adrenalectomized patients and in 41 normal volunteers but were very low in 4 patients with severe diabetic neuropathy. Planz and Planz (1979) found that in normal human subjects basal plasma DBH activity in adrenal vein blood (suprarenal vein) was as high as in the periphery of the circulation (iliac vein). This indicates that DBH which is stored in the chromaffin cells of the adrenal medulla is not released into adrenal blood.

Removal of the adrenal gland or the adrenal medulla does not result in a significant decrease in serum DBH activity in the rat (Weinshilbaum & Axelrod,1971a; Weinshilbaum, Kvetnansky, Axelrod, & Kopin, 1971). Treatment of rats with 6-hydroxydopamine (Weinshilbaum & Axelrod, 1971a) or guanethidine (Grobecker et al,1977) which destroy sympathetic nerves in heart and spleen and nerve terminals respectively result in large reductions of serum DBH activity. These results are compatible with the conclusion that a large part of the serum DBH activity originates from sympatetic nerve terminals and not the adrenal medulla.

Since DBH is a large protein, it should have difficulty in passing through the membranes of the blood vessels. There is evidence that the enzyme depends on the lymphatic system to reach circulation (Aberg et al,1974; Foss et al, 1974; Ngai et al, 1974; Geyer et al,1977; Velasquez & Alexander,1979).

Velasquez (1979) demonstrated that carotid artery occlusion in rabbits resulted in an increased interstitial lymph DBH and norepinephrine levels. A time lag was present before they peaked in the plasma, probably due to a delayed entry of newly released enzyme and catecholamine into the bloodstream. Ngai (1974) found that sympathetic stimulation in dogs resulted in an increase in lymph flow possibly due to an increase in the volume of lymph. The "total lymph DBH output" reflected the DBH released into the lymphatic system, however the amounts of DBH present in the lymph were too small to produce significant alteration in serum DBH activity. In Aberg's (1974) study DBH is present in human lymph in concentrations approximately one third that of serum. The ratio of DBH activity in lymph and serum were positively correlated but it was not possible to determine whether the DBH in blood was derived from lymph or vice versa. Geyer (1977) later suggested that the DBH released from the sympathetic neurons is normally delivered to the general circulation via the lymphatics and also rapidly recirculates through the lymphatics.

Of interest, there is no known function for DBH in the blood (Rush & Geffen, 1980). The enzyme is quite stable and does not lose its activity following prolonged exposure of the serum to room temperature (Eshel et al, 1978). Weiss et al (1980) have demonstrated that storing frozen blood samples for up to two years, and that thawing and refreezing them do not affect the DBH activity. The activity of the enzyme is present in the plasma but not in the cellular elements of the blood and there is no difference between the serum and plasma when heparin is used as the anticoagulant (Weinshilboum & Axelrod, 1978).

It has been indicated that DBH activity in human blood has a twenty-four hour rhythm with its activity being lower at night and highest during the day (van Cauter & Mendlewicz, 1978). As well serum DBH values in normal subjects are quite stable over long periods of time (Weinshilboum & Axelrod, 1971b; Nagatsu & Udenfriend, 1972; Horwitz et al, 1973; Ogihara & Nugent, 1974; Lamprecht, Andres, & Kopin, 1975; Grant et al, 1976). The levels of the enzyme are low in newborns and increase in most subjects by several orders of magnitude during growth and development (Perkulova & Lozovskii, 1979; Vincent, 1980). Lamprecht (1975) showed that the levels of DBH vary with age but in older adults the levels of the enzyme activity remain constant over intervals of up to seven years.

Studies measuring human blood DBH have employed different assay methods. It is therefore important to recognize these varying methods when comparing data from independent studies. Nagatsu and Udenfriend (1972), using a spectrophotometric method with tyramine as substrate found a range in human plasma DBH of 3-100 umol/min/L. Weiss et al (1979), using the same method, observed a similar range of 0-79 umol/min/L. On the other hand Planz and colleagues (1975), using a coupled radiochemical assay with tyramine as substrate found human plasma DBH to range in value from 66 to 492 nmol/20 min/ml.

It is clear from the above values that the normal levels of DBH in human plasma may vary by several orders of magnitude between individuals (Planz, & Palm, 1973). Several studies utilyzing sibling and twin pairs (mono- and dizygote) have established that human plasma DBH is to a very significant extent genetically determined (Ross, Wetterberg & Myrhed, 1973; Levitt & Mendlewicz, 1975; Weiss et al,1980). Examination of the distributions of enzyme activities in large populations has included a subgroup of 3-4 % with very low DBH enzymatic activity. The orders of magnitude differences in the levels of enzyme activity among individuals and the trait of low activity is inherited in a simple monogenic (mendelian) fashion (Weinshilbaum et al ,1975; Weiss et al,1980). Subjects with very low plasma activity should be excluded from studies where changes in DBH

levels are to be determined because of the difficulty in the assay of such small amounts of enzyme.

The levels of DBH are affected by several disorders. Hypertension, renal, cardiovascular, neurological, psychiatric, neoplasmic and endocrine diseases all have an effect on the DBH levels in humans (Weinshilboum, 1978). It is therefore important in recognizing disorders, basal plasma enzyme levels, the twenty four hour rhythm, the age and genetic characteristics of the subjects when homogeneous samples are required for testing.

2.4 DBH AS AN INDEX OF SYMPATHETIC ACTIVITY

The catecholamines released during mass sympathetic discharge are of extreme value during acute physical exertion (Kranerbuhl, 1975). The resultant physiologic changes will be discussed in Section 2.7. The changes in the circulating levels are well documented and show that catecholamines (especially NE) can be used as an index of sympathetic activity. However, upon release into the synaptic cleft, norepinephrine is subject to rapid degradation by monoamine oxidase and catechol-o-methyl transferase (Geyer, Schanberg, & Kirshner, 1977) but the same has not been observed for DBH (Rush & Geffen, 1980), which has been found to be released together with norepinephrine from nerve terminals in a simi-

lar proportion as it is stored in the large dense-cored vesicles (Smith & Winkler, 1972; Wooten & Cardon, 1973). In addition plasma DBH is more easily measured than norepinephrine (Iseki et al, 1979a) and has a longer biological half-life in the circulation (Axelrod, 1972; Bush & Geffen, 1980).

From his studies, Planz (1973, 1974, 1975) concluded that acute increases in sympathetic activity in man can be determined precisely by measurement of the rise in DBH activity in plasma.

Other clinical studies have demonstrated that DBH levels in plasma are increased when there is stimulation of the SNS (Takishita et al,1977; Iseki et al, 1979b). However the magnitude of the changes of the enzyme levels are small when compared with baseline levels of DBH activity (Horwitz, 1973). As there are some individual variations in plasma DBH activity, it is more suitable to examine the responses of the enzyme to stimuli than to simply record the basal activity.

The handgrip isometric exercise studies of Leon et al (1974), and Ogihara and Nugent (1974), and the bicycle ergometer study of Wirth et al (1980) concluded that DBH could not be used as an index of acute sympathetic activity. However, counter to these authors, the body of the literature has demonstrated that acute stress mediated through exercise

of sufficient intensity does result in a significant elevation of plasma DBH. In addition, the intensities of exertion in the negative studies were probably not sufficient to elevate the serum DBH levels.

Hartnagl et al (1976) studied patients with various diseases characterized by prolonged overactivity of the SNS, i.e. head injuries, tetanus and multiple injuries. It was observed that sustained increases in NE and/or E levels which were present for several days or weeks, were associated with a gradual decline in plasma DBH activity. The author suggested that the decline in DBH activity was a result of a depletion in releasable soluble DBH from the nerve vesicles. It was also indicated that DBH activity estimation should be accompanied by concomittant measurement of catecholamines for meaningful interpretation of the results.

Roth and Mulrow (1976) and Kopin (1979) are of the opinion that the strong genetic determinant of the levels of plasma DBH make it difficult for its activity to be employed to determine acute changes in sympathetic nerve activity (Roth & Mulrow,1976; Kopin,1979). According to Kopin (1979), plasma levels of DBH may be useful as a genetic marker for some disorders and may reflect, in a single individual, prolonged changes in the level of sympathetic nerve activity. The presence of large amounts of circulating DBH coupled with a long biological half-life in plasma (Weinshil-

bcum,197e) would require a large change in the SNS tone over a long period of time before alterations in plasma DBH could be detected (Geyer et al,1977)

The exercise studies (Section 2.8) have demonstrated that DBH can be an accurate index of acute increases in sympathetic activity. On the other hand, prolonged sustained overactivity of the SNS results in a decline of resting plasma DBH, probably due to a depletion of the soluble enzyme in the nerve vesicles.

2.5 PRODUCTION OF LACTIC ACID

Different methods have been employed in order to quantify the intensity of exercise as a measure of physical stress. Percent VO2 max (Ekblom et al, 1968; Costill, Thomason, & Roberts, 1973) and heart rate (Hunter, & Critz, 1971) have been used in this way to equate exercise intensities between individuals. More recently, the anaerobic threshold (AT) has been found to be effective in equating relative workload intensities between subjects (Kindermann, Simon, & Keul, ~~1979~~) since it takes into consideration the oxygen consumption at the point where there is an accumulation in blood lactate (Wasserman and Whipp, 1975). This point (AT) can be determined quite accurately by obtaining serial blood

lactate samples during physical exertion (Reinhard et al, 1979). Prior to a more detailed discussion of the anaerobic threshold, the production of lactic acid during exercise will be reviewed since it plays a major role in the determination of the threshold.

One pathway the muscle uses to produce ATP is anaerobic glycolysis. This mechanism is important when oxygen supplies are inadequate. The substrate the muscle utilizes for anaerobic glycolysis is stored glycogen. The glucose-6-phosphate resulting from glycogen breakdown is oxidized to two molecules of pyruvic acid in the pathway of glycolysis. When oxygen is not sufficient the NADH2 produced in glycolysis cannot transfer its 2H to the respiratory chain for oxidative phosphorylation. As well the pyruvic acid molecules cannot enter the mitochondria to be completely oxidized in the Kreb's cycle. To get around this problem, pyruvic acid can accept 2 hydrogens from NADH2, freeing NAD and forming lactic acid. Lactic acid production thus allows glycolysis to proceed when hypoxic conditions in the muscle cell retard NADH2 oxidation in the mitochondria (Armstrong, 1979).

Lactate dehydrogenase (LDH) is the enzyme responsible for the catalysis of the reversible conversion of pyruvic acid to lactic acid. The production and removal of lactic acid are influenced by the content of LDH in the sarcoplasm

of muscle fibres (Skinner & McLellan,1980). LDH can be present as heart specific (H-LDH) or muscle specific (M-LDH) isozymes where M-LDH facilitates the reduction of pyruvate to lactate, whereas H-LDH favours the oxidation of lactate to pyruvate for subsequent utilization in the Kreb's cycle (Kaplan & Pverse,1972; Sjodin, 1976). Studies by Tesch, Sjodin, and Karlsson (1978) and Karlsson et al (1974) have presented evidence that fast twitch (FT) muscle fibres are characterized by great total-LDH activity and a high proportion of M-LDH while slow twitch fibres (ST) have a predominance of H-LDH.

The production of lactic acid is also influenced by catecholamines and glucagon. Glycogen phosphorylase in the liver is activated by E and NE to break down glycogen to glucose which enters the circulation to be carried to muscle (Hartley,1975). Muscle glycogen phosphorylase is also affected by epinephrine and possibly norepinephrine via beta adrenoceptors (Himms-Hagen, 1972), hence with increased concentrations of these hormones the rate of glycogen breakdown in liver and muscle should increase (Hartley, 1975). The concentration of glucagon in the blood is known to increase during prolonged physical exertion (Galbo, Holst, & Christensen, 1975) in order to maintain the blood glucose level constant. During exercise both E and NE secretions into the circulation are increased. Thus, it is not surprising that a correlation between CA and lactate during exercise in humans

has been demonstrated (Pequignot et al,1980). This is in line with the findings that beta-adrenergic blockade reduced the plasma lactate levels in running dogs due to the inhibition of muscle glycogenolysis (Issekutz, 1978).

Jorfeldt (1970) has suggested that FT fibres produce lactate during activity and that, simultaneously, the ST fibres take up lactate from the blood as well as directly from the FT fibres for oxidation. Tesch et al (1978 and 1980) concluded that FT fibres are better than ST fibres at forming lactate because of their high total-LDH and M-LDH activities. As well, lactate production was related to the percent of FT fibres present (Tesch et al,1980). In accordance with Jorfeldt (1970), Essen et al (1978) observed a moderate relationship (r=0.54) between the percent ST fibres of the human vastus lateralis and the lactate removal rates from the blood. This probably reflects the high capacity of ST fibres to oxidize lactate due to its predominant H-LDH content.

During exercise, there appears to be a preferential recruitment of specific muscle fibre types (Skinner and McLellan,1980). Secher et al (1976), Essen (1978) and Thomson et al (1979) postulated that glycogen depletion patterns during exercise follow muscle fibre recruitment. During work at low intensities there is a primary reliance upon oxidative fibres (i.e. ST fibres) for contractile activity and

that a major use of anaerobic fibres (i.e. FT fibres) only occurs at high work levels or when the aerobic fibres are depleted of glycogen during prolonged low intensity work (Armstrong et al, 1974). The fibre type recruitment is preferential but not exclusive. For example, it is probable that during intense short-term exercise the predominant fibres recruited may be FT but that ST fibres are probably recruited as well. The duration of exertion is short enough that little glycogen is depleted from the ST fibres and thus do not appear to be recruited..

During exercise both intracellular lactate production and lactate release increase (MacDougall,1977). Marked differences between skeletal muscle and blood lactate concentrations during heavy exercise have been observed, demonstrating a concentration gradient from the muscle to the blood (Karlsson, Diamant, & Saltin ,1968; Karlsson,1971). Lactate formed in the muscle during exercise is released at low and moderate workloads but accumulates in the tissue at heavy workloads when the rate of formation is high (Jorfeldt et al,1978). Thus there is a levelling off of lactate release from exercising muscles with increased muscle lactate concentrations. After the termination of exhaustive exercise, the lactate content of blood continues to increase and reaches a peak after 5-8 minutes into the recovery period (Sahlin et al,1976; Hermansen & Vaage, 1977; Tzankoff et al,1979).

2.6 ANAEROBIC THRESHOLD

There appear tc be three stages cf physiclogic and metabclic charges accerpanying exercise prcgressing from lcw tc maximal intensity (Kindermann et al, 1980, Skinner, & McLellan, 19c0). These stages will be discussed, followed by the definitien cf the anaerobic thresbold and its prcpcsed use in equating exercise intensities between individuals.

As the levels cf lcw intensity exercise increase, a greater amcunt cf oxygen is extractec from the blccd by the wcrking tissues. This results in a lower fraction of oxygen in the expired air (FeC2) and a greater amount of C02 is produced and expired (FeCC2) as well (Skinner & McLellan,198C). Cbserved during this stage are linear increases in oxygen ccnsurptien (V02) and heart rate (HR) (Wasserman & Whipp,1975; Kindermann et al,1979). Little cr nc lactate is fcrmed during this stage and aerobic metabclism is primarily involved (Skinner & McLellan,1980).

With further increases in worklcad intensity, a nonlinear increase in minute volume expired (Ve) and minute volume of carbcn dicxide expired (VeC02) (Wasserman & Whipp,1975), an increase in FeO2 without a correspcnding decrease in FeC02 (Clcce & Campbell, 1969), plus a rise in blcod lactate from approximately 2 mmcl/l (Kindermann et al,1979) are observed. This initial rise in blcod lactate

does not result in a drop in blood pH (Reinhard, Muller, & Schmulling, 1979). Ve is increased because of the stimulation of the respiratory center by the higher levels of CO2 and lactate. A higher VeCO2 results from the combined effect of the elevated Ve and blood lactate content (Wasserman et al,1967). Therefore, this stage is characterized by a rise in blood lactate without an accompanied fall in pH (Reinhard et al, 1979) because the impending metabolic acidosis is compensated by the increase in VeCO2 owing to buffering by the bicarbonate system (Wasserman & Whipp,1975). This second stage can be termed "compensated metabolic acidosis" and blood lactate can go up to about 3-4 mmol/l before a drop in pH occurs (Reinhard et al,1979).

VO2 and HR rise linearly with increasing intensity to about 65-90 % VO2 max but at near maximal workloads, both begin to plateau (Skinner & McLellan,1980). Rapid increases in blood lactate occur at intensities which raise the level above 4 mmol/l (Kindermann et al, 1979). Ve increases further and a continuous rise in VeCO2 is observed in an attempt to compensate for the large rise in lactate; FeCO2 thus declines while FeO2 continues to rise (Skinner & McLellan,1980). In this stage, the rise in blood lactate is accompanied by a fall in blood pH and can be termed "uncompensated metabolic acidosis" (Reinhard et al,1979).

The term "anaerobic threshold" (AT) has been utilized to describe the onset of both compensated metabolic acidosis (Reinhard et al,1979) and uncompensated metabolic acidosis (Kindermann et al,1979; Skinner & McLellan,1980). For purposes of this study, the former point will defined as the anaerobic threshold. At this point the lactic acid production in skeletal muscle increases and diffuses into the circulation, resulting in non-metabolic production of carbon dioxide which serves as an immediate ventilatory stimulus. The lactate response appears to be due mainly to anaerobiosis since the increased energy requirements occur at the same time as a possible reduction or occlusion of muscle blood flow, an increased recruitment of FT fibres; which are better suited for anaerobic glycolysis, and a decreased utilization of free fatty acids (i.e. aerobic metabolism) (Skinner & McLellan,1980). Due to the stresses imposed on the physiological and metabolic systems, the anaerobic threshold can be used as a method of equating an individual's exercise intensity (Sady, Katch, & Weltman, 1980). As well, people with different capacities for work can exercise at equivalent intensities when the workloads are determined as a proportion of the anaerobic threshold (eg. 80 % of the oxygen consumption at the AT).

It is better to define the AT in terms of VO2 than workrate, because the AT occurs at the same VO2 independent of the duration of the work increments (Wasserman &

Whipp,1975) used in the testing procedure. As a matter of convenience, the VO2 at the anaerobic threshold will be termed "TVO2" in this review. Whipp, Koyal, and Wasserman (1974) studied subjects who performed cycle ergometer exercise with 15 watt increments to exhaustion. The duration of each increment was 0.08, 0.17, 0.25, 0.5, 1.0 or 4.0 minutes, each being randomly assigned to the subjects on different days. It was found that both VO2 max, and TVO2 were independent of the work increment duration. On the other hand the maximal workrate and AT measured in watts both decreased exponentially as the increment duration increased. It was concluded that the duration of increments do not appreciably affect VO2 max or TVO2, but that the AT in watts is overestimated as increment duration decreases.

Wasserman et al (1973) compared lactate and bicarbonate changes for a 1 and 4 minute workload incremental test. The magnitude of the lactate increase and bicarbonate decrease were less for the 1 minute test than the 4 minute test. However, changes from control values occurred at the same workrate. Based on Wasserman (1973), a 2 minute workload duration is a good compromise between a duration that is too short to get an accurate exercise response and too long to prematurely end the test due to fatigue. The lactate diffusion from the working muscles to the vascular space take from 2 minutes to reach peak values at lower work intensities to 5 minutes at higher intensities (Skinner &

McLellan, 1980). In addition the VO2 reaches steady state within 2-3 minutes at lower workrates and progressively later at higher workrates (Wasserman et al, 1973). The assumption can be made that the delay in lactate to peak in the blood and VO2 to reach steady state would coincide even if the subject was performing at the next stage.

Weltman and Katch (1979) demonstrated that VO2 max is related to the TVO2. Individuals who ranked high in VO2 max correspondingly ranked high in the TVO2. According to MacDougall (1977), the AT occurs at approximately 55 % of the VO2 max in the typical untrained subject, 75 % in the physically active non-endurance athlete and at a level in excess of 85 % VO2 max in the highly trained endurance athlete.

It has been demonstrated that the AT is influenced by endurance training (Williams et al,1967). Sedentary males who trained 45 minutes per day, 4 times a week for 9 weeks showed an increase in their AT of 44 % when expressed as absolute VO2 and by 15 % as expressed relative to VO2 max (Davis, Frank, Whipp, & Wasserman,1979). In a recent study (Sady et al,1980), two groups of overweight females trained on a bicycle ergometer 4 times a week for 8 weeks. The high intensity group trained at a level above the onset of metabolic acidosis (i.e. AT) corresponding to 80 % of their VO2 max. The low intensity group trained at 40 % VO2 max (i.e. below AT). The main finding of this experiment was that the

increase in VO2 was in the high intensity group. Apparently a major effect of training intensity is to increase the amount of work which is performed aerobically before the onset of metabolic acidosis.

Sady (1980) feels that one must train above the level of metabolic acidosis (i.e. AT) in order to cause changes in the VO2. However, MacDougall (1977) presents evidence that long duration sub-AT training elevates the anaerobic threshold as well. On the other hand, Kindermann (1979) maintains that the optimal load intensity for endurance training should be in the range of the compensated metabolic acidosis. Under these conditions there is a high stimulation of the oxidative metabolism in skeletal muscle cells, with only little use being made of mechanisms that would lead to lactate production. It is possible that any training which leads to an increase in the capacity of muscle to oxidize pyruvate and fatty acids would result in an elevation of the anaerobic threshold (MacDougall, 1977).

The traditional measure of exercise intensity is the use of workloads relative to VO2 max (i.e. %VO2 max). However the use of the anaerobic threshold as a measure of exercise intensity is emerging. At the present, it appears that studies using both parameters would present the best method of measuring the intensities. By employing both parameters, the intensities can be determined relative to both a maximal (VO2 max) and submaximal (AT) reference point.

2.7 THE INFLUENCE OF EXERCISE ON PLASMA CATECHOLAMINES

The influence of catecholamines on metabolism is wide in range and beyond the scope of this review. However background information on the various effects of catecholamines will be presented. The importance of exercise as a stress which modifies the levels of both E and NE in the circulation and their relationship to the intensity of the workload and anaerobic threshold will be reviewed. The changes in the catecholamine response to exercise due to training will also be investigated.

Catecholamines stimulate alpha and beta adrenergic receptors to mediate their effects (Harris et al,1965). In general alpha adrenoceptors are associated with vasoconstriction and beta adrenoceptors with vasodilation, positive cardiac inotropic and chronotropic effects, increased metabolism, glycogenolysis and lipolysis (Turner & Bagnara,1976). Norepinephrine primarily stimulates the alpha receptors and epinephrine operates through both alpha and beta receptors (Lefkowitz,1976). Therefore epinephrine increases oxygen consumption, lipolysis, and lactate and pyruvate pro-

duction by activating beta adrenoceptors (Harris et al,1965). Epinephrine as well elevates the blood sugar by increasing the rate of glycogenolysis and gluconeogenesis in the liver (Felig & Wahren,1975; Turner & Bagnara,1976) by stimulating the beta receptors (Arnold & Selbris,1968; Higgins et al,1978; Issekutz,1978).

Both epinephrine (E) and norepinephrine (NE) are found in the blood. Post-ganglionic adrenergic nerve endings are known to secrete NE while the adrenal medulla secretes both E and NE (Guyton,1971). In man, epinephrine accounts for approximately 75 percent of the adrenal secretion, NE for 25 percent, although the relative proportions change under various physiological conditions (Krahenbuhl,1975).

NE secretion appears to be related to physiological stress, or the amount of work attempted by the organism, while E secretion seems to be more directly related to mental stress and emotional response (Daniels & Chosy,1972). As the emotional involvement increases, E secretion from the adrenal medulla increases (Krahenbuhl, 1975). Dimsdale and Moss (1980) found that plasma E was greatly elevated after public speaking (emotional stress) while NE was not, and that the opposite was apparent after exercise (physical stress). Pierce, Kupprat and Henry (1976) studied women athletes during training and competition and found a rise in the urinary levels of NE following training sessions and

competition. The levels of epinephrine in the urine however were significantly higher after competition than after training, suggesting that anticipation of competition imposes a mental stress on the athlete.

The degree of elevation of plasma catecholamine due to exercise is related to the workload intensity. Studies by Haggendal et al (1970), Ketchen et al (1971), Hartley et al (1972a) and Saltin et al (1975) have determined that NE levels increase more rapidly at workloads above 70-75 % VO2 max than below. On the other hand, significant elevations of E appear only near 100% VO2 max. At maximal intensities, NE was found to be elevated approximately 6 fold above resting values, while E increased 4 fold in these studies. However the absolute amounts of NE at maximum (3.2 ng/ml) were much greater than E (0.4 ng/ml).

Davies et al (1974) found that the change in CA is closely related to circulatory stress and relative energy expenditure, expressed as a percent of VO2 max. Thus with larger oxygen requirement during graded exercise, elevated circulatory adjustments mediated by an increased sympathetic impulse flow result in a rise in circulating NE (Haggendal et al, 1970). The percentage of NE which would escape inactivation and reuptake mechanisms in the synaptic cleft would increase disproportionately at higher workloads and overflow into the blood (Haggendal et al,1970; Blanz et al,1975).

The above studies indicate that the disproportionate rise in NE may be related to the anaerobic threshold. The AT is in the vicinity of 60-70 % of the VO2 max of the subjects and it is near these workloads that the rise in NE becomes most apparent. These elevated levels of plasma catecholamines may be a factor in the rise in blood lactate occuring near these work intensities (i.e. AT) since a relationship between CA and lactate production has been demonstrated (Pequignot et al, 1979).

The response of CA to exercise is considerably modified by training. Hartley et al (1972a and 1972b), Pennie et al (1978), Cousineau (1977), and Winder et al, (1979) showed that an endurance training effect is associated with a blunting of the catecholamine response to exercise, so that increases in plasma E and NE are smaller during submaximal exercise of the same relative and absolute intensities following a training programme. For example, Winder (1979) demonstrated that bicycle ergometer work at 58 % VO2 max following 9 weeks of training elevated the plasma NE to 1.55 ng/ml compared to 1.61 ng/ml before conditioning. Similarly, E increased only to 0.42 ng/ml after compared to 0.64 ng/ml before training. As well at 850 Kp (submaximal exercise), in both situations, E and NE were significantly lower after the conditioning period.

LeBlanc et al (1977) observed that NE perfusion in trained subjects caused a greater increase in plasma glucose than in untrained subjects. As well, blood lactate was found to increase only in the untrained subjects. Bloom et al (1976) found that the rise in blood glucose concentration was much greater in athletes than in non-athletes after 16, 24 and 32 minutes of bicycle exercise at respective work-rates of 45 %, 60 %, and 75 % maximum. The rise in lactate, pyruvate and alanine were lower in the trained subjects. As well, the increase in plasma glycerol and free fatty acids were much greater in the athletes. These metabolic responses were associated with lower circulating CA levels in the trained cyclists. LeBlanc (1977) and Bloom (1976) suggest that physical training in humans produces an increased sensitivity of the tissues to circulating catecholamines, especially with regards to beta receptor mediated actions such as mobilization and utilization of substrates.

2.8 DOPAMINE-B-HYDROXYLASE AND EXERCISE

The following discussion will include the response of DBH to exercise, its relation to catecholamines, and finally the effects of training on the changes of its levels due to exertion.

There are similarities in the responses of DBH and catecholamines to physical exertion. As with norepinephrine, the activity of plasma DBH has been shown to increase in response to exercise (Bennett et al,1973; Horwitz et al,1973; Wooten & Gordon,1973; Planz et al,1973; 1974; 1975; Rysanek et al,1975; Klepping et al,1976; Guilland et al, 1979). Similar to plasma catecholamines, the enzyme activity appears to be dependent on the intensity performed. Planz (1975) demonstrated that during physical exercise which elevated stepwise from 12.5 to 100, 200, and 300 Watts, plasma DBH was not affected by the lowest workload, but rapid almost linear elevation to 110, 120, and 130 % resting levels occurred thereafter. Bennett et al (1973) found similar increases in DBH at workloads of 100 Watts and greater. In Planz's study, peak DBH values of 130 % Resting DBH were observed after the highest workload (300 Watts) which was maintained for one minute. The DBH value obtained 5 minutes after the termination of exercise was not significantly different from that obtained immediately after the exercise bout (Planz et al, 1975). This suggests that at higher intensities, above 100 Watts, the peak enzyme activity can be obtained during or very soon after the workload is achieved. This is supported by Bennett (1973) who found that a peak in enzyme activity of 126 % Resting DBH levels occurred during exercise at 200 Watts, or immediately after exercise.

Planz (1975) observed a direct correlation between DBH activity and the logarithm of catecholamine (CA) ; the increase in DBH was almost linear with exercise intensity while the slope of the curve for plasma CA concentration became steeper with increasing workload. A possible explanation for this may be that at low intensities of work the small amounts of catecholamines released are taken up or quickly degraded so that little rise in the plasma levels are observed. On the other hand DBH is not subject to these effects and the released enzyme appears in the circulation. As well, there appears to be a good correlation between the elevation of DBH and norepinephrine but not DBH and epinephrine during graded physical exercise (Klepping et al,1976). This may be attributed to the simultaneous release of DBH and norepinephrine from the nerve terminals, while mainly epinephrine and little DBH is released from the adrenal medulla.

In a study by Frewin et al (1973), treadmill walking at a speed of 3.5 mph and 8.6 % grade for 20 minutes at an ambient temperature of 40 degrees C elevated the plasma DBH by only 10.3 %. However, the blood sample was taken 45 minutes after the termination of exercise. Planz (1973 and 1975) has reported the half-life of plasma DBH after exercise to be 20-30 minutes. Thus Frewin may have seen more significant elevations if samples had been taken sooner after exercise. Lecn et al (1974) studied the effect of hand-

grip isometric exercise on serum DBH activity. The subjects were asked to sustain handgrips at various levels 25 to 100 % of maximum. Only small increases in DBH activity were found. Similar results were reported by Ogihara and Nugent (1974). It is possible that handgrip, though it elevates the blood pressure, is not strenuous enough to elicit a DBH response.

Unlike the catecholamines, little is known about the effects of training on the response of DBH to exercise. Wirth and colleagues (1980) did not determine any increase in plasma DBH activity in either trained or untrained subjects when working for 45 minutes on a bicycle ergometer at 40 % of each individual's previously determined maximal workload (in watts). However, this workload may not have been intense enough to elicit an observable response. Athletes exercised only at 150 watts and the untrained subjects at 100 watts. Planz (1973) and Bennett (1973) found significant elevations in the enzyme over resting values at workloads of 100 watts or more in untrained subjects. Thus Wirth used only borderline intensities, which may explain why no change in DBH was observed. To date only one study has compared the effect of exercise on plasma DBH activity between trained and untrained subjects (Planz et al,1974) In this case it was observed that the increases in DBH and CA were smaller in trained than untrained subjects. However the protocol and the method used to compare the two groups in

this study were not clearly reported. Another study by Geffen and colleagues (1975) demonstrated divergent trends in two groups of subjects after a training period of four months. The physical work capacity (PWC) increased in both groups over the training duration, but resting DBH decreased by 71 % in one group and increased by 50 % in the other. As well in response to an acute bout of exercise consisting of a PWC determination measured by heart rate responses to standardized ergometer workloads, the rise in DBH was greater in both groups after the training period. DBH was elevated by 40.1 % during exertion after 4 months training compared to an 11.8 % rise before the fitness programme was initiated. These results do not agree with those of Planz with regards to the rise in DBH due to exercise in trained and untrained subjects. Geffen suggested that physical training may result in an induction of DBH synthesis leading to an enhanced sympathetic response in fit individuals. This would result in larger increments in serum DBH activity in response to exercise after a training regimen. The results of these two sets of experiments do not provide solid evidence either way regarding the circulating DBH response in trained and untrained subjects or before and after a period of training.

None of the results were corrected for the changes in DBH concentration due to blood volume changes that accompany sympathetic activation. Also the wide range of variation in

basal levels between subjects made the statistical evaluation of changes in small groups very difficult. Probably the best controlled studies are those of Planz (1973, 1974, and 1975), who concluded that "acute increases in sympathetic activity in man can be determined precisely by measurement of the rise in DBH activity in plasma".

In view of the similarities of the release of DBH and catecholamines with exercise stress, it would not be surprising to observe a similar behaviour of the enzyme with CA in relation to fitness. Expected results would be that during submaximal exercise, a smaller elevation of plasma DBH should occur in athletes compared to untrained subjects (Planz et al, 1974). This would be due to the smaller sympathetic response to submaximal exercise in athletes.

2.9 SUMMARY

In summary, DBH is a catecholamine biosynthetic enzyme whose synthesis can be induced by stress, much the same as catecholamines. Along with NE it is released from the sympathetic nerve terminals to the circulation where it may serve as an index of sympathetic activity. The blood levels of both DBH and catecholamines are elevated with exercise. Training is known to lower the catecholamine response to submaximal exercise. However little is known about the effect of training or relative fitness of the subject on the plasma levels of DBH. The use of the anaerobic threshold as determined by the rise in lactate is important when equating submaximal workload intensities between fit and unfit individuals. The use of VO2 max may be more appropriate for higher workload intensities. By using both the AT and VO2 max, the comparison of the response of DBH between these two groups of subjects to a bout of exercise can be made more accurate and meaningful.

Chapter III

RESEARCH METHOD

3.1 INTRODUCTION

This study was performed in order to investigate the difference between trained and untrained subjects with regards to the acute exercise response of plasma dopamine-β hydroxylase. This chapter will present the subjects, the experimental procedures followed, and finally the statistical methods used to analyse the data.

3.2 ORIENTATION SESSION

In order to familiarize the subjects with the experimental setting and procedures, they were called to an orientation meeting. The aims of the study and the methods to be employed during the tests were explained in detail. Each individual was then given the opportunity to walk or run on the treadmill. An informed consent form (APPENDIX M) delineating the aims, procedures, and risks involved in the project was then signed by the participants.

3.3 SUBJECTS

A trained and an untrained group, each consisting of 7 male volunteers ranging in age from 19-33 years, were employed in the study. The trained subjects were middle-distance runners from local athletics clubs and were characterized by maximal oxygen consumptions above 60 ml/kg/min. The untrained group consisted of individuals characterized by maximal oxygen consumptions near and below 50 ml/kg/min. They were not necessarily sedentary but may have been habitual or occasional exercisers at intensities much lower than the trained subjects. The maximal oxygen consumptions of the two groups were consistent with the normal data presented in the Standardized Test of Fitness (1979) manual where VO2 max of 50 ml/kg/min were at the upper end of the 'minimum' (average) group and VO2 max above 60 ml/kg/min were in the 'excellent' category (APPENDIX A).

3.4 PROCEDURE

Two testing procedures were performed with approximately one week between each. In both cases, the subjects appeared at the laboratory in a two-hour post-absorptive state and had not exercised for at least twenty-four hours prior to the administration of the test. The first test consisted of a graded treadmill running protocol to determine the VO2 max. The subjects performed the protocol outlined in Table 1, all at 0.0% grade, according to their group.

STAGE	SPEED (KM/H)		DURATION (MIN)
	TRAINED	UNTRAINED	
Warm-up	9.0	6.0	3
1	12.0	8.0	2
2	15.0	10.0	2
3	17.0	12.0	2
4	19.0	14.0	2
5	21.0	15.0	2
6	22.0	16.0	2
7	23.0	17.0	2

Table 1: Treadmill running protocol for the determination of the maximal oxygen consumption.

The expired gases were collected during the last 30 seconds of each workload. An open system with a two-way valve mouthpiece through which the expired gases were collected into a Tissot chamber was used in this situation. Expired oxygen and carbon dioxide were analyzed by Godart Oxygen and Capnograph (CO2) gas analyzers respectively.

The second test performed was the determination of the anaerobic threshold, which also doubled as the acute exercise situation from which DBH values were examined. A catheter was inserted into the antecubital vein at the onset of a fifteen minute bedrest period. At the end of the rest period a blood sample was removed via the catheter.

Subsequent to the rest period, the subjects performed the graded treadmill running protocol according to their group as listed in Table 2. For reasons previously presented (p. 36), the duration of each workload was set at 2 minutes. This was adequate time for the collection of the blood samples. The selection of the 2 minute interval also made it possible to use a protocol with many small increments to get multiple measurements over the range of exercise intensities.

STAGE	SPEED (km/h)		DURATION (min)
	TRAINED	UNTRAINED	
Warm-up	8.0	6.0	3
1	9.5	8.0	2
2	12.0	9.5	2
3	14.5	12.0	2
4	17.0	14.5	2
5	19.5	17.0	2
6	22.0	18.0	2
7	23.0	19.0	2

Table 2: Treadmill running protocol for the determination of the anaerobic threshold and doubling as the acute exercise bout.

The expired gases were collected during the last 30 seconds of each workload in order to calculate the oxygen consumption of the effort. Blood samples were also collected during the last 30 seconds of each stage and 5 minutes post-exercise. All blood samples were analysed for lactate, dopamine-B-hydroxylase, pH, and hematocrit.

3.5 COLLECTION AND STORAGE OF BLOOD

Each sample consisted of 5.0 ml of blood drawn from the antecubital vein into a syringe via the catheter. 1.0 ml was used immediately for the pH analysis using an automated pH/blood gas analyser. 4.0 ml of blood was placed in a heparinized vacutainer and stored on ice until 0.5 ml could be removed and deproteinized for subsequent use in the lactate assay (deproteinized samples were frozen). The remaining 3.5 ml was used for hematocrit determinations and centrifuged so that the plasma could be separated, frozen, and stored for later DBH analyses.

Following the collection of each blood sample, an equal volume of saline (5.0 ml) was infused in order to keep the catheter patent and to replace the blood fluid lost. Heparin was not used to keep the catheter patent in this study.

3.6 DOPAMINE-B-HYDROXYLASE ASSAY

DBH was assayed by the method of Nagatsu and Ucenfriend (1972) with the single modification that 2.0 ml rather than 1.0 ml ammonium hydroxide was used to elute octopamine from the Dowex-50 microcolumn.

To determine the reproducibility of the assay, 6 replicates of the DBH assay were performed on a resting blood sample. The mean value for DBH activity was found to be 26.73 umol/min/l with a standard deviation of 1.35 umol/min/l (S.D. = 5.1 %). This compares with a standard deviation of 1.8 % for 5 replicates in the study performed by Nagatsu and Udenfriend (1972). In order to ensure that the octopamine production was due to DBH in the blood, another replicate was performed on a boiled plasma preparation of the same sample. The optical density for this boiled preparation was determined as 0.071 (0.66 umol/min/l) compared to a mean O.D. of 1.48 (26.73 umol/min/l) for the other 6 replicates.

Because of the reported wide range of interindividual variation of DBH activity and that increases in DBH activity in absolute units are correlated with the absolute resting levels (Planz and Palm, 1973), changes have been expressed in units relative to the individual resting values (% Resting DBH).

3.7 LACTATE ASSAY

Blood lactate was assayed by the Boehringer-Mannheim Biochemica Test-Combination kit method. This is a spectrophotometric procedure which measures the absorbtion at 340 nm. for NADH formed in the reaction:

L-lactate + NAD+ ----------> pyruvate + NADH + H+ .

To determine the reproducibility of the assay, 6 replicates of the same blood sample collected 5 minutes after a submaximal bout of exercise were performed. The mean lactate value was found to be 21.86 mg % with a standard deviation of 1.44 mg % (S.D. = 6.6 %).

3.8 CRITERIA FOR THE DETERMINATION OF AT AND TVO2

The AT was defined as the first exercise stage prior to that at which a significant accumulation in blood lactate was apparent. The oxygen consumption at this stage was defined as TVO2.

To determine the AT, an analysis of variance was performed for each group on the lactate values across the exercise stages. This was followed by a Scheffe post-hoc com-

parison of the means to identify the first exercise stage at which a statistically significant elevation in blood lactate above resting level was apparent. By subtracting the mean resting blood lactate value from the first significantly elevated mean lactate value, the "criterion difference" in blood lactate was obtained for each group. This technique to calculate the criterion difference in blood lactate in trained and untrained subjects has been used previously (Reid, 1980). This criterion difference was added to the resting lactate values of each subject in the appropriate groups. The last exercise stage having the lactate level less than the sum of the resting lactate value and criterion difference was termed the AT. The oxygen consumption at this stage was termed TVO2. %TVO2 was then simply the percent of the oxygen consumption at the anaerobic threshold.

3.9 DESIGN

Two statistical designs were employed to analyse the data collected during this experiment:

1. DBH response with exercise intensities relative to the AT (i.e. %TVO2). The design of the experiment was a two-way analysis of variance with 6 repeated measures. The two levels of factor A were trained and untrained subjects

and the 8 levels of factor B (repeated measures) were the plasma DBH activities at rest, 50, 70, 80, 90, 100, 105 %TVO2, and the maximum workload achieved.

2. DBH response with exercise intensities relative to the VO2 max (i.e. %VO2 max). The design was a two-way analysis of variance with 7 repeated measures. The two levels of factor A were the trained and untrained groups and the 7 levels of factor B were the plasma DBH activities at rest, 50, 60, 70, 80, 90, and 100 %VO2 max.

3.10 ANALYSIS OF DATA

The following statistical techniques were used for the analysis of the data:

1. Statistics including means and standard deviations of the physiological parameters describing the subjects.

2. One-way analysis of variance with Scheffe post-hoc analysis to determine the criterion difference in lactate concentrations in each group. The AT and TVO2 were then determined using this data.

3. Following the determination of the TVO2 of each individual, the rise in DBH activity expressed as % Resting

DBH , was determined for each workload achieved and subsequently plotted against the corresponding oxygen consumptions (% Resting DBH vs VO2). Due to the small sample size and the exploratory nature of the study, statistical regression was not used to generate the DBH values at the workloads relative to AT. Interpolation of this curve (% Resting DBH vs VO2) was employed to estimate the change in DBH activities associated with rest, 50, 70, 80, 90, 100, 105 %TVO2, and at the maximum workload achieved.

Once the change in DBH activities of the relative intensities were determined for each subject , the values belonging to the members of the trained group were combined and compared to the corresponding results of the untrained group using a two-way ANOVA with 8 repeated measures statistical analysis. This was followed by a Scheffe post-hoc comparison of the means in order to isolate the intensities between which the significant differences occurred.

Although percentile data may not be suitable for analysis of variance due to distributions which may violate the normality and homogeneity of variance assumptions, ANOVA is particularly robust to deviations from these two assumptions (Kirk, 1982). As a precaution all percentile data were analysed by Cochran's test for homogeneity of variance and subsequently found to be homogeneous. Frequency distribution plots were used to verify normality of distribution.

In doing these plots it was noted that the scores fell in the 80-160 range. Due to the fact that values above and below resting values were observed, the distribution did not represent the 0-100 range often associated with the need for data transformation. Therefore ANOVA was selected as the most appropriate statistical method to test for differences between the trained and untrained groups.

4. Interpolation of the curve (% Resting DBH vs VO2) was used to estimate the change in DBH activities associated with rest, 50, 60, 70, 80, 90, and 100 %VO2 max. A two-way ANOVA with 7 repeated measures followed by a Scheffe post-hoc comparison of the means was performed to isolate the intensities between which the significant differences occurred.

5. A one-way ANOVA on the hematocrit values across the exercise stages was performed for each group in order to determine the hemoconcentration effects of the exercise bout.

Chapter IV

RESULTS AND DISCUSSION

The main area of investigation was the comparison of the exercise response of the blood enzyme DBH, to exercise of the same relative intensity between trained and untrained subjects. The exercise intensities were determined relative to both the anaerobic threshold (i.e. %TVO2) and the maximal oxygen consumption (i.e. %VO2 max). In this respect, the results and discussion will be presented in the seven following sections:

1. Physiological characteristics of the subjects groups.
2. The determination of AT and TVO2.
3. Hematocrit and blood volume changes.
4. Plasma DBH exercise response between trained and untrained groups.
5. Plasma DBH response to exercise of various intensities.
6. Discussion of the DBH response to exercise.
7. The use of plasma DBH as an index of sympathetic activity.

4.1 PHYSIOLOGICAL CHARACTERISTICS OF THE SUBJECT GROUPS

PARAMETER	TRAINED		UNTRAINED	
	Mean	S.D.	Mean	S.D.
Age (yrs)	26.8	5.6	24.0	3.0
Height (cm)	177.8	3.4	181.9	5.9
Weight (kg)	65.6 *	4.1	83.4	7.4 *
VO2max (ml/kg/min)	69.6 *	5.6	50.6	1.7
VO2max (l/min)	4.5	0.5	4.2	0.4
TVC2 (ml/kg/min)	62.6 *	4.9	42.0	3.1
AT (%VO2 max)	90.1	5.9	83.2	7.5
lactate 5 min post ex. (mg %)	89.3	38.4	86.1	19.0
Resting DEH (umol/min/l)	79.1 *	21.3	59.4	20.9

* Significant at 0.05 level

Table 3: Physiolcgical characteristics of trained and untrained subjects.

The means and standard deviations of the parameters describing the physiological characteristics of the trained and untrained subjects are presented in Table 3.

Significant differences with respect to weight and VO2 max expressed in ml/kg/min were determined, the trained individuals being lighter and possessing superior aerobic capacities relative to body weight. This is in agreement with Rusko et al (1978) who reported higher VO2 max in endurance trained persons. The maximal oxygen consumptions of 50.6 and 69.6 ml/kg/min in the untrained and trained groups respectively were within the criterion defined at the outset concerning the selection of subjects. In addition to being a widely used selection parameter, the relative measure of oxygen consumption was employed in order to avoid ruling out subjects from participating due to their body weight.

The significantly greater values for TVO2 in the trained group is in agreement with the findings of Weltman and Katch (1979) who reported that the oxygen consumptions at the anaerobic threshold (TVO2) were directly correlated with the maximal oxygen consumption (VO2 max).

No significant differences with respect to age, height, and absolute VO2 max in l/min could be determined between the groups. In addition, the lack of significant

difference in the AT expressed as %VO2 max contradicts McDougall (1977), who presented evidence of such a disparity between trained and untrained subjects. The high absolute oxygen consumptions of the untrained subjects may be partially explained by their larger body mass requiring a greater amount of oxygen to oxidize the fuel supplies during exercise. However, this group may be considered trained by many researchers and their fitness may have dampened the expected disparity in the AT expressed as %VO2 max. In relative terms, 50.6 ml/kg/min is in the 'minimum' (average) range in the Canadian population and 69.6 is 'excellent' (Standardized Test of Fitness, 1979; n=5576). Thus compared to the Canadian population, the trained and untrained groups are two standard deviations apart. These findings suggest that not all subjects or groups of subjects respond in the classical manner of higher proportions of maximal oxygen consumptions at the AT with training. The results are confounding, especially when the trained subjects selected for this investigation followed very rigorous training regimens compared to the untrained subjects. Since there is a range in the appearance of AT expressed as %VO2 max in the general population, the untrained group may represent a chance selection of such subjects with genetically high threshold values.

In the present study, the untrained subjects met the criterion of lower VO2 max relative to body weight, but only

after the second treadmill run, the AT test doubling as the acute bout of exercise, were the high anaerobic threshold values found in this group. The lactate values obtained 5 minutes post-exercise were not significantly different between the groups suggesting that the end-point of exercise was similar in relative terms for both sets of subjects. The peak values were lower than expected, that is, lower than 100 mg %, in the trained group. The 24 hour period without exercise prior to the test may not have been sufficient for complete glycogen repletion to occur from the previous training bout in the trained subjects. In addition the two trained subjects (FS and MJ) with the lowest lactate values had fasted overnight. Another factor to be considered is the greater oxidative capacity of the muscle fibres in trained subjects. Since well trained endurance athletes tend to have higher percentages of ST fibres which possess a predominant H-LDH profile, they are capable of metabolizing the lactate produced by the FT fibres, thus resulting in lower peak lactate values. The test protocol may have caused a selective glycogen depletion in the FT fibres due to the high running speeds, thus reducing their capacity to produce lactate. It is also possible that both selective FT muscle glycogen depletion and the greater capacity of trained muscle to metabolize and remove lactate were simultaneous factors resulting in the lower than previously reported post-exercise values. This would reduce the expected

disparity in post-exercise lactate between the groups in this study.

The mean absolute resting values of plasma DBH for the two groups of subjects are outlined in Table 3. The levels along with the large standard deviations are in the same range as those described by Nagatsu and Udenfriend (1972). The resting levels were significantly greater in the trained group. This difference is probably due to genetic determinants of basal plasma DBH activity (Geffen et al, 1975) and to the relatively small group sizes.

4.2 DETERMINATION OF AT AND TVO2

The means of lactate and pH values at each stage (increasing workloads) for trained and untrained subjects are presented in Figures 1 and 2 respectively. These results are in accord with those described by Reid (1980). As expected, the drop in blood pH lagged behind the accumulation in lactate, indicating that compensatory mechanisms such as increased Ve and VeCO2 were in operation (Skinner & McLellan, 1980) in order to compensate for the impending metabolic acidosis. As the lactate production in the working muscles and the accumulation in the circulation increased further, the buffering mechanisms were no longer adequate and the drop in pH occurred.

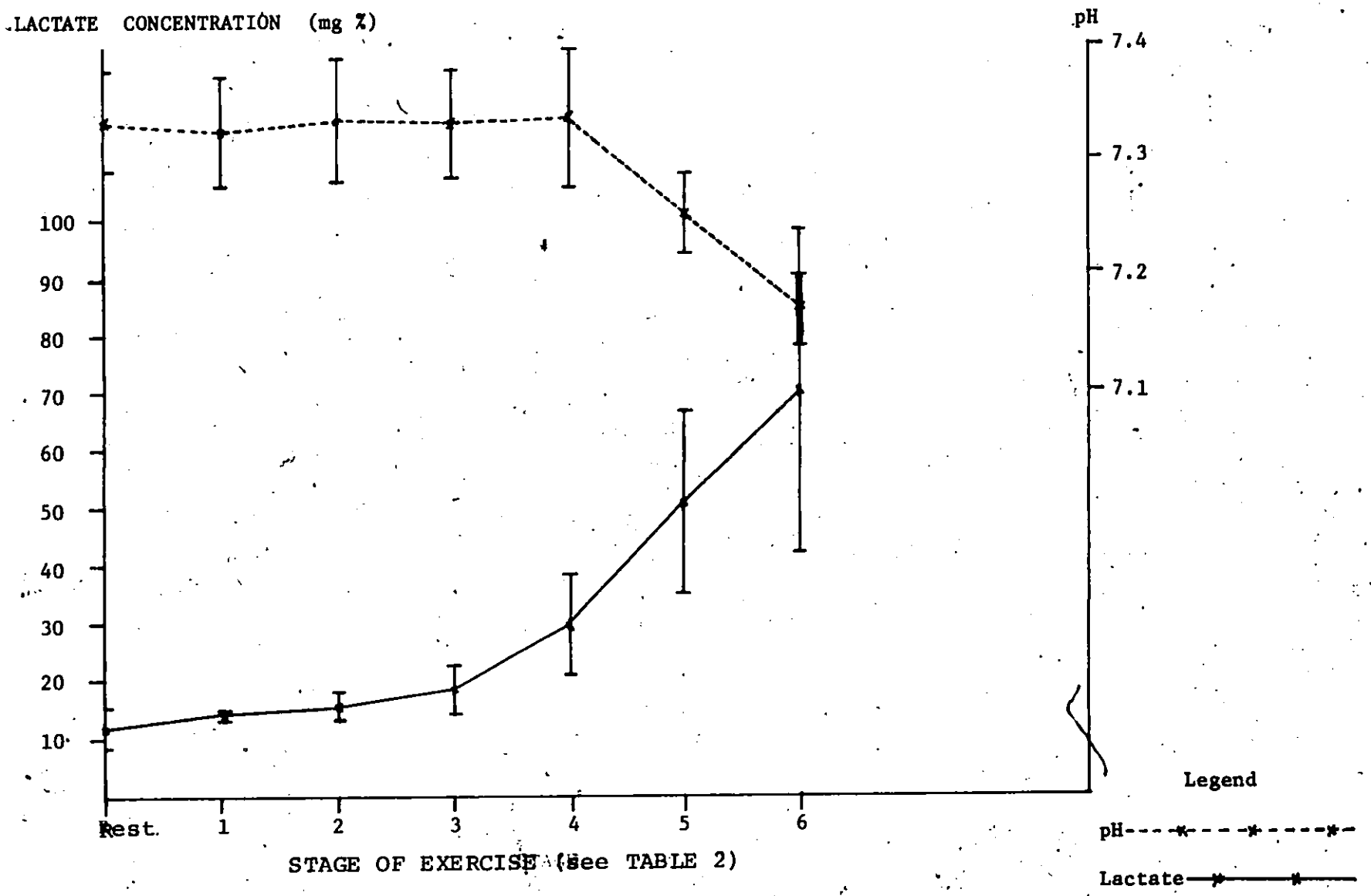

Figure 1: Mean values of pH and lactate concentration at stages of increasing treadmill speed in trained subjects.

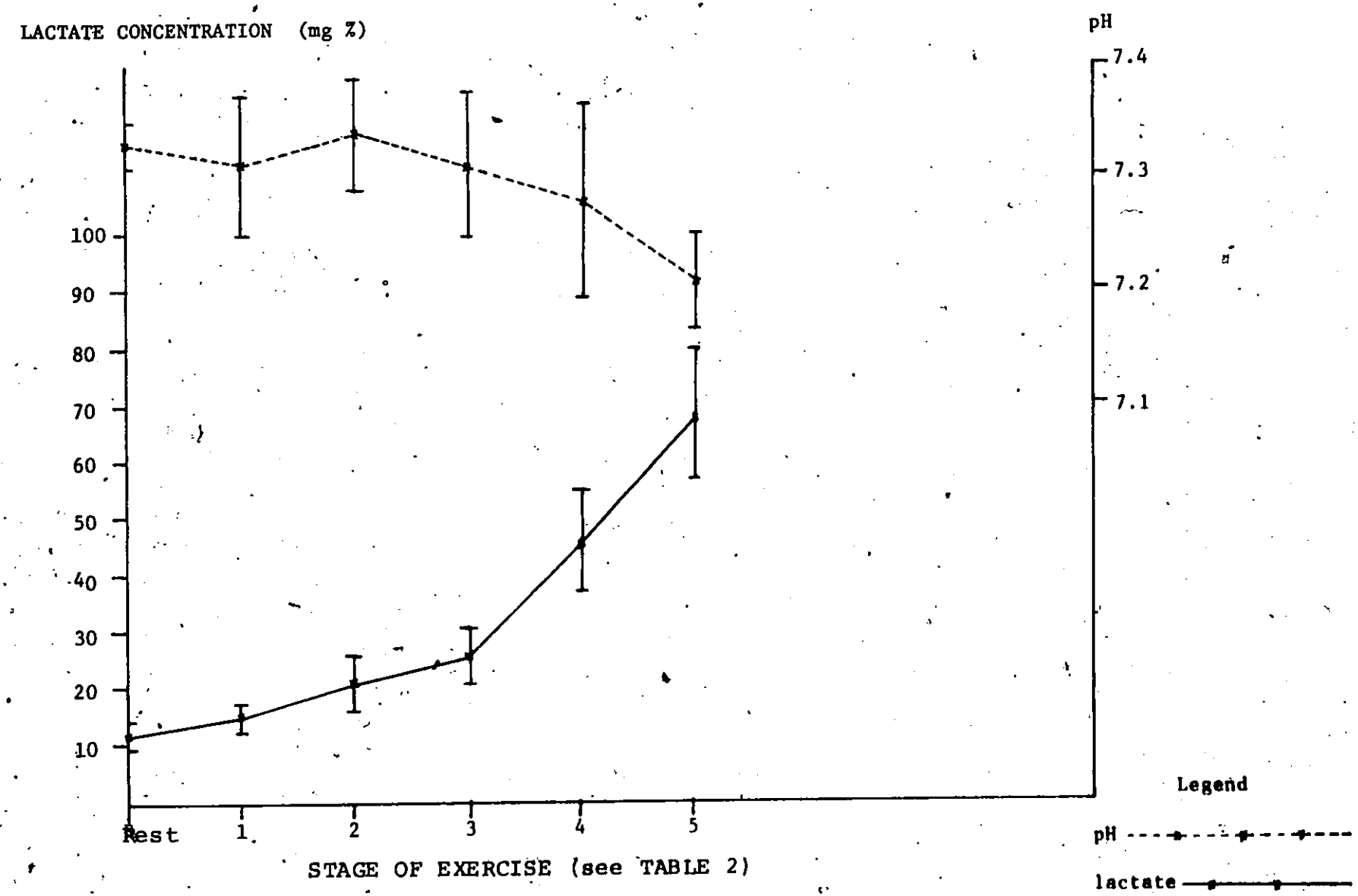

Figure 2: Mean values of pH and lactate concentration at stages of increasing treadmill speed in untrained subjects.

To determine the AT, an analysis of variance was performed for both trained and untrained groups on the lactate values at rest and across each exercise stage. This was followed by a Scheffe post-hoc comparison of the means, performed in order to isolate the first exercise stage at which the mean lactate levels were significantly elevated above those at rest. From the Scheffe analysis, the criterion difference in lactate was calculated by subtracting the mean resting lactate value from the first significantly elevated lactate value.

The summary of ANOVA for lactate values at rest and across each exercise stage in the trained group is presented in Table 4. The Scheffe post-hoc comparison of the mean blood lactate values for the trained group is presented in table 5. The corresponding summary of ANOVA and Scheffe analysis for the untrained group are presented in Table 6 and Table 7 respectively.

SOURCE OF VARIATION	SS	df	MS	F
Between subjects	13851.66	6	2306.64	13.12*
Within subjects	5456.55	31	176.02	

* Significant at 0.05 level

Significant differences in the mean blood lactate levels observed across the exercise stages.

Table 4: Summary of ANOVA comparing blood lactate values at successive stages in trained subjects.

Exercise Stage	Rest	1	2	3	4	5	6
Mean lactate conc. (mg %)	12.46	16.05	15.39	16.46	29.54	50.67	69.89
Rest(C)	12.46	1.57	2.91	5.98	17.07	38.19*	57.41*
1	16.05		1.34	4.41	15.49	36.62*	55.84*
2	15.39			3.07	14.15	35.28*	54.50*
3	16.46				11.08	32.21*	51.43*
4	29.54					21.13	40.35*
5	50.67						19.22*

*Significant at 0.05 level; S (.05)= 38.19

First significant elevation above resting lactate levels occurred at exercise stage 5.

Criterion difference in lactate = 38.19 mg %.

Table 5: Scheffe post-hoc comparison of mean blood lactate values at successive stages in trained subjects for the determination of criterion difference in lactate required for the anaerobic threshold.

SOURCE OF VARIATION	SS	df	MS	F
Between subjects	13956.92	5	2791.78	50.52*
Within subjects	1657.80	30	55.26	

* Significant at 0.05 level

Significant elevation in the mean blood lactate levels observed across the exercise stages.

Table 6: Summary of ANOVA comparing blood lactate values at successive stages in untrained subjects.

Exercise Stage	Rest	1	2	3	4	5
Mean lactate conc. (mg %)	12.08	15.14	20.58	25.29	45.73	67.86
Rest(C) 12.08		3.06	8.50	13.21	33.65*	55.78*
1 15.14			5.44	10.15	30.59*	52.72*
2 20.58				4.71	25.15*	47.28*
3 25.29					20.44*	42.57*
4 45.73						22.13*

*significant at C.05 level; S(.05)= 33.65

First significant elevation above resting lactate levels occurred at exercise stage 4.

Criterion difference in lactate = 33.65 mg %.

Table 7: Scheffe post-hoc comparison of mean blood lactate values at successive stages in untrained subjects for the determination of criterion difference in lactate required for the anaerobic threshold.

The criterion differences in lactate concentrations over rest, required for significance in the Scheffe analysis were determined to be 38.19 mg % in the trained and 33.65 mg % in the untrained subjects. These values were added to the resting lactate values for each subject in their respective groups, to identify the stage at which a significant increase in blood lactate had occurred. With this procedure, the AT was defined as the first stage prior to that which a significant accumulation in blood lactate had occured (i.e. the last exercise stage having the lactate level less than the sum of the resting lactate value and the criterion difference in lactate. TVO2 was then defined as the oxygen consumption at the AT. The raw data presenting the stage at which the AT and TVO2 occurred in each subject can be found in APPENDICES C and D (VO2, Ve, Lactate, and pH Observed at Each Stage in Trained and Untrained Subjects.)

For two subjects (NJ and DS) in the trained group, the addition of 38.19 mg % to their respective resting lactate levels resulted in lactate values greater than those achieved during exercise. In these two cases, the stage previous to the largest drop in in pH was used to determine the TVO2. This criterion was chosen because it was the pat-

tern observed in 6 (BE, RC, DD, PD, LR, BS) of the nine subjects whose pH values were available. In addition, the Ve became disproportionately large at these same points.

In the present study, seven subjects in each group were tested. Two subjects (PS and GO), one in each group, were withdrawn from further statistical analyses. With regards to subject PS, no breakaway lactate response, sharp drop in pH, or disproportionate increase in expired ventilation could be determined. Since the TVO2 was required for further data determination but could not be isolated in this subject, his initial data was removed from further analysis. Subject GO could only perform three stages and produced low lactate values compared to the other members in the untrained group. The oxygen consumption attained during the treadmill performance was also quite low compared to the others, therefore, his data was removed from further analysis.

4.3 HEMATOCRIT AND BLOOD VOLUME CHANGES

In order to control for changes in blood volume that often accompany exercise, an analysis of variance was performed on the hematocrit values of both groups recorded at each stage during exercise. The summaries of ANOVA for the trained and untrained groups are presented in Table 8. Since no significant differences were revealed with this analysis, hemoconcentration was not a factor influencing the enzyme values.

Trained group

SOURCE OF VARIATION	SS	df	MS	F	Fc(.05)
Between subjects	35.63	6	5.94	0.25	2.92
Within subjects	308.00	13	23.69		

Untrained group

SOURCE OF VARIATION	SS	df	MS	F	Fc(.05)
Within subjects	35.71	5	7.14	1.57	2.77
Within subjects	81.67	18	4.54		

Fc(.05): critical F value at the .05 level

Differences in mean hematocrit values across the stages not statistically significant in trained or untrained groups.

Table 8: Summaries of ANOVA comparing hematocrit values at successive stages in trained and untrained groups.

4.4 PLASMA DBH EXERCISE RESPONSE BETWEEN TRAINED AND UNTRAINED SUBJECTS

	RELATIVE EXERCISE INTENSITY (%TVO2)							
Group	Rest	50	70	80	90	100	105	max
Untrained	100.0	103.6	105.0	105.9	107.1	110.5	114.8	119.8
TRAINED	100.0	100.7	101.2	102.2	104.0	106.3	108.3	112.6

Table 9: Mean DBH levels expressed as % Resting DBH at rest and during exercise intensities from 50 %TVO2 to maximum workload.

The means of the DBH activities, expressed as % Resting DBH, at rest, 50, 70, 80, 90, 100, 105 %TVO2 and at the maximum workload achieved are presented in Table 9 and graphed in Figure 3. The raw data can be found in APPENDICES G, H, and I (Values for % Resting DBH at Rest, %TVO2, and at the Maximum Workload Achieved in Trained and Untrained Subjects and the Pooled Data).

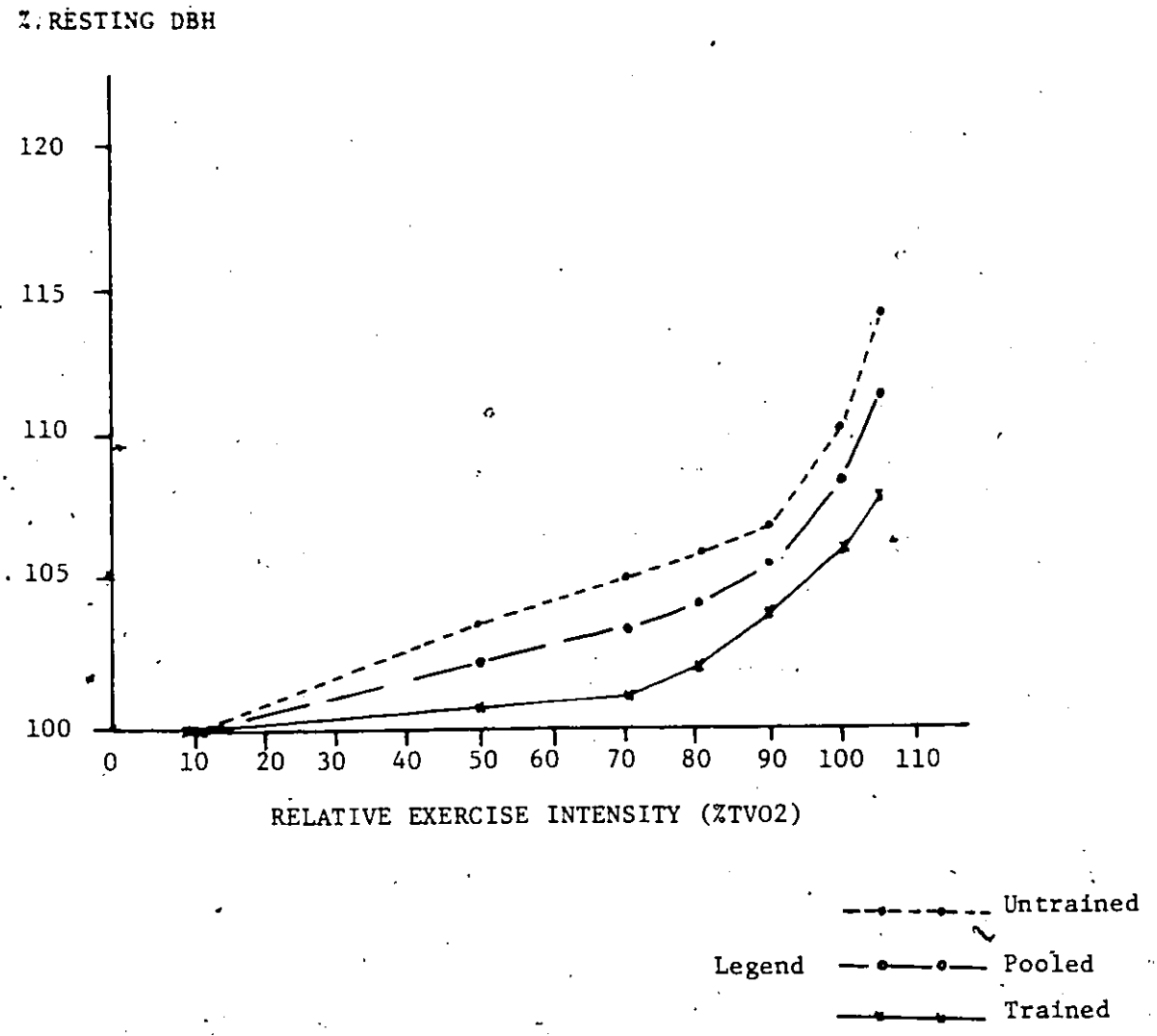

Figure 3: Mean values of DBH (% Resting DBH) with increasing intensity (%TVO2) in trained and untrained subjects and in pooled (trained + untrained) data.

For means and standard deviations of the three data groups, see APPENDICES G, H, and I.

	Relative Exercise Intensity (%VO2 max)						
Group	Rest	50	60	70	80	90	100
Untrained	100.0	104.3	105.1	106.0	107.7	111.7	120.7
Trained	100.0	100.8	101.2	102.1	103.8	106.8	112.6

Table 10: Mean DBH levels expressed as % Resting DBH at rest and during exercise intensities from 50 to 100 %VO2 max.

Table 10. outlines the means of DBH activities expressed as % Resting DBH at rest, 50, 60, 70, 80, 90, and 100 %VO2 max and are graphed in Figure 4 (% Resting DBH vs %VO2 max). The raw data can be found in APPENDICES J, K, and L (Values for % Resting DBH Obtained at Rest and %VO2 max Achieved in Trained and Untrained Subjects and Pooled Data).

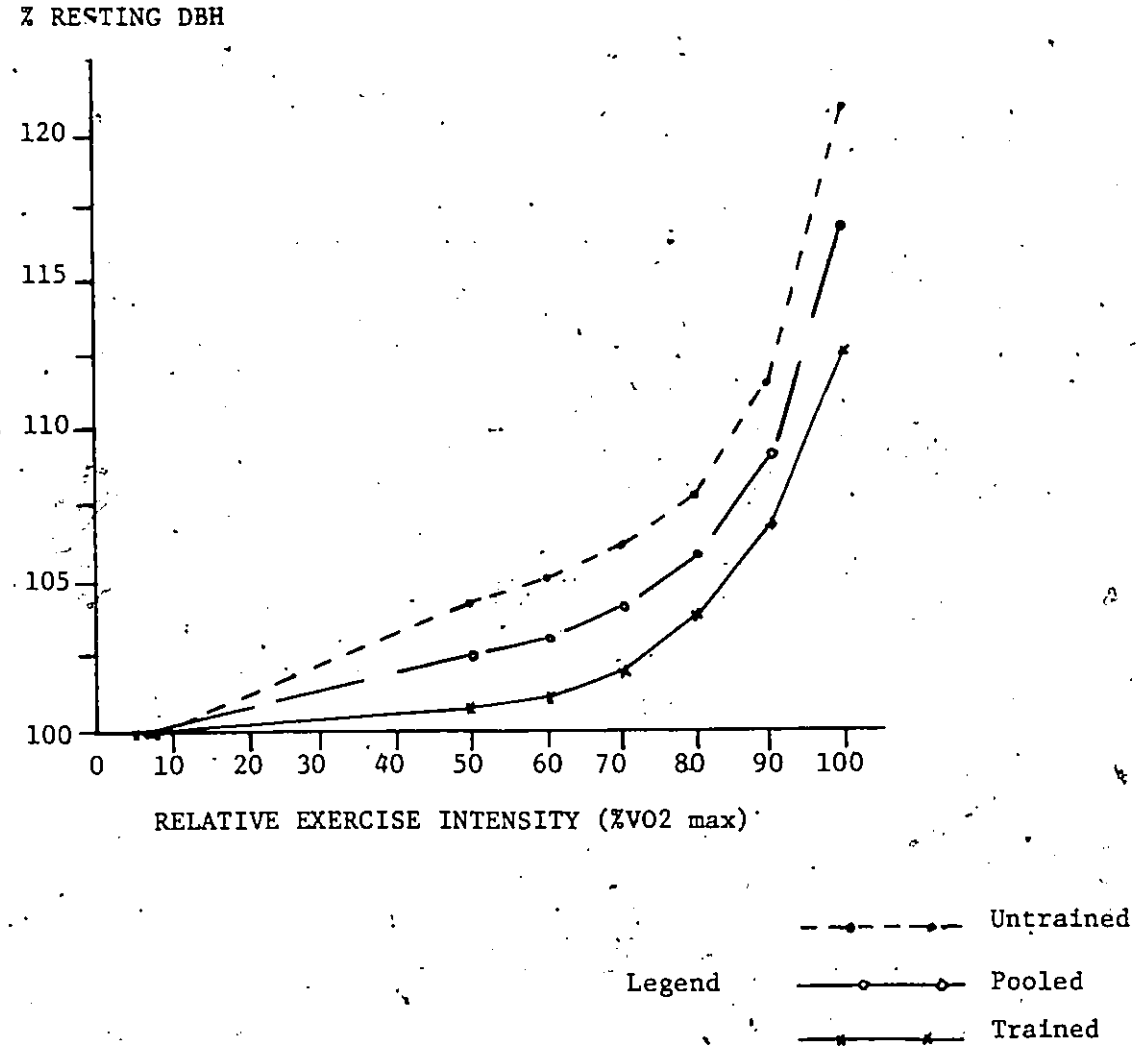

Figure 4: Mean values of DBH (% Resting DBH) with increasing intensity (%VO2 max) in trained and untrained subjects and in pooled (trained + untrained) data.

For means and standard deviations of the three data groups, see APPENDICES J, K, and L.

Table 11 outlines the summary of ANCVA with repeated measures performed on the data in Table 9 (Mean DBH levels expressed as % Resting DBH at rest and during exercise intensities from 50 %TVC2 to maximum).

SOURCE OF VARIATION	SS	df	MS	F
Between subjects				
G	373.67	1	373.67	0.40
error	9445.62	10	944.56	
Within subjects				
E	2424.17	7	346.31	5.14*
E X G (Interaction)	104.33	7	14.90	0.22
error	4717.06	70	67.39	

* Significant at 0.05 level

Significant differences observed between the mean DBH values along the selected exercise intensities (%TVC2).

G: Group (Trained, Untrained)

E: Relative intensity of exercise (%TVC2)

Table 11: 2-way ANOVA with 8 repeated measures on % Resting DBH at rest and during relative exercise intensity as determined by %TVC2.

Table 12 outlines the summary of ANOVA with 7 repeated measures on the data in Table 10 (Mean DBH levels expressed as % Resting DBH at rest and during exercise intensities from 50 to 100 %VO2 max).

SOURCE OF VARIATION	SS	DF	MS	F
Between subjects				
G	340.01	1	340.01	0.48
error	7044.39	10	704.44	
Within subjects				
E	2207.40	6	367.90	5.79*
E X G (interaction)	100.36	6	16.73	0.26
error	3814.16	60	63.57	

* Significant at .05 level

Significant differences observed only between the mean DBH values along the selected exercise intensities (%VO2 max).

G: Group (Trained, Untrained)

E: Relative intensity of exercise (%VO2 max)

Table 12: 2-way ANOVA with 7 repeated measures on % Resting DBH at rest and during relative exercise intensity as determined by %VO2 max.

From the analyses of variance with repeated measures on the plasma DBH values obtained at exercise intensities relative to the AT (%TVO2) and VO2 max (%VO2 max) (Tables 11 and 12 respectively), no significant differences could be determined between the trained and untrained groups. Thus the null hypothesis presented in the 'Statement of the Problem' in Chapter I was found to be true. However, significances at the 0.05 level were achieved only in the DBH values across the selected intensities within subjects both when the intensities were determined relative to the AT (Table 11) and the VO2 max (Table 12). Scheffe post-hoc analyses to find the sources of the intensity effects were then performed (see next Section 4.5).

4.5 PLASMA DBH RESPONSE TO EXERCISE OF VARIOUS INTENSITIES

Because there were no significant differences in the trained and untrained groups, the Scheffe post-hoc comparisons of the mean DBH values across the selected intensities were performed on the pooled data to identify the intensity effects. This was done on the data pertaining to the DBH values at exercise intensities relative to the AT and VO2 max (Tables 13 and 14 respectively).

%TVO2	rest	50	70	80	90	100	105	max
DBB (% rest)	100	102.3	103.1	104.0	105.6	108.4	111.5	116.2
rest	100.0	2.3	3.1	4.0	5.6	8.4	11.5	16.2*
50	102.3		0.8	1.7	3.3	6.1	9.2	13.9*
70	103.1			0.9	2.5	5.3	8.4	13.1*
80	104.0				1.6	4.4	7.5	12.2
90	105.6					2.8	5.9	10.6
100	108.4						3.1	7.8
105	111.5							4.7

* Significant at 0.05 level

Significant differences observed between the mean DBB value at the maximum workload achieved and those at rest, 50, and 70 %TVO2.

max: maximum workload achieved

Table 13: Scheffe post-hoc comparison of mean DBB levels with increasing exercise intensity (%TVO2) in the pooled (Trained + Untrained) data.

%VO2 max	rest	50	60	70	80	90	100
DBH (% rest)	100.0	102.5	103.1	104.0	105.8	109.2	116.6

rest	100.0	0.0	2.5	3.1	4.0	5.8	9.2	16.6*
50	102.5		0.0	0.6	1.5	3.3	6.7	14.1*
60	103.1			0.0	0.9	2.7	6.1	13.5*
70	104.0				0.0	1.8	5.2	12.6*
80	105.8					0.0	3.4	10.8
90	109.2						0.0	7.4

*Significant at 0.05 level

Significant differences observed between the mean DBH value at 100 %VO2 max and those at rest, 50, 60, and 70 %VO2 max.

Table 14: Scheffe post-hoc comparison of the mean DBH levels with increasing exercise intensity (%VO2 max) in the pooled (Trained + Untrained) data.

The Scheffe post-hoc analyses on the DBH values pertaining to exercise intensities relative to both the AT and VO2 max (Tables 13 and 14) in the pooled data show that only at the maximum workload achieved and 100 %VO2 max respectively are the DBH values significantly different than those at rest.

As well, the DBH values at rest, 50, and 70 %TVO2 were significantly different than that found at the maximum workload achieved (Table 13).

The DBH values at rest, 50, 60, and 70 %VO2 max were significantly different than that found at 100 %VO2 max.

4.6 DISCUSSION OF THE DBH RESPONSE TO EXERCISE

The results obtained using exercise intensities relative to AT (%TVO2) and VO2 max (%VO2 max) were very similar. In either method, no significant differences were determined between the trained and untrained groups with regards to the exercise response of plasma DBH. However, significant differences were found in the DBH response to exercise of increasing intensities. The intensity effect was due in both cases to the significant increase in DBH occurring only at

the maximum relative intensity compared to rest. Also, only intensities below 70 %VO2 max were significantly less than maximum values.

Wirth et al (1980) did not find any difference between trained and untrained groups as well as a lack of significant increases in plasma DBH with exercise in either group. The present study also showed no significant difference between trained and untrained groups, however an increase in plasma DBH with exercise intensity was shown in the pooled data.

Even though no significant differences in the plasma DBH exercise response between the trained and untrained groups were observed, trends were apparent in Figure 3 (% Resting DBH vs %TVC2) and Figure 4 (% Resting DBH vs %VO2 max). In both figures, the elevations in DBH with intensity were not as great in the trained subjects compared to the untrained. Such trends are in accord with Planz (1974) who claimed smaller increases in DBH activity during graded exercise in trained subjects.

It is possible that DBH follows responses similar to catecholamines with exercise stress, that is, reduced elevations with exercise in trained subjects (Bloom et al, 1976) although this reduction was not statistically significant in the present study. Since both DBH and norepinephrine are released from sympathetic nerve endings, DBH may follow the

same reduced sympathetic response to exercise as does norepinephrine. This trend is not in agreement with Geffen et al (1976) who found larger increases in DBH activity during graded exercise in the same subjects after several months of training. Two distinctly different groups of subjects were used in the present study and may yield different results than those found by Geffen. The fact that the groups were distinct and not one group studied over a period of time limits the comparison that can be made with Geffen's results.

The curvilinear pattern of increase in plasma DBH activity with graded exercise (see Figure 3: % Resting DBH vs %VO2 and Figure 4: % Resting DBH vs %VO2 max) obtained in this study are comparable to the linear increase observed by Planz et al (1975). The peak DBH values achieved in the pooled data of this study were 116.8 % Resting DBH at the maximum workload achieved, much lower than the 130 % Resting DBH in Planz's study.

As discussed in Section 4.1 (PHYSIOLOGICAL CHARACTERISTICS OF THE SUBJECT GROUPS), even though the relative VO2 max (ml/kg/min) were much higher in the trained subjects, the absolute VO2 max (l/min) and AT expressed as %VO2 max were not significantly different between the groups. This suggests that the disparity in the fitness level between the two groups were not as great as anticipat-

ed. As a result the enzyme response may have been quite similar in the trained and untrained subjects. Therefore no significant differences were found in the exercise response of DBH between the groups.

4.7 PLASMA DBH AS AN INDEX OF SYMPATHETIC ACTIVITY

An additional area of interest of this study was the use of plasma DBH as an index of exercise intensity. The significant increases in plasma DBH seen only at the maximum workload achieved and at 100 %VO2 max in the pooled data indicated that the use of the enzyme for this purpose is limited. The results also indicated that once a moderate exercise intensity was reached (i.e. 70 %VO2 max) the DBH response was not statistically distinct from any higher intensity. To be a valuable indicator of exercise intensity, plasma DBH values would have to be demonstrated to be more sensitive to exercise intensity than were shown in the present study. It is probable that the presence of large amounts of circulating DBH coupled with a long biological half-life would require a large change in the SNS tone over a long period of time before an alteration in plasma DBH could be detected (Geyer et al, 1977). Other authors (Roth & Mulrow, 1976; Kopin, 1979; Wirth et al, 1980) have come to similar conclusions. This is in disagreement with Planz et

al (1975) who concluded that DBH could be used as a sensitive index of changes in sympathetic activity. In addition, other biochemical parameters such as heart rate and catecholamines are much more sensitive (i.e. increasing several fold) than DBH and would preclude its use as an index of acute changes in sympathetic activity due to physical stress.

Chapter V

CONCLUSIONS AND RECOMMENDATIONS

From the results of the present study the following may be concluded:

(1)There was no significant difference in the response of plasma DBH to an acute bout of exercise of intensities relative to either AT or VO2 max between trained and untrained subjects.

(2)There was a significant elevation in plasma DBH with increasing exercise intensity. However, the enzyme was significantly elevated above resting levels only at the maximum intensity as measured by either TVO2 or VO2 max methods. Therefore DBH does not appear to be as sensitive an index of acute sympathetic changes induced by exercise as published reports of other parameters such as heart rate and catecholamines.

From the above conclusions, the following recommendations for further research can be warranted:

(1)The use of the AT as a point of relative exercise intensity between individuals of varying fitness should be further examined.

(2)Studies with large samples are required to investigate the effects of exercise on circulating DBH due to the large variations existing in resting levels.

(3)Longitudinal studies are needed to elucidate the effects, both at rest and during exercise, of training on plasma DBH.

(4)Further studies monitoring the levels of both catecholamines and DBH in the circulation and comparing these parameters between trained and untrained subjects would do much to determine whether they respond in a similar manner to exercise in the two groups.

(5)The use of DBH as an index of acute sympathetic changes would not be recommended.

(6)Further studies should include the measure of both VO2 max and AT in the selection of subjects. This is to avoid using subjects that will not meet the criteria set out for the experiment.

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APPENDIX A

Physical Characteristics of Subject Groups

Subject	Age (yrs)	Height (cm)	Weight (Kg)
Trained Subjects			
AB	19	179.5	63.90
TB	23	179.5	62.50
RB	24	177.0	59.76
NJ	31	174.0	64.75
BS	31	174.0	69.60
DS	33	182.5	71.10
$\bar{X}$	26.8	177.75	65.59
PS*	20	180.0	60.80
Untrained Subjects			
RC	25	179.5	71.20
PD	25	192.0	87.09
DD	23	185.0	85.96
TM	29	180.0	79.40
NM	21	180.0	92.76
TS	21	175.0	83.90
$\bar{X}$	24.0	181.92	83.39
GO*	22	185.5	74.12

* Data not used in subsequent statistical analyses.

APPENDIX B

Values for Maximal Oxygen Consumption and Blood Lactate at 5 Minutes Post-exercise.

Subject	Maximal Oxygen Consumption (ml/kg/min)	Blood Lactate 5 minutes Post-exercise (mg %)
Trained Subjects		
AB	77.18	71.06
TB	62.50	131.47
RB	66.50	144.31
NJ	67.90	54.29
BS	75.53	63.27
DS	67.80	71.46
$\bar{X}$	69.57	89.31
PS*	72.98	32.14
Untrained Subjects		
RC	52.50	92.61
PD	50.00	97.80
DD	50.07	60.48
TM	49.76	113.98
NM	52.90	80.04
TS	48.37	66.87
$\bar{X}$	50.60	86.10
GO*	40.14	34.93

* Data not used in subsequent statistical analyses.

APPENDIX C

Oxygen Consumption (VO2), Expired Ventilation (Ve), Lactate, and pH Observed at Each Stage in Trained Subjects.

Subject	Stage	VO2 (ml/kg/min)	Ve (l/min)	Lactate (mg %)	pH
AB	0			13.77	7.298
	1	48.43	62.72	14.17	7.269
	2	49.45	68.84	17.96	7.277
	3	55.86	80.43	23.95	7.292
	4	60.85	97.45	40.72	7.286
	5	77.18	119.15	65.87	7.277
	6*			71.06	7.235
TB	0			6.44	
	1	37.50	64.30	13.18	
	2	43.60	81.70	16.48	
	3	52.29	95.80	19.56	
	4	55.62	116.50	41.28	
	5	60.15	129.00	75.49	
	6	62.50		111.88	
	7*			131.47	
RB	0			12.77	7.327
	1	48.98	64.47	13.97	7.319
	2	52.41	74.18	16.77	7.347
	3	48.54	85.80	23.15	7.314
	4	56.99	102.15	25.35	7.297
	5	62.45	111.45	41.92	7.228
	6	66.50	117.93	61.48	7.197
	7*			144.31	7.140
NJ	0			13.57	7.409
	1	41.70	62.76	13.57	7.391
	2	50.20	76.83	13.97	7.418
	3	59.20	88.55	16.17	7.408
	4	63.72	101.12	24.95	7.439
	5	67.90	112.07	36.93	7.206
	6*			54.29	7.324

Subject	Stage	VO2 (ml/kg/min)	Ve (l/min)	Lactate (mg %)	pH
BS	0			11.78	7.332
	1	47.64	52.53	15.35	7.359
	2	47.25	62.38	11.78	7.326
	3	60.76	84.66	14.37	7.345
	4	65.80	104.36	22.16	7.339
	5	70.77''	123.25	45.71	7.293
	6	75.53	134.52	52.30	7.176
	7*			63.27	7.198
DS	0			16.57	7.316
	1	36.60	54.55		7.297
	2	39.70	64.34		7.307
	3	51.20	86.34	13.57	7.304
	4	54.50	107.86	22.75	7.328
	5	61.90''	127.38	38.11	7.263
	6	67.80	147.77	53.89	7.137
	7*			71.46	7.219
PS**	0			15.57	7.291
	1	48.35	59.38	16.97	7.290
	2	50.66	68.01	15.57	7.334
	3	61.09	86.54	16.37	7.296
	4	63.40	103.89	23.35	7.310
	5	72.98	118.67	32.14	7.265
	6*			32.14	7.340

* 5 minutes post-exercise

** Data not used in subsequent analyses

'' TVO2

APPENDIX D

Oxygen Consumption (VO2), Expired Ventilation (Ve), Lactate, and pH Observed at Each Stage in Untrained Subjects.

Subject	Stage	VO2 (ml/kg/min)	Ve (l/min)	Lactate (mg %)	pH
RC	0			16.37	7.323
	1	28.37	37.29	20.16	7.265
	2	34.80	48.22	27.35	7.341
	3	39.60''	64.37	32.33	7.298
	4	52.50	93.11	53.69	7.258
	5	50.41	109.41	74.25	7.223
	6*			92.61	7.228
PD	0			10.18	7.366
	1	27.03	46.91	14.17	7.415
	2	31.81	58.09	15.97	7.426
	3	34.97	71.29	20.36	7.429
	4	42.62''	97.93	31.34	7.429
	5	45.87	121.64	73.25	7.268
	6*			97.80	7.311
DD	0			9.58	7.304
	1	31.30	55.81	13.97	7.229
	2	38.50	69.76	15.37	7.274
	3	43.28	86.37	20.76	7.245
	4	47.17''	111.45	37.92	7.248
	5	50.07	133.79	65.27	7.200
	6*			60.48	7.250
TM	0			13.80	7.323
	1	28.41	50.89	15.18	7.315
	2	32.42	63.32	25.64	7.316
	3	43.45''	79.52	30.37	7.328
	4	46.66	88.87	53.05	7.165
	5	49.76	115.41	83.42	7.139
	6*			113.98	7.172

Subject	Stage	VO2 (ml/kg/min)	Ve (l/min)	Lactate (mg %)	pH
NM	0			12.38	7.334
	1	30.08	50.89	13.77	7.326
	2	32.13	63.32	17.37	7.346
	3	39.28''	79.52	22.75	7.281
	4	46.48	88.87	47.70	7.297
	5	46.94	115.41	58.48	7.232
	6			64.47	7.139
	7*			80.04	7.157
TS	0			10.18	7.328
	1	33.97	57.36	13.57	7.308
	2	35.04	66.60	21.76	7.334
	3	39.87''	88.17	25.15	7.273
	4	45.77	106.42	50.70	7.279
	5	48.27	114.71	52.49	7.208
	6*			66.87	7.227
GO**	0			13.97	7.317
	1	30.48	47.16	15.57	7.318
	2	34.95	57.35	27.35	7.331
	3	38.07	64.46	39.32	7.281
	4*			34.93	7.277

* 5 minutes post-exercise

** Data not used in subsequent analyses

'' TVO2

APPENDIX E

Hematocrit, DBH (absolute values), and % Resting DBH Observed at Each Stage in Trained Subjects.

Subject	Stage	Hematocrit	DBH (umol/min/l)	% Resting DBH
AB	0	47	62.2	100.0
	1	50	72.5	116.6
	2	51	55.5	89.3
	3	51	52.2	84.0
	4	51	67.3	108.2
	5	51	68.9	110.7
	6	51	70.1	122.3
TB	0		77.7	100.0
	1		82.3	106.0
	2		80.1	103.2
	3		81.1	104.4
	4		77.7	100.0
	5		93.9	121.0
	6		72.1	92.8
	7		92.4	119.0
RB	0	38	80.7	100.0
	1	43	98.9	122.5
	2	43	85.0	105.3
	3	43	86.4	107.1
	4	44	73.0	90.5
	5	45	80.6	99.9
	6	45	82.4	102.1
	7	47	85.6	106.1
NJ	0		114.2	100.0
	1		138.7	121.5
	2		115.6	101.3
	3		110.4	96.7
	4		122.0	106.9
	5		115.6	101.3
	6		121.3	106.2

Subject	Stage	Hematocrit	DBH (umol/min/l)	% Resting DBH
BS	0	43	53.0	100.0
	1	44	44.0	83.1
	2	43	46.3	87.4
	3	44	62.8	118.5
	4	46	51.3	96.8
	5	44	48.8	92.1
	6	46	63.6	119.9
	7	45	48.1	90.9
DS	0		86.6	100.0
	1		88.8	102.5
	2		86.6	100.0
	3		89.2	103.1
	4		85.7	98.9
	5		83.1	95.9
	6		71.1	82.0
	7		81.8	94.5

APPENDIX F

Hematocrit, DBH (absolute values), and % Resting DBH Observed at Each Stage in Untrained Subjects.

Subject	Stage	Hematocrit	DBH (umol/min/l)	% Resting DBH
RC	0		91.3	100.0
	1		101.6	111.3
	2		84.7	92.3
	3		91.4	100.1
	4		96.6	105.8
	5		106.8	117.0
	6		109.3	119.7
PD	0	40	73.2	100.0
	1	40	72.2	98.6
	2	41	65.8	89.9
	3	42	69.8	95.4
	4	43	75.6	103.3
	5	44	56.6	77.3
	6	44	75.5	103.2
DD	0		58.1	100.0
	1		60.5	104.1
	2		59.8	102.9
	3		57.7	99.3
	4		73.8	127.0
	5		82.4	144.0
	6		62.0	106.7
TM	0	38	31.4	100.0
	1	40	39.5	125.7
	2	40	49.9	158.8
	3	40	45.0	143.4
	4	41	48.5	154.5
	5	42	46.9	149.2
	6	42	44.0	140.2

Subject	Stage	Hematocrit	DBH (umol/min/l)	% Resting DBH
NM	0	42	56.0	100.0
	1	44	46.0	82.2
	2	44	47.3	84.5
	3	44	50.3	89.9
	4	46	49.9	89.1
	5	46	45.7	81.7
	6	46	60.2	107.5
	7	48	48.4	86.0
TS	0	41	46.3	100.0
	1	43	46.3	100.0
	2	43	48.5	104.7
	3	43	46.5	100.5
	4	44	51.4	111.0
	5	44	49.2	106.2
	6	44	43.3	93.6

APPENDIX G

Values for % Resting DBH Obtained at Rest, %TVO2, and at the Maximum Workload Achieved in Trained Subjects.

	RELATIVE EXERCISE INTENSITY (%TVO2)							
	Rest	50	70	80	90	100	105	Max
Subject								
AB	100.0	100.0	100.6	101.7	104.0	107.1	109.4	119.3
TB	100.0	101.8	102.2	102.9	103.2	107.5	112.0	127.0
RB	100.0	100.0	100.1	101.2	103.3	106.8	109.5	110.3
NJ	100.0	102.0	102.9	103.1	103.8	104.8	105.9	106.7
BS	100.0	100.0	100.5	103.6	109.9	118.1	123.7	127.6
DS	100.0	100.5	100.8	100.9	100.0	93.5	89.0	84.8
$\bar{X}$	100.0	100.7	101.2	102.2	104.0	106.3	108.3	112.6
S.D.	0.0	0.9	1.1	1.1	3.2	7.8	11.24	16.1

APPENDIX H

Values for % Resting DBH Obtained at Rest, %TVO2, and at the Maximum Workload Achieved in Untrained Subjects.

	RELATIVE EXERCISE INTENSITY (%TVO2)							
	Rest	50	70	80	90	100	105	Max
Subject								
RC	100.0	101.0	101.2	101.6	101.8	102.3	103.0	114.0
PD	100.0	99.5	96.7	94.5	92.0	89.3	88.0	86.8
DD	100.0	101.8	102.2	102.8	105.5	119.4	140.0	144.0
TM	100.0	120.5	130.0	135.5	141.1	146.8	149.7	155.2
NM	100.0	100.0	100.0	100.2	101.0	102.2	103.4	108.5
TS	100.0	100.0	100.1	100.5	101.2	103.0	104.3	110.5
$\bar{X}$	100.0	103.8	105.0	105.9	107.1	110.5	114.8	119.8
S.D.	0.0	8.2	12.3	14.8	17.2	20.2	24.4	25.2

APPENDIX I

Values for % Resting DBH Obtained at Rest, %TVO2, and at the Maximum Workload Achieved in the Pooled (Trained + Untrained) Data.

	RELATIVE EXERCISE INTENSITY (%TVO2)							
	Rest	50	70	80	90	100	105	Max
$\bar{X}$ n = 12	100.0	102.3	103.1	104.0	105.6	108.4	111.5	116.2
S.D.	0.0	5.8	8.6	10.2	11.9	14.8	18.4	20.5

APPENDIX J

Values for % Resting DBH Obtained at Rest and %VO2 max Achieved in Trained Subjects.

	RELATIVE EXERCISE INTENSITY (%VO2 max)						
	Rest	50	60	70	80	90	100
Subjects							
AB	100.0	100.1	101.3	103.7	108.0	113.9	119.3
TB	100.0	102.0	102.1	102.8	103.1	108.6	127.0
RB	100.0	100.0	100.0	100.6	102.2	105.0	110.3
NJ	100.0	102.0	102.7	103.0	103.2	104.0	106.7
BS	100.0	100.0	100.3	101.5	105.8	114.3	127.6
DS	100.0	100.5	100.6	100.8	100.5	94.9	84.8
$\overline{X}$	100.0	100.8	101.2	102.1	103.8	106.8	112.6
S.D.	0.0	1.0	1.1	1.3	2.7	7.3	16.1

APPENDIX K

Values for % Resting DBH Obtained at Rest and %VO2 max Achieved in Untrained Subjects.

	RELATIVE EXERCISE INTENSITY (%VO2 max)						
	Rest	50	60	70	80	90	100
Subject							
RC	100.0	101.2	101.5	101.8	103.8	108.5	114.0
PD	100.0	98.6	96.5	94.0	91.0	87.6	86.8
DD	100.0	101.8	102.0	102.5	103.0	111.0	144.0
TM	100.0	124.0	129.9	135.5	142.0	148.3	155.2
NM	100.0	100.0	100.2	101.3	104.1	109.1	114.8
TS	100.0	100.0	100.3	100.9	102.3	105.5	110.5
$\bar{X}$	100.0	104.3	105.1	106.0	107.7	111.7	120.7
S.D.	0.0	9.7	12.3	14.8	17.5	19.9	26.1

APPENDIX L

Values for % Resting DBH Obtained at Rest and %VO2 max Achieved in the Pooled (Trained + Untrained) Data.

	RELATIVE EXERCISE INTENSITY (%VO2 max)						
	Rest	50	60	70	80	90	100
$\bar{X}$	100.0	102.5	103.1	104.0	105.8	109.2	116.6
S.D.	0.0	6.8	8.6	10.2	12.1	14.5	21.1

APPENDIX M

INFORMED CONSENT

The present study is designed to compare the response of the blood enzyme, dopamine-B-hydroxylase, to an acute bout of exercise, between trained and untrained individuals.

Two treadmill running procedures will be involved in this investigation. In both cases, the subjects will not have eaten for at least two hours prior to testing. The first test will be the maximal oxygen consumption determination. The trained subjects will warm-up at a speed of 9.0 Km/h for 3 minutes and immediately perform each 2 minute stage at the following speeds: 12, 15, 17, 19, 21, 22, 23 Km/h until exhaustion. Similarly, the untrained subjects will warm-up at 6.0 Km/h for 3 minutes and immediately perform each 2 minute stage at the following speeds: 8, 10, 12, 14, 15, 16, 17 Km/h until exhaustion. Expired gases will be collected through a mouthpiece attached throughout the test.

The second test, performed approximately one week later, will be the anaerobic threshold determination which will double as the acute bout of exercise necessary to investigate the enzyme response. It will also consist of treadmill running to exhaustion. The trained subjects will warm-up at a speed of 8 Km/h for 3 minutes and immediately perform each 2 minute stage at the following speeds: 9.5, 12, 14.5, 17, 19.5, 22, 23 Km/h until exhaustion. Similarly the untrained subjects will warm-up at 6 Km/h for 3 minutes and immediately perform each 2 minute stage at the following speeds: 8, 9.5, 12, 14.5, 17, 18, 19 Km/h until exhaustion. A 5.0 ml blood sample will

be collected from each subject initially at rest, during the last 15 seconds of each stage, and 5 minutes following termination of exercise. These samples will collected through a catheter inserted in a vein located in the arm, prior to exercise. The catheter will remain in the vein throughout the procedure.

Physical discomfort and a certain amount of risk of injury associated with exercise fatigue will likely accompany both treadmill procedures but is expected to be shortlasting. Some discomfort may also result from the insertion of the catheter as well as its presence in the vein during exercise. There are risks, however small, such as infection, collapsed vein, or blood coagulating in the needle, that accompany catheterization and blood sampling. A qualified technician will insert the catheter and will be present for sampling.

The information obtained from this study will aid in assessing the use of dopamine-B-hydroxylase as an index of acute stress. The subjects will benefit by obtaining their maximal oxygen consumption and anaerobic threshold measurements.

All information and data derived from each subject will be presented in such a manner that the individual cannot be identified. Any release of information will be done only with his knowledge and consent.

Inquiries concerning the procedures or any other aspect involved in this investigation will be willingly answered by the researcher.

I, (name)_____, understand the nature of the study and the testing procedures to be undertaken. I am also confident that the risks will be minimal and any discomfort involved will be short-lasting. I consent to participate as a subject in this research project knowing that I am free to withdraw consent and discontinue participation at any time.

Date_____

Signature_____

Witness_____

Parent's signature (under 18)

_____

Norms and Percentile Scores by Age Groups for Predicted Maximal Oxygen Consumption (ml/kg/min)

Males*

Age (Yr.)	17-19	20-29	30-39	40-49	50-59	60-65
Excellent	⩾ 62	⩾ 57	⩾ 49	⩾ 43	⩾ 40	⩾ 36
Good	55-61	51-56	45-48	40-42	36-39	32-35
Minimum	49-54	45-50	40-44	36-39	32-35	28-31
Below minimum	43-48	39-44	35-39	32-35	28-31	24-27
Poor	⩽ 42	⩽ 38	⩽ 34	⩽ 31	⩽ 27	⩽ 23

	Age groups					
Percentile	17 - 19	20 - 29	30 - 39	40 - 49	50 - 59	60 - 65
100	67.9	63.1	54.0	47.4	43.8	40.1
95	60.8	56.2	48.7	42.9	39.2	35.6
90	59.4	54.9	47.6	42.0	38.3	34.7
85	57.7	53.2	46.3	40.9	37.2	33.6
80	56.6	52.2	45.5	40.3	36.5	32.9
75	55.7	51.4	44.8	39.7	35.9	32.3
70	54.9	50.6	44.3	39.2	35.4	31.8
65	54.3	50.0	43.7	38.8	35.0	31.4
60	53.5	49.3	43.2	38.3	34.5	30.9
55	52.9	48.7	42.7	37.9	34.1	30.5
50	52.2	48.0	42.2	37.5	33.6	30.1
45	51.5	47.4	41.7	37.0	33.2	29.6
40	50.9	46.8	41.2	36.6	32.8	29.2
35	50.2	46.1	40.7	36.2	32.3	28.7
30	49.5	45.4	40.2	35.8	31.9	28.3
25	48.7	44.7	39.6	35.3	31.4	27.8
20	47.8	43.8	38.9	34.7	30.8	27.2
15	46.7	42.8	38.1	34.0	30.1	26.6
10	45.0	41.1	36.8	32.9	29.0	25.4
5	43.6	39.8	35.8	32.0	28.1	24.5
0	36.5	33.0	30.4	27.5	23.5	20.0