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**Racial Differences in Cardiovascular and Sympathetic
Reactivity to Dynamic Exercise**

A Dissertation

Presented for the

Doctor of Philosophy

Degree

The University of Tennessee, Knoxville

Ava J. Walker

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DEDICATION

To my dad, who taught me to work really hard for what I wanted.

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To my loving friends in the Land of PhysiOZ. To Ed, that wonderful Wizard who imparts his knowledge with a unique wisdom and humility. To Dave, the Good Witch of the North, for magically appearing from time to time, just when I was most lost. To Bill, the Scarecrow, a wonderful, lifetime friend, who has that special kind of "smarts" you can't find in books. To Don, the Tin Man, who carries his own oil can. To Wendy, that not so Cowardly Lion, who lives by the "courage" of her convictions. To Pete, Toto, a faithful friend. Thanks for making my journey an experience I'll never forget. I have learned: If you can't find your "heart's desire" in your own backyard, maybe it's time to move!

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ABSTRACT

The purpose of this study was to assess possible racial differences in cardiovascular (CV) and sympathetic nervous system (SNS) responses to a graded exercise test. A biracial group of normotensive college-age males (15 blacks, 15 whites) were tested for maximal oxygen uptake (VO_{2max}), resting blood pressure (BP) and heart rate (HR). Subjects performed graded exercise on a cycle ergometer at 25, 50 and 75% of VO_{2max} (6 min at each stage). BP and HR were measured during supine rest, bike rest, and at each stage using a Colin STBP 780 blood pressure monitor. Cardiac output was measured using the CO_2 rebreathing technique, and expressed as cardiac index (CI). At each stage, venous blood was sampled to allow determination of plasma norepinephrine (NE) and epinephrine (E). The results indicated that resting BP was similar for blacks and whites (114/68 vs. 115/68, respectively). However, blacks had a greater BP response to exercise, evidenced by higher systolic blood pressure (SBP) and diastolic blood pressure (DBP) at 50 and 75 % VO_{2max} . Racial differences did not exist for HR, but blacks had higher values for systemic vascular resistance (SVR), while whites had higher CI values, across all conditions. Though no overall racial differences were seen for plasma catecholamine concentrations at rest, blacks had significantly lower levels of NE (1275.6 vs. 1556.0 pg/ml) and higher levels of E (306.9 vs. 216.9 pg/ml) than whites at 75% VO_{2max} . The present study confirms previous findings of an increased BP response to exercise in normotensive blacks, due to an elevation in SVR. However, the results of the plasma catecholamine analysis failed to provide clear evidence of a racial difference in sympathetic drive.

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CHAPTER I

INTRODUCTION

Hypertension is one of the most prominent health issues facing Americans in this century [1-3]. Reports from the Hypertension Detection and Follow-up Program study [4], and a recent update from the National Health and Nutrition Examination Survey [5], place the incidence of essential hypertension in blacks at approximately twice that of whites. In addition, mortality and morbidity from hypertensive cardiovascular disease are elevated in the black population. Black Americans have increased rates of myocardial infarction, stroke [6], and renal disease and failure [7, 8] compared to whites.

Recently a great deal of research has focused on the possible mechanisms responsible for the elevated incidence of essential hypertension among blacks in the United States. While a specific cause for essential hypertension in any race has not been determined, it is likely that the mechanism is related to a variety of variables, rather than from any one source [9]. Possible contributors to racial differences in hypertension include disparities in renal physiology [10-12], potassium and calcium intake [6, 13, 14], endocrine factors [12, 15, 16], autonomic nervous system function [17], and socioeconomic status [18, 19].

Racial differences in hypertension may also be related to the finding that blacks have an increased cardiovascular response to physical and psychological stressors [20]. An increased "cardiovascular reactivity" is thought to play a role in the development of essential hypertension. This is

partly based on retrospective studies showing that an exaggerated BP response is a significant predictor of hypertension upon 5-10 year follow-up [21-23]. Additionally, borderline hypertensives [11, 24] and children of hypertensive parents [25] exhibit exaggerated BP responses to stress.

Several studies have documented an exaggerated BP response to exercise in normotensive black children [26-28] and black adults [29, 30]. Blacks have higher BP values during exercise than whites, without accompanying increases in HR, suggesting an elevation in peripheral resistance. This coincides with the finding of Anderson et al. [19] and Treiber et al. [28] who demonstrated that blacks have greater increases in BP and peripheral resistance in response to cold face stimulus than whites. The mechanism of the increased vascular resistance in blacks, however, is uncertain.

The present study was designed to determine if the increased BP reactivity of blacks during dynamic exercise is due to increased sympathetic nervous system (SNS) reactivity. Cardiovascular responses were assessed during a graded exercise test on a cycle ergometer, including blood pressure (BP), heart rate (HR), cardiac index (CI), and systemic vascular resistance (SVR). In addition, SNS activity was assessed by measuring plasma norepinephrine (NE) and epinephrine (E).

Hypotheses

Establishing whether there are racial differences in cardiovascular and sympathetic nervous system reactivity may help identify possible mechanisms that could predispose blacks to develop hypertension. In order to assess the

differences between black and white normotensive males for cardiovascular and sympathetic nervous system reactivity to dynamic exercise, the following hypotheses will be tested:

Hypothesis 1: Heart rates measured at supine rest, sitting rest, and at the end of each stage of a dynamic graded exercise test at 25, 50 and 75% of VO_{2max} are not different in black and white normotensive males.

Hypothesis 2: Systolic blood pressures and diastolic blood pressures measured at supine rest and sitting rest are not different in black and white normotensive males.

Hypothesis 3: Systolic blood pressures and diastolic blood pressures measured at the end of each stage of a dynamic graded exercise test at 25, 50 and 75% of VO_{2max} are higher for black normotensive males than for white normotensive males.

Hypothesis 4: Cardiac index calculated for sitting rest, and at the end of each stage of a dynamic graded exercise test at 25, 50 and 75% of VO_{2max} is not different in black and white normotensive males.

Hypothesis 5: Systemic vascular resistance calculated for sitting rest, and at the end of each stage of a dynamic graded exercise test at 25, 50 and 75% of VO_{2max} is higher for black normotensive males than for white normotensive males.

Hypothesis 6: Plasma epinephrine concentration measured at supine rest, sitting rest, and at the end of each stage of a dynamic graded exercise test at 25, 50 and 75% of $\text{VO}_2 \text{ max}$ is not different in black and white normotensive males.

Hypothesis 7: Plasma norepinephrine concentration measured at supine rest and sitting rest is not different in black and white normotensive males.

Hypothesis 8: Plasma norepinephrine concentration measured at the end of each stage of a dynamic graded exercise test at 25, 50 and 75% of $\text{VO}_{2\text{max}}$ is higher for black normotensive males than for white normotensive males.

Hypothesis 9: The incidence of positive parental history will be greater for blacks than whites, but is not expected to correlate with cardiovascular reactivity to dynamic exercise.

Significance

Cardiovascular and sympathetic hyper-reactivity has been associated with an increase in susceptibility to hypertension [24, 31]. Considering reports of prevalence and the severity of hypertension in blacks, early detection is of the utmost importance. Despite multiple interacting factors (race, age, sex, body weight, diet, etc.), a hypertensive response to stress, especially exercise, in normotensives could prove to be of significant value for the early detection of

sustained hypertension [23]. This study could contribute to the identification of mechanisms in the pathogenesis of essential hypertension in blacks. With this information researchers may be able to develop better preventive measures and a more effective treatment.

Delimitations of the study

This study is delimited to:

- 1) Thirty, apparently healthy, normotensive, young men between 18-35 years of age
- 2) Men who are of average fitness but are not currently involved in varsity athletics, high intensity aerobic activities, or participate in weight-lifting workouts on a regular basis.

Limitations of the study

This study is limited as follows:

- 1) The subjects for this study are not randomly selected but recruited from volunteers meeting specified criteria.
- 2) Differences in resting BP measures may be influenced by factors other than those controlled for in this study such as pre-test anxiety, undiagnosed disease, or other pathology.

CHAPTER II

REVIEW OF LITERATURE

The purpose of this review is to consider research in the area of hypertension, specifically that which is concerned with cardiovascular reactivity and sympathetic nervous system responses to stress as they relate to the hemodynamics of resting BP. Finally, the review considers previous research into the differences in black-white responses to various types of stressors, both physical and psychological.

Hypertension

The problem of hypertension is highly complex. Blood pressure is the product of the concerted action of the pumping of blood by the heart (cardiac output) and the vascular tone (total peripheral resistance) of the arteries that carry that blood.

In Figure 1 we see that hemodynamic analysis extends beyond measures of cardiac output (CO) and the calculation of total peripheral resistance (TPR). Neural and humoral regulatory mechanisms, the magnitude and distribution of intravascular volume, indexes of aortic distensibility, ventricular ejection velocity, and cardiac and renal performance must be measured and interrelated. Hypertension has been attributed to abnormalities in each of these integral factors [1].

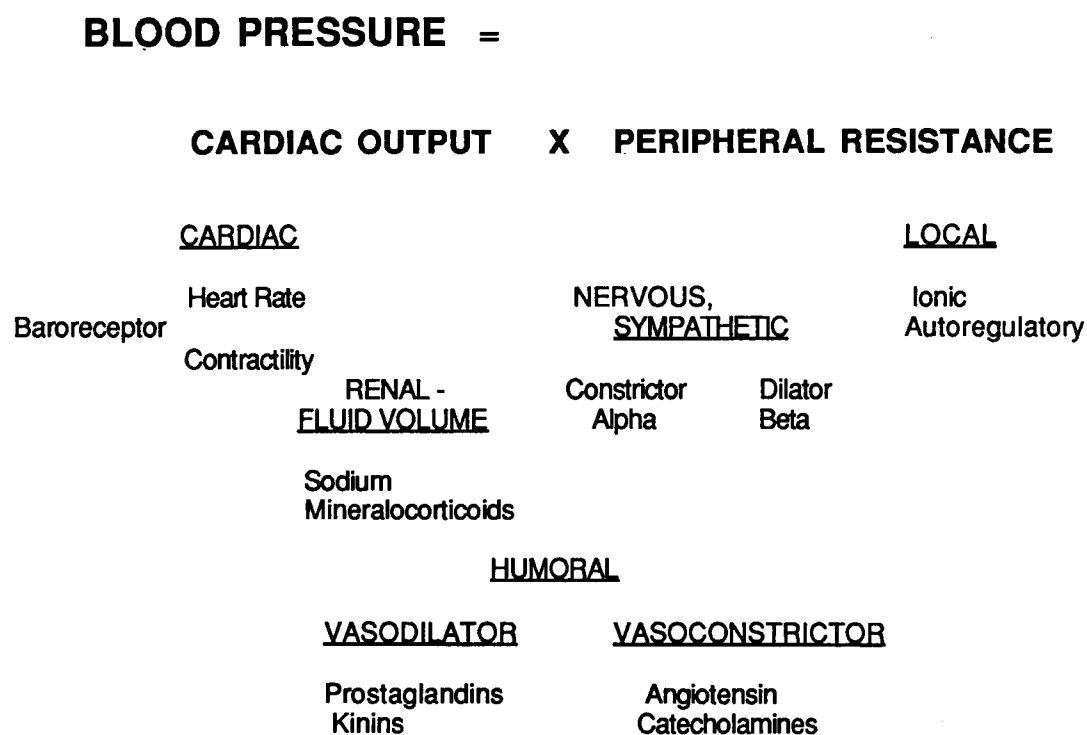


Figure 1. Interacting mechanisms of cardiac output

(From Kaplan, 1986 *Clinical Hypertension 4 ed.* Williams & Wilkins, Baltimore, p 58)

There are generally two classifications of hypertension identified, one being essential hypertension (sometimes referred to as primary, or idiopathic), and the other, secondary hypertension. Most cases of hypertension seem to fall into the essential category where the cause of the condition is unknown (idiopathic). Secondary hypertension accounts for less than 10% of the reported cases and is due to some identifiable mechanism (i.e., renal, endocrine) [9].

Defining the parameters of hypertension is controversial and many disagree with an arbitrary dividing line between normal and high BP. Pickering [32] argued that there is no "dividing line" and warned there was no critical hypertensive level, the higher the level, the higher the risk of developing cardiovascular disease. Rose [33] defined hypertension as "the level at which the benefits ... of action exceed those of inaction". However, the "official" criteria of the World Health Organization is as follows:

- * normal BP measures, or what is termed normotension, is a systolic pressure less than 140 mm Hg and a diastolic pressure less than 90 mm Hg;
- * borderline hypertension is a systolic pressure between 141 - 159 mm Hg and a diastolic between 91 - 94 mm Hg;
- * hypertension, is defined as a systolic pressure greater than 160 mm Hg and/or a diastolic pressure greater than 95 mm Hg [34].

Prevalence

The National Center for Health Statistics' latest National Health and Nutrition Examination Surveys II (NHANES II) 1976 to 1980, using a criteria of SBP of 160 mm Hg or higher or a DBP of 95 mm Hg or higher, or as taking

antihypertensive medication, reported the prevalence of hypertension at over 30 million people from ages 25 to 74 [3]. In 1985 the Subcommittee on Definition and Prevalence of the Joint National Committee on Detection, Evaluation, and Treatment of High Blood Pressure (JNC III) [35] re-analyzed the NHANES II data, defining hypertension as a blood pressure of 140 mm Hg or higher for the SBP and 90 mm Hg or higher for the DBP. Their report estimated the number of Americans at risk of morbidity and premature mortality associated with high blood pressure at an astounding 58 million.

The U.S. black population has an extremely high prevalence of essential hypertension, in fact, it is among the highest of any group in the world [36]. Adams [37] was perhaps the first to show significant differences in blood pressure between blacks and whites. He examined workers in New Orleans, 6,000 blacks and 8,000 whites, and reported higher systolic and diastolic blood pressures in blacks for all ages. Subsequent studies such as Comstock in Muscogee County [38] and McDonough [39] in Evans County, Georgia, Stamler's report on the Community Hypertensive Evaluation Clinic Program (CHEC), [40] and the Hypertension Detection and Follow-up Program (HDFP) Cooperative Group's Multicenter Cooperative Study [4] have also shown very distinct patterns of higher pressures among adult blacks when compared to adult whites. These studies also demonstrate a more rapid rise of blood pressure with age in blacks and increased susceptibility to the consequences of hypertension.

Morbidity/Mortality

Actuarial data from the 1979 Build and Blood Pressure Study [41] showed that mortality increases with each increment of both systolic and diastolic BP. This particular study has received much criticism due to its select population of largely white, upper-middle class insurance policy holders.

Many epidemiological studies have looked at the relationship of BP to cardiovascular morbidity and mortality. The Framingham Study, on-going since 1948, has made observations every 2 years on 5,200 men and women aged 30 to 62 at entry. Kannel and Kreger [42] reported a number of important points relating BP to cardiovascular morbidity and mortality. The study points out: (a) the incidence of cardiovascular disease and mortality rises progressively with systolic and diastolic blood pressures, more for males than females, (b) risks apply to the incidence of all major types of CVD with stroke becoming proportionately more common with increasing pressures, and (c) elevated blood pressures are independent risk factors for CVD.

The Pooling Project Research Group [43], observing 7,000 white men aged 40 to 59, showed a doubling of the rate of major coronary events over an 8.6 year interval among those men whose initial diastolic BP levels were above 95 mm Hg. Although most of the data from the studies mentioned above were derived primarily from white, middle-class Americans, the findings from the Evans County, Georgia study [44] showed similar relations between BP and both morbidity and mortality in blacks.

U. S. blacks suffer more overall mortality than whites at all BP levels, as evidenced by the 5-year Multiple Risk Factor Intervention Trial (MRFIT) which involved over 23,000 black men and 325,000 white men. Although black men

with DBP above 90 mm Hg did have lower mortality rates than white men from coronary heart disease, they had higher mortality from cerebrovascular disease [45].

Evans County [44, 46], a predominantly rural community in Georgia, was the site of a large population-based study in an area where cerebrovascular disease was among the highest in the nation for black males. Despite an extremely high prevalence rate of hypertension, the incidence of ischemic heart disease (IHD) was reportedly not excessive in comparison with the white population of Evans County, nor had the mortality attributed to IHD among the black residents of Evans County been higher for black population elsewhere in the United States. The increased levels of physical activity in blacks has been cited as possibly having protective qualities in this population.

The early investigators of Evans County hypothesized that differences in risk factors for CVD were partially due to differences in lifestyle and access to medical resources. They measured social status using the McGuire-White Index and divided whites into two groups, higher and lower social status. Since almost all blacks (95%) were below the median of whites, they were analyzed as an aggregate for comparison with the two white status groups.

Each risk factor studied (age, SBP, cholesterol, Quetelet's Index, pulse, smoking, abnormal ECG) showed a graded response among hypertensives from black to white lower status, down to white higher status groups. The SBP was highest in black hypertensives and lowest in the white high-status group.

Factors Related to Racial Differences

Genetics: Several researchers have attempted to associate BP and darkness of skin color. A Charleston County, South Carolina, population-based study of blacks [47] reported a significant association between BP and skin darkness among black men and women.

Renal/sodium: The renal retention of sodium may be a fundamental defect in the evolution of essential hypertension in blacks. Dahl et al. [49] first proposed a theory that permanent hypertension could follow this path: genetic defect in renal sodium excretion --> increase in body fluid volume--> increase in a "sodium excreting hormone"-->hypertension. Another hypothesis has evolved from these and other animal studies, linking an inhibitor of the sodium pump to an increased intracellular sodium concentration to an increase in free intracellular calcium that, in turn, is responsible for heightened contraction of vascular tissue [50]. Curtis et al. [51] observed six male kidney transplant patients and reported long-term remission of hypertension in these patients who likely developed renal failure solely as a consequence of essential hypertension.

Diet (sodium, potassium, calcium): Cushman and Langford studied 407 black and 216 white hypertensive males. They used 24-hour urine collections and determined that, while sodium and creatinine levels were similar for both races, the black males excreted only 62% as much potassium as the white males. This study demonstrated a negative correlation between serum potassium level and systolic blood pressure.

Grim et al. [10], using a random sample of the Evans County data found that blacks took in less and excreted significantly less sodium than whites.

More importantly, black men ate 23 mEq/24 hours of potassium versus 54 mEq/24 hours for white men, and they excreted 24 mEq/24 hours compared with 40 mEq/24 hours for white men.

Watson et al. [13] first suggested that calcium might play a role in the development of hypertension. Several of the national surveys (DISH, NANES) were analyzed by McCarron [52] who reported that calcium intake is much greater in whites than blacks and that calcium intake is greater in normotensives than hypertensives. He proposed that part of the explanation of the differences in calcium intake between blacks and whites is because of an increased incidence of lactose intolerance in blacks.

Stress: It has been suggested that frequent episodes that elicit an exaggerated pressor response, which manifests itself as part of the "survival" reaction, may eventually lead to permanent physiological changes (i.e., elevated blood pressure). Research in the biobehavioral area has focused on the significance of increased cardiac output (CO) due to what has been termed "increased beta-adrenergic reactivity" [19].

In 1981, Obrist [24] proposed that the repeated elicitation of this beta-adrenergic response pattern, especially during behavioral challenges that evoked active coping, might ultimately lead to sustained high BP. He reported that stressors (i.e., mental arithmetic, shock avoidance, reaction time) which require coping, an increase in CO, and blood and oxygen supply to bodily tissues. This frequently occurs in excess of metabolic needs. When this occurs, autoregulatory processes are activated that eventually result in an elevated total peripheral resistance (TPR) and higher BP.

Biobehavioral researchers have also explored the role of short-term increases in sympathetic nervous system-mediated cardiovascular activity during behavioral or environmental stress in the development of cardiovascular disorders, including hypertension [53]. This so-called "reactivity", as these acute cardiovascular changes have come to be known, may be characterized by any number of adjustments occurring in a variety of patterns and combinations. These behaviorally and environmentally induced changes in sympathetic nervous system activity, caused by factors such as territorial conflict, crowding, and other psychosocial stressors, produced sustained high BP in animals. This sustained high BP has been shown to be preceded by transient activation of the "fight or flight" mechanism of beta-adrenergically mediated sympathetic outflow. Stimulation of the beta-adrenergic receptors of the sympathetic nervous system produces increases in heart rate (HR), BP, and muscle blood flow, as well as increases in renin release [54].

Harburg et al [18] analyzed each of the 382 census tracts in Detroit and identified two factors, "socioeconomic status" (SES) and "social instability" (SIS). They found that chronic exposure to socioenvironmental stressors was consistently related to mean BP in blacks. Further analyses confirmed the effect of living in a high stress area on BP in black men under 40 years of age. The percentage of hypertensives in this young, high-stress, group of blacks was two and one half times that of the younger, low-stress, black men [48].

Exaggerated Responses to Laboratory Stressors

Research on racial group differences in reactivity to stress has been done in various laboratory situations and with a wide range of stressors. These studies have involved different age groups, from healthy children to adults that are either normotensive or have established essential hypertension. Several different types of challenges have been utilized in these studies and are usually categorized as either physical (i.e., static or dynamic exercise, postural change, cold pressor, sodium loading) or psychosocial (i.e., mental arithmetic, aversive reaction time, video games). Many of the studies reviewed in this section have used a combination of both physical and psychosocial stressors.

The early epidemiological surveys reported differences in resting BP measures. In the mid-fifties, researchers began to investigate risk factors for the disease in young normotensive individuals in hopes that some kind of intervention might be developed. It was during this time that Drs. John Thomas of Meharry Medical School and Caroline B. Thomas of Johns Hopkins University School of Medicine developed the Meharry Cohort Study (MCS) [55]. It seemed a reasonable hypothesis that there could be racial differences in precursors of hypertension in young adults that could lead to a greater rate of development of the disease in black adults. The MCS was one of the first of its kind to study populations of young black and white adults with similar education and occupational characteristics.

The Meharry Cohort Study [55] observed statistically significant differences in resting SBP and DBP between black and white male medical students, while resting HR were identical. The investigators reported a strong

racial difference in pulse pressure at peak exercise (double Masters exercise test).

In an effort to determine if racial differences exist early in life, several researchers have examined cardiovascular measures in biracial populations of children and adolescents, both at rest and during laboratory stressors. While higher resting heart rates have frequently been observed in black infants when compared to white infants, this trend typically disappears or even reverses itself by 5 years of age.

Several studies have focused on racial differences in cardiovascular reactivity of children and adolescents in response to physical stressors. Alpert et al. [26] measured systolic BP and heart rate responses in 184 black and 221 white subjects aged 6-15 during dynamic exercise (cycle ergometer testing at maximum workload). Subjects were classified by age, sex, and four categories of body surface area (I-IV; IV being largest) in an attempt to account for racial differences in reactivity. No significant differences were observed between groups at rest, however, black boys in the three largest body surface area groups exhibited higher exercise-induced peak systolic BP and a greater change in systolic BP across all body surface area groups. Since there were no racial group differences shown in maximum heart rate, the authors speculated that the BP differences were due to higher systemic vascular resistance in the blacks.

Recently Arensman et al. [27] studied 10-year-old boys, 19 blacks and 31 whites, who underwent maximum supine graded exercise tests with Doppler determination of cardiac output and measurement of systolic and diastolic BP and systemic vascular resistance. No differences were noted for blood

pressures during rest, maximum exercise, or recovery. Whites had a significantly higher cardiac index during all phases, and blacks had a greater systemic vascular resistance as evidenced by the similar BP responses and a lower cardiac index. The authors speculate that these racial differences may serve as potential markers for a "prehypertensive state".

Treiber et al. [28] used dynamic upright exercise (maximal effort running on a treadmill) with seventy-five (51 white, 24 black) children between the ages of 4 to 6-years to determine if racial and sex differences existed in BP values. Black children had significantly lower heart rate values for both preexercise and maximum effort measures, higher SBP values at peak exercise, and greater SBP increases in response to exercise than did white children. Again the authors discussed these differences in terms of possible mechanisms that may be responsible for racial difference in essential hypertension.

Anderson et al. [20] examined the influence of parental history of hyper-responsivity on cardiovascular stress reactivity to a competitive mental arithmetic task on a group of young black, normotensive females (ages 18-22). The subjects performed a stressful mental arithmetic task, frequently used in a research setting as a beta-adrenergic sympathetic nervous system stimulus. Physiological measurements taken during the trials included SBP, DBP, HR, and forearm blood flow (FBF). Forearm vascular resistance (FVR) was calculated by dividing the average mean arterial pressure (MAP) by the average FBF.

Results showed that, without regard to parental history, the mental arithmetic task produced the expected "defense pattern" of responsivity:

increases in HR, SBP, DBP, and FBF, with a corresponding decrease in FVR. The surprising finding of this study was that black women with a parental history of hypertension showed significantly smaller SBP and FBF responses than those without a parental history. There was also a trend for those with a positive parental history to display smaller diastolic BP responses; and heart rate was consistently lower in this group throughout each condition of the experiment. This finding is contradictory to previous studies which have demonstrated that both white males and females with a parental history of hypertension generally evidence the more pronounced cardiovascular adjustment to behavioral stressors.

Anderson et al. [19] proposed two arguments suggesting perhaps a less critical role of beta-adrenergic reactivity in the etiology of hypertension in blacks as opposed to whites. The first important point relates to the typically low plasma renin levels in blacks (beta adrenergic hyperactivity is usually associated with high renin levels). Next, they point out the decreased effectiveness of beta-blocking drugs in the treatment of hypertension in blacks, which are frequently effective in reducing BP in whites.

Anderson and his colleagues suggest other mechanisms that could be more critical in mediating stress responses. Enhanced peripheral vasoconstrictive responses, such as those exhibited by essential hypertensives during the cold pressor test, may point towards other mechanisms associated with the development of hypertension. Hypertension in blacks could, in fact, develop more from vasoconstrictive pressor responses and not so much from pressor responses mediated by cardiac factors as is often observed in whites in the early phases of hypertension. Using ten black

and ten white normotensive males, they specifically tested potential racial differences in pressor and vascular reactivity by comparing the magnitude of cardiovascular responses to a stimulus known to produce a pattern of response that includes peripheral vasoconstriction --the application of cold to the forehead. This particular stimulus slows the heart rate, a pressor response due to active increases in peripheral vascular resistance, including resistance in skeletal muscle.

Results from this study support the hypothesis of racial differences in cardiovascular responses to a vasoconstrictive stimulus. The Cold Face Stimulus (CFS) produced significant BP responses that were larger in the black subjects. The patterns of BP increases, which were greater for diastolic than SBP and which occurred despite a decrease in the heart rate, provide further evidence of a generally enhanced peripheral vasoconstriction response. They believe this evidence does suggest that blacks may show greater sympathetic nervous system mediated pressor and vasoconstriction responses than whites in response to certain stimuli, and this could be associated with the development of essential hypertension.

Neuroendocrine Indicators

The measurement of plasma norepinephrine (NE) has typically been used to assess the role of the sympathetic nervous system in essential hypertension [56]. Norepinephrine, released primarily at sympathetic nerve endings and to a slight extent from the adrenal medulla, acts to cause the smooth vascular muscle in blood vessels to contract. This vasoconstriction

results in the increase in total peripheral resistance which causes elevated blood pressure.

Plasma NE levels have been studied in groups of black and white hypertensive and normotensive subjects in an attempt to determine racial differences in sympathetic nervous system reactivity. Studies to date do not provide adequate evidence for a sympathetic nervous system reactivity difference between blacks and whites. Rowlands et al. [16] measured resting NE levels in 16 blacks with untreated mild to moderate hypertension and 16 white hypertensives matched for age, gender, casual blood pressure, and socioeconomic status. No differences were found between black and white subjects. Sever et al. [57] were unable to observe any differences in NE activity between black and white hypertensive subjects, or between black and white normotensive factory workers.

A recent study by Tischenkel et al. [29] measured plasma E and NE responses to a battery of four physical and mental stressors. They used a cold pressor test, video game, structured interview, and a cycle ergometer test with eighty-three 24-44 year old normotensive black and white men and women. No significant race differences were found in the NE or E reactivity during the exercise session.

Epinephrine, the catecholamine known as the mediator of the "fight or flight" response to stress, may induce greater effects on blood pressure and for a longer period of time than originally thought [1]. Several investigators [58, 59] have proposed an uptake of circulating epinephrine at the adrenergic synaptic cleft that is subsequently rereleased into the cleft as a co-transmitter during subsequent stimulation of the sympathetic nerves. Musgrave et al. [60]

infused E in man at a rate too low to cause changes in HR or BP, but high enough to increase the blood levels of norepinephrine. Over a long period of time, perhaps repeated episodes of low levels of E could eventually elicit changes in the smooth vascular muscle in the periphery. This may lend support to Folkow's theory that intermittently elevated blood pressure could lead to structural changes in the vasculature, and in doing so, induce permanent hypertension.

Summary

This review attempts to discuss many of the variables associated with racial differences in essential hypertension. Discrepancies in factors such as renal function, diet, heredity, and socioeconomic status make this topic, to say the least, multidimensional. A strong possibility exists that this complex array of variables interrelate in such a way that one specific cause may be extremely difficult to identify. It is vital to the research in this area that as many of these variables as possible be considered within the parameters of each experimental design.

CHAPTER III

METHODS

Subjects

Subjects were a biracial group of 30 normotensive, college-aged males (15 whites, 15 blacks) who volunteered to participate in the study. Volunteers were solicited from the university campus and surrounding community by newspaper articles and posters. Prospective subjects were screened on the basis of race (using self-definition) and resting BP in the normotensive range. They then completed a health questionnaire, and informed consent was obtained in accordance with the university's human subjects committee.

BP and HR were measured in the right arm using the Colin automated blood pressure monitor (STBP 780) after 20 min of supine rest. The STBP 780 monitors for Korotkoff sounds using electrocardiographic R-wave gating, thereby reducing noise artifact. A mercury sphygmomanometer was connected to the cuff, and a stereo headset was used by the investigator to simultaneously monitor the STBP 780 recordings. Subjects were excluded from the study if, during the initial screening or, on the two subsequent test days, their supine resting BP values were greater than 140 mmHg SBP and /or 90 mmHg DBP.

Anthropometric measurements were taken on each subject included height, weight, and sum of skinfolds. A three-site skin fold measurement was made, using Harpenden calipers [61]. Subjects were given a questionnaire on parental history of hypertension [62], which they returned at a later date.

Maximal Oxygen Uptake ($\text{VO}_{2\text{max}}$) Test

Percentage of expired oxygen (O_2) and carbon dioxide (CO_2) were measured during a maximal graded exercise test on a cycle ergometer. The subject began by exercising at 30 W, and the work rate was increased by 30 W each minute until they were no longer able to sustain the pedal cadence. A computerized system connected to Applied Electrochemistry S3- AO_2 and CD-3A CO_2 analyzers provided minute by minute analysis during the test. These analyzers were calibrated against known gases analyzed by the Scholander technique. Oxygen uptake (VO_2) and carbon dioxide production (VCO_2) was calculated using the Haldane transformation of the Fick equation. Additional criteria for subjects in the study included a $\text{VO}_{2\text{max}}$ value in the range of 40-55 $\text{ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$.

Dynamic Exercise

For the dynamic, submaximal exercise test the subjects reported to the lab in the early morning, prior to breakfast, strenuous physical activity, or caffeine ingestion. After 15 min of supine rest, a teflon catheter was inserted into a forearm vein to allow for multiple sampling throughout the exercise test. Following the insertion of the catheter and an additional 15 min rest period, a supine resting blood sample (3cc) was taken.

The trial began with 6 min of seated rest with the subject in position on the cycle ergometer. The subject's VO_2 and VCO_2 were measured at 30-second intervals and the value obtained at minute 4 was determined to be the steady-state value. A 3 cc venous blood sample was then obtained and

measurements of cardiac output were completed by minute 6, when the subject was taken to the next stage.

Cardiac output was measured non-invasively by the CO₂ rebreathing technique. Arterial pCO₂ was estimated from end-tidal pCO₂, measured through the use of a rapid-response infrared CO₂ analyzer. The subject then rebreathed into a latex bag containing CO₂ and O₂ to permit rapid equilibration with venous pCO₂ [63]. Arterial and venous CO₂ content (CaCO₂ and CvCO₂) were determined using a standard dissociation curve.

CO was calculated from the indirect Fick equation:

$$CO = VCO_2 / (CaCO_2 - CvCO_2)$$

Cardiac output values were corrected for body surface area (BSA) by the method of DuBois [64] and expressed as Cardiac Index (CI) (L·min⁻¹·m⁻²). Systemic vascular resistance (SVR) was calculated from the formula: SVR = MABP/CI, where SVR is expressed as units, MABP as mm Hg, and CI as L·min⁻¹·m⁻².

Plasma epinephrine (E) and norepinephrine (NE) were sampled five times during the dynamic exercise test (supine rest, seated rest, 25, 50, and 75% VO_{2 max}). Blood samples for the catecholamine analysis were placed in cold tubes containing ethyleneglycol-bis (Beta-amino-ethylether)-N,N'-tetraacetic acid and glutathione to give a final concentration of 1.8 and 1.2 mg/ml blood, respectively. The plasma was separated and stored frozen (-70° C) until analysis. The E and NE samples were measured in duplicate by a modification of the radioenzymatic technique of Cryer et al. [65]. Catechol-O-methyl transferase was extracted from rat liver by the method of Axelrod and Tomchick [66]. Tritium-labeled S-adenosyl methionine was purchased from

New England Nuclear. All samples from a subject were analyzed within a single assay, and 7 or 8 pairs (white/black) of subjects were analyzed in each assay. An internal standard (100-pg) for E and NE were run with each sample. The coefficient of variation for the internal standards averaged 9.3% for E and 10.9% for NE.

Data Analysis

Means and standard errors were calculated for all variables under each condition. An unpaired Student's t-test was used to compare values for the subjects' physical characteristics (height, weight, sum of skinfolds, age, resting SBP and DBP). Parental history of hypertension (PHH) was analyzed by a Chi-square nonparametric analysis.

A two-way ANOVA with repeated measures in one direction was used to analyze HR, SBP, and DBP data for the $\text{VO}_{2\text{max}}$ test. When appropriate, a test for simple main effects was used to determine if there were significant racial differences for HR, SBP, and DBP. An unpaired Student's t-test was used to test for differences in $\text{VO}_{2\text{max}}$ ($\text{L}\cdot\text{min}^{-1}$) and $\text{VO}_{2\text{max}}$ ($\text{ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$).

A two-way ANOVA, Race x Intensity (2 x 5) with repeated measures on the second factor, was used to analyze hemodynamic and catecholamine data for measures obtained during pre-exercise rest and three exercise intensities (25, 50, and 75% $\text{VO}_{2\text{max}}$). Where appropriate, tests for simple main effects were used to determine if there were significant differences in the means between blacks and whites for each variable at each intensity.

CHAPTER IV

RESULTS

Descriptive and Physical Characteristics of Subjects

Characteristics of the thirty normotensive subjects, fifteen blacks and fifteen whites are shown in Table 1. The Student's t-test indicated no significant differences between group means for blacks and whites on the selected physical variables, with the exception of resting heart rates, for which whites were higher (61.67 ± 1.7 b/min) than blacks (56 ± 1.7 b/min), $p = 0.0275$. Of those subjects able to report on parental history of hypertension (PHH), the incidence of PHH was not significantly higher for blacks (8 of 14) than for whites (4 of 14), $p = 0.085$.

Maximal Oxygen Uptake (VO_{2max}) Test

Results of the VO_{2max} tests are shown in Table 2. Mean scores for VO_{2max} were indicative of average values for their age group [67] (blacks $M = 44.09$ ml·kg⁻¹·min⁻¹; whites $M = 47.06$ ml·kg⁻¹·min⁻¹). No significant differences were found for absolute ($p = .16$), or relative VO_{2max} ($p = .07$), nor in maximal mean HR values ($p = .94$). Mean values for maximal SBP during the VO_{2max} test were not significantly different between blacks and whites. While DBP values demonstrated a trend toward being higher for black subjects, the groups were not significantly different ($p < 0.06$). The 2 x 2 (Race x Intensity) repeated measures ANOVA for HR, SBP, and DBP responses (rest to maximal effort)

TABLE 1. Descriptive and Physical Characteristics of the Subjects

Variable	Blacks	Whites
n	15	15
Age (years)	21 ± .8	22.4 ± .7
Ht. (cm)	177.5 ± 2.0	178.3 ± 1.5
Wt. (kgs)	75.39 ± 2.2	76.8 ± 2.7
BMI (kg/m ²)	23.9 ± .47	24.1 ± .59
BSA (m ²)	1.92 ± .04	1.94 ± .04
Rest HR (beats/min)	56.0 ± 1.7	61.7 ± 1.7*
Rest SBP (mm Hg)	118.8 ± 2.0	118.0 ± 2.0
Rest DBP (mm Hg)	68.6 ± 2.5	67.1 ± 1.8
PPH	8 of 14	4 of 14

Values are listed as mean ± SE

* significant at $p < 0.05$

Table 2. Maximal Oxygen Uptake (VO_{2max}) Test Data

Variable	Blacks	Whites
VO_{2max} (L/min)	3.33 ± .13	3.62 ± .15
VO_{2max} (ml·kg ⁻¹ ·min ⁻¹)	44.1 ± 1.2	47.1 ± 1.1
Max HR (beats/min)	83.6 ± 3.1	183.9 ± 2.1
Max SBP (mm Hg)	214.7 ± 5.1	209.1 ± 4.2
Max DBP (mm Hg)	86.5 ± 2.6	82.9 ± 1.9

Values are listed as mean ± SE

during the $\text{VO}_{2\text{max}}$ tests failed to show a significant interaction effect (Race * Intensity), and post hoc analysis showed no statistically significant difference for either rest or $\text{VO}_{2\text{max}}$ for these variables.

Dynamic Exercise

Table 3 presents changes in cardiovascular measures for the graded exercise test. Overall, racial differences for SBP, DBP, MABP, and SVR were all statistically significant ($p < 0.05$) and all, with the exception of CI, showed a significant interaction (Race x Intensity), $p < 0.05$. Overall, HR was not different for blacks and whites, $p = .197$ (Figure A-1). ANOVA tables can be seen in Appendix A.

There were significant changes experienced by blacks and whites for SBP and DBP across conditions for the graded exercise test. The analysis for SBP yielded a significant Race x Intensity interaction ($p < 0.0001$). Post hoc analysis for SBP showed mean values for supine rest, sitting rest and 25% $\text{VO}_{2\text{max}}$ were not significantly different, while mean values for 50% and 75% $\text{VO}_{2\text{max}}$ were different at a statistical significance of $p < .015$ and $p < .001$, respectively (Figure A-2).

The interaction term for DBP was also statistically significant, $p < 0.0018$. A post hoc analysis of DBP responses revealed a similar pattern as SBP, with supine rest, bike rest, and 25% $\text{VO}_{2\text{max}}$ mean values showing no significant difference. Means were significantly different for blacks and whites at 50% ($p < 0.001$) and 75% $\text{VO}_{2\text{max}}$ ($p < 0.001$).

Blacks had a smaller mean CI values than whites over all conditions that were examined ($p = .02$), with no Race x Intensity interaction ($p = .80$) (Figure

Table 3. Cardiovascular Measures at Rest and During Submaximal Exercise

Variable	INTENSITY				
	REST		% VO ₂ max		
	Supine	Bike	25	50	75
SBP (mm Hg)					
Blacks	113.9 ±2.4	127.1 ±3.2	146.4 ±4.4	173.9 ±4.7*	199.1 ±5.7**
Whites	114.9 ±2.3	121.7 ±2.3	136.7 ±3.1	160.5 ±4.1	180.1 ±3.8
DBP (mm Hg)					
Blacks	68.3 ±1.9	82.9 ±2.1	85.8 ±2.3	85.0 ±2.3*	88.6 ±3.7**
Whites	67.7 ±1.8	80.9 ±1.9	79.9 ±1.4	73.8 ±1.6	77.0 ±1.8
HR (beats/min)					
Blacks	57.6 ±1.6	72.9 ±3.1	100 ±1.9	129.9 ±3.0	164.9 ±2.6
Whites	57.9 ±1.3	71.1 ±1.6	95.1 ±2.7	122.7 ±2.7	161.5 ±2.6
MABP (mm Hg)					
Blacks	83.5 ±1.9	97.7 ±2.2	105.7 ±2.7*	114.3 ±2.8**	125.2 ±3.9**
Whites	83.3 ±1.6	94.5 ±1.9	98.7 ±1.8	102.5 ±2.2	111.0 ±2.3
% VO ₂ (ml·kg ⁻¹ ·min ⁻¹)					
Blacks	—	.37±.02	.96±.04	1.67±.07	2.59±.10
Whites	—	.36±.02	1.03±.07	1.82±.10	2.82±.13
CI (L·min ⁻¹ ·m ⁻²)					
Blacks	—	2.3 ±1	3.9 ±1	5.9 ±2	7.2 ±2
Whites	—	2.7 ±2	4.5 ±2	6.3 ±2	7.6 ±3
SVR (units)					
Blacks	—	43.7 ±1.9**	27.2 ±.6*	19.7 ±.7*	17.3 ±.6
Whites	—	35.4 ±1.8	22.2 ± 1.0	16.4 ±.6	14.6 ±.4

Values are listed as mean ± SE

* p < 0.05

** p < 0.01

A-3). Differences in SVR for blacks and whites are clearly evident, blacks having greater overall vascular resistance, with a significance of ($p = .0001$), and a significant interaction of Race x Intensity ($p = .02$). Blacks showed the greatest SVR at rest ($p < 0.0001$) and 25% VO_{2max} ($p < 0.002$) (Figure A-4).

Epinephrine and norepinephrine responses to dynamic exercise are presented in Table A-1. Plasma epinephrine levels were statistically different between groups, with blacks having higher overall values than whites. Post hoc analysis revealed that blacks and whites did not differ significantly at rest or dynamic exercise at 25% or 50% VO_{2max} ; however, blacks had higher plasma epinephrine levels at 75% VO_{2max} ($p < 0.007$).

The overall mean values for plasma concentrations of norepinephrine were significantly different ($p < .02$) and, while the mean values were not statistically different for blacks and whites at rest, 25%, or 50% VO_{2max} , the mean values at 75% VO_{2max} were statistically significant ($p < 0.001$) (Figure 2). Figure 3 shows NE changes from rest to 75% VO_{2max} (delta NE) for all subjects plotted over changes in SBP (delta SBP) across exercise conditions. There was no significant relationship between these variables, as evidenced by $r^2 = 0.005$.

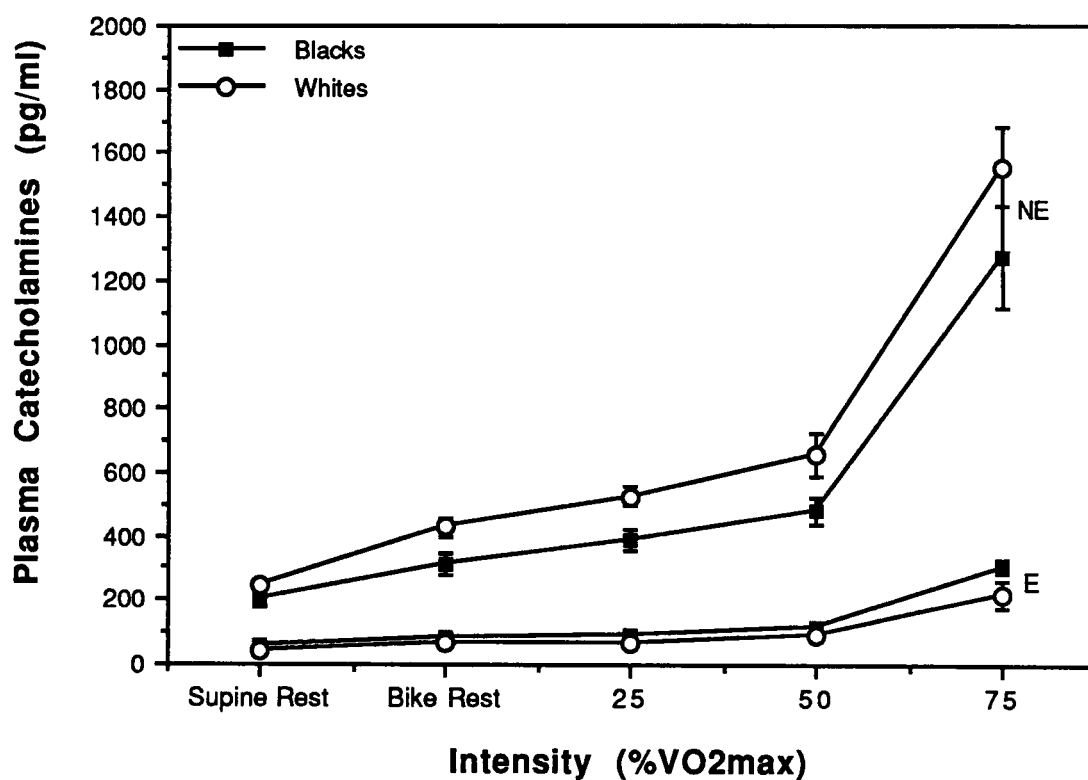


Figure 2. Plasma catecholamines, epinephrine (E) and norepinephrine (NE), response to dynamic exercise. A significant main effect of Race was found for E ($p < 0.05$) and NE ($p < 0.02$).

Values are means $\pm$ SE

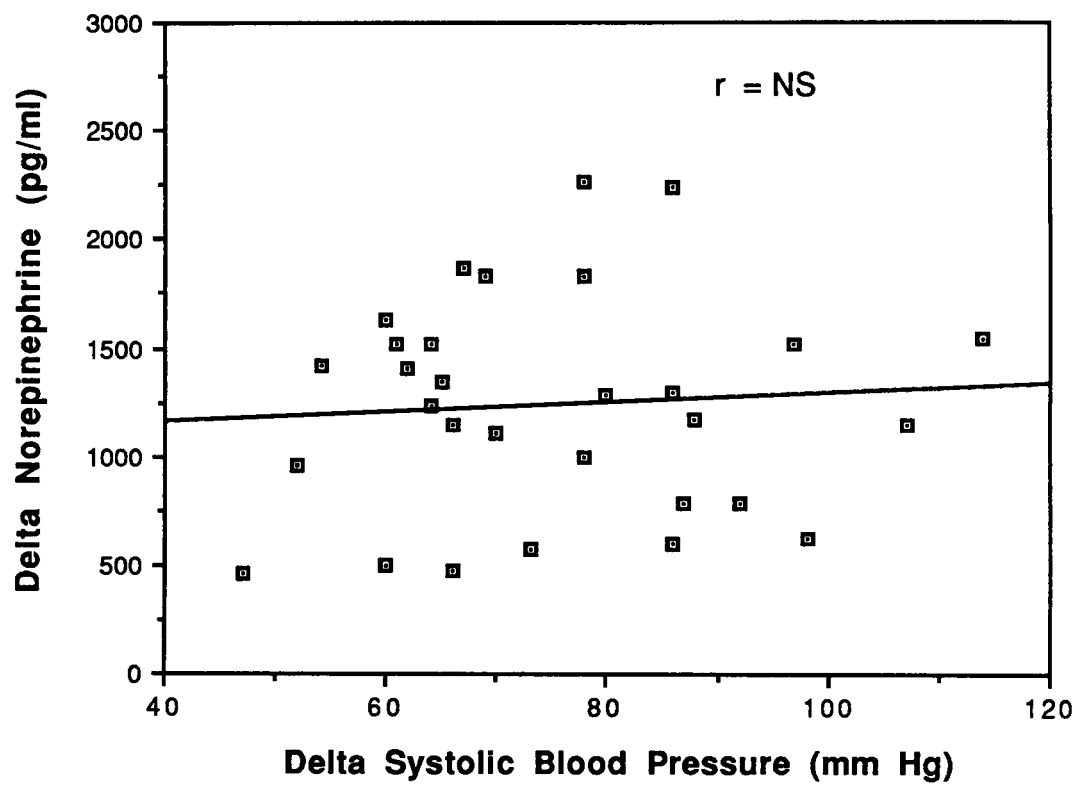


Figure 3. Scatter plot of changes from baseline to 75% $\text{VO}_{2\text{max}}$ in norepinephrine (NE) over changes in systolic blood pressure (SBP)

CHAPTER V

DISCUSSION

Subject Characteristics

The subjects who volunteered to participate in this study were screened for normal BP and fitness level. Previous work has shown that individuals with mildly elevated pressures have an exaggerated BP response to exercise [11, 24]. However, this study was designed to examine racial differences in BP reactivity prior to the onset of hypertension. Aerobic fitness has also been shown to influence the BP response to exercise [68, 69]. Thus, we wanted to ensure that subjects in both groups had normal levels of aerobic fitness.

The use of a questionnaire to assess parental history of hypertension has previously been validated by Anderson et al. [20]. Given the two-fold increase in the prevalence of hypertension among blacks, it was expected that positive parental history (PHH) would be higher for our black subjects. 65.5 % of the black subjects in this study reported a positive PHH, compared to 28.6% of the white subjects. However, due to the sample size, this difference was not statistically significant ($p=.085$). In a larger population of 433 black and 573 white medical students, Thomas [55] reported a significant correlation between race and positive parental history of stroke or hypertension ($p < .01$).

Resting supine BP was nearly identical for blacks and whites at the time of the initial screening, as well as on the day of the submaximal exercise test. At the initial screening, the blacks had a lower HR (56.0 ± 1.7 bpm) than the

whites (61.7 ± 1.7). On the day of the test, however, resting HR was not different (57.6 ± 1.6 vs 57.9 ± 1.3 , respectively). The literature is not clear on this topic, with some studies reporting lower resting HR in blacks [11, 28, 55] and others reporting no difference [19, 20, 25, 29, 70].

Hemodynamic Measures during Dynamic Exercise

The increased reactivity in SBP for blacks, across all exercise conditions, was consistent with the findings of other studies [28, 30, 26]. Treiber et al. [28] found that black children had greater increases in SBP during exercise than white children. They confirmed an earlier report by Alpert et al. [26] that racial differences in cardiovascular reactivity exist in normotensive children. Ekelund et al. [30] recently examined a biracial population of 4263 adults (20-69 yrs). Despite similar resting pressures, black men had higher SBP values than white men during the second stage of a Bruce treadmill test (174 vs 163 mm Hg). This difference was not seen, however, in black and white women (153 vs 152 mm Hg).

Diastolic BP measures also reflected a greater reactivity to exercise in the black subjects. Several investigations that have reported racial differences in DBP during exercise concur with our findings [25, 27, 28, 72]. Perhaps due to the difficulty of measurement during exercise, many studies failed to report changes in DBP across exercise conditions [29, 30, 55, 71]. In addition to the SBP and DBP differences in reactivity, the values for MABP response to exercise were found to be greater in blacks than in whites.

Previous studies often speculated that blacks have an increased peripheral resistance in exercise, due to the existence of elevated pressures

without accompanying increases in HR [26, 28, 30]. However, these reports of differences in cardiovascular reactivity often did not examine the immediate determinants of mean arterial blood pressure (MABP) - CI and SVR. In the present study blacks had an increased SVR at rest and throughout exercise, compared to whites. These findings are supported by Arensman et al. [27] who found that black children had a greater SVR (31.2 vs 24.1 at rest, and 20.0 vs 15.3 at maximal exercise) and lower CI (2.24 vs 2.89 L·min⁻¹·m⁻² at rest and 4.47 vs 5.77 L·min⁻¹·m⁻² at maximal exercise).

Other researchers have found that, in response to a cold-face stimulus, blacks have greater increases in SBP, DBP, SVR, and forearm vascular resistance than whites [19, 20, 72]. These studies differ from the exercise studies mentioned above in that blacks did not exhibit higher resistance values at rest. In any case, it is apparent that racial differences in BP during physical stress result from an elevated vascular resistance in blacks, compared to whites.

Researchers have speculated that cardiovascular hyper-reactivity to stress could be due to SNS influences [19, 73, 74]. Few studies, however, have attempted to examine racial differences in SNS activity during stress [29, 75, 76].

Catecholamine Response to Dynamic Exercise

Despite a number of factors that can influence plasma catecholamine levels, there is good evidence that they are valid indices of SNS activity [77-80]. The resting values in our black and white subjects for NE (199.6 vs 241.8 pg/ml respectively) and E (81.0 vs 63.4 pg/ml, respectively) are in agreement

with other studies [16, 29, 57, 75]. Sever et al. [57] and Rowlands [16] also found no difference in NE levels between blacks and whites at any given level of BP. Two other indices of SNS activity, plasma renin and resting HR, are normally lower in blacks. From these data, it would not appear that blacks have a greater SNS activity than whites at rest [56].

During the graded exercise test, the black subjects in our study had lower NE levels and higher E levels than the whites at 75% $\text{VO}_{2\text{max}}$. Norepinephrine causes alpha-mediated systemic vasoconstriction to all vascular beds, whereas E causes a decrease in total systemic vascular resistance [81]. Thus, the differential catecholamine response in the present study was in the opposite direction to explain the racial differences observed in SVR.

Two other studies have examined plasma catecholamine levels during exercise. Hohn et al. [75] studied adolescents 10 to 17 years old for possible racial differences in plasma catecholamine levels across pre- and post treadmill exercise conditions. They found NE values 1 minute post exercise to be higher for whites (2051 ± 100 pg/ml) compared to blacks (1779 ± 97 pg/ml), however these values were not found to be significantly different.

Tischenkel et al. [29] recently examined catecholamine responses to various stressors, as a function of race and sex. After 4 min of cycle exercise (65% age-predicted HR_{max}), they found no significant effects of race or sex for either NE or E. It should be noted, however, that their study reported no racial difference in SBP reactivity for males. Black females (111 to 171 mm Hg) were found to be more reactive than white females (105 to 134 mm Hg). However, in agreement with the present study, both of these studies showed

a trend toward lower NE responses in blacks. Thus it seems unlikely that the greater BP reactivity in blacks is due to an increase in sympathetic drive.

There is some evidence that racial differences in alpha-receptor sensitivity may exist. Dimsdale et al. [76] reported that black hypertensives on a high-sodium diet have an increased BP response to NE infusion. However, these results must be "taken with a grain of salt" because 1) NE exerts β -effects on CO in addition to its alpha effects on the vasculature, and 2) differences in baroreflex sensitivity may influence the pressor response. Regional infusion studies using alpha-specific agonists (i.e.-phenylephrine) are needed to more closely examine this question.

The cardiovascular response to exercise is often viewed as being sympathetically-mediated [29]. However, factors other than SNS activity may influence pressures. Racial differences in parasympathetic tone, vascular structure, vasoactive hormones, and interactions with sodium sensitivity, could potentially influence the exercise pressor response.

In conclusion, the current data do not support the idea that racial differences in BP responsiveness are due to differences in SNS reactivity. However, a consistent finding in this and other studies [19, 20, 27, 28, 30, 71, 72] is that blacks have an elevated peripheral vascular resistance during stress. This alteration in vascular structure and/or function may precede the development of essential hypertension [12]. Therefore, studying the mechanisms involved in the exercise pressor response may help clarify the pathogenesis of hypertension in blacks.

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APPENDIXES

APPENDIX A.

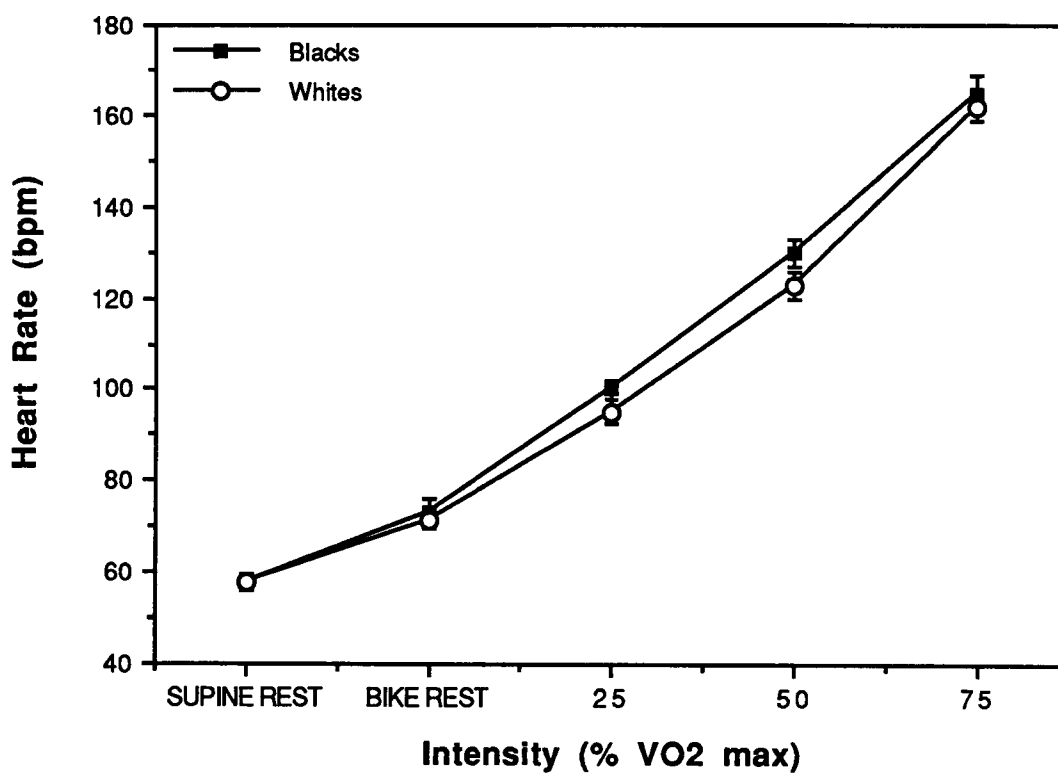


Figure A-1. Heart rate responses to dynamic exercise. No significant main effect for Race was found.

Values are means $\pm$ SE

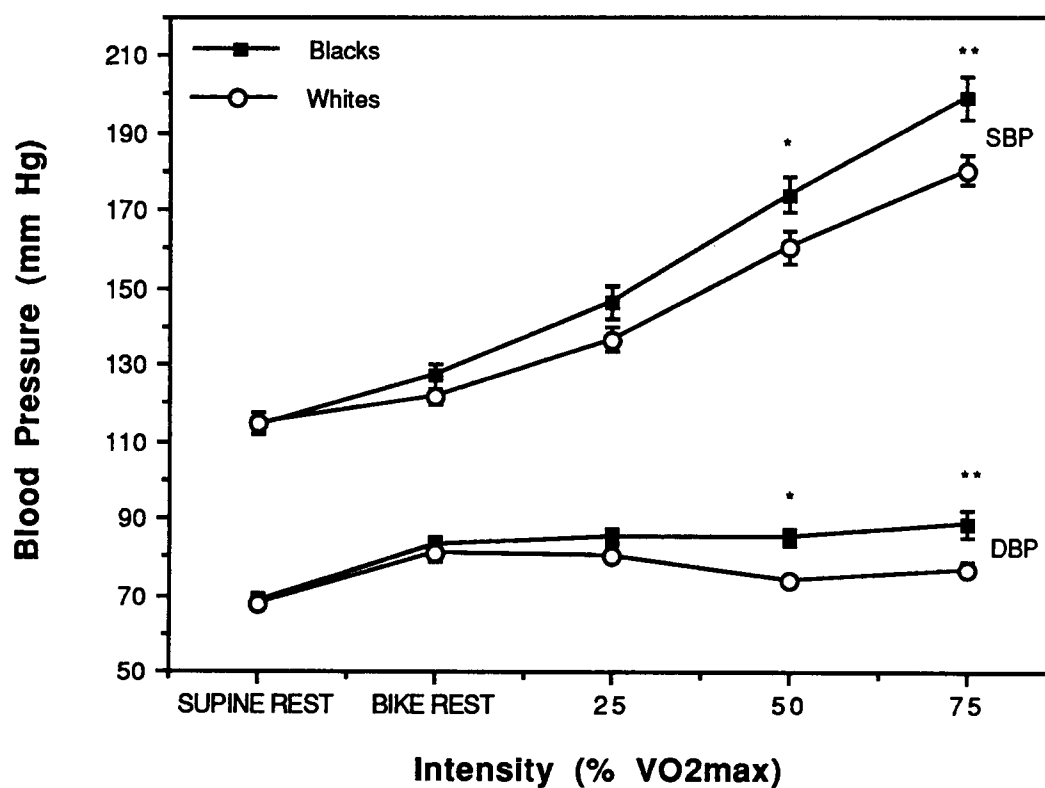


Figure A-2. Systolic blood pressure (SBP) and Diastolic blood pressure (DBP) responses to dynamic exercise. Significant main effects for Race were found for SBP ($p = .05$) and DBP ($p = .01$). Significant interaction for Race x Intensity were found, SBP ($p = .0001$) and DBP ($p = .002$).

Values are means $\pm$ SE

** $p < 0.001$

* $p < 0.02$

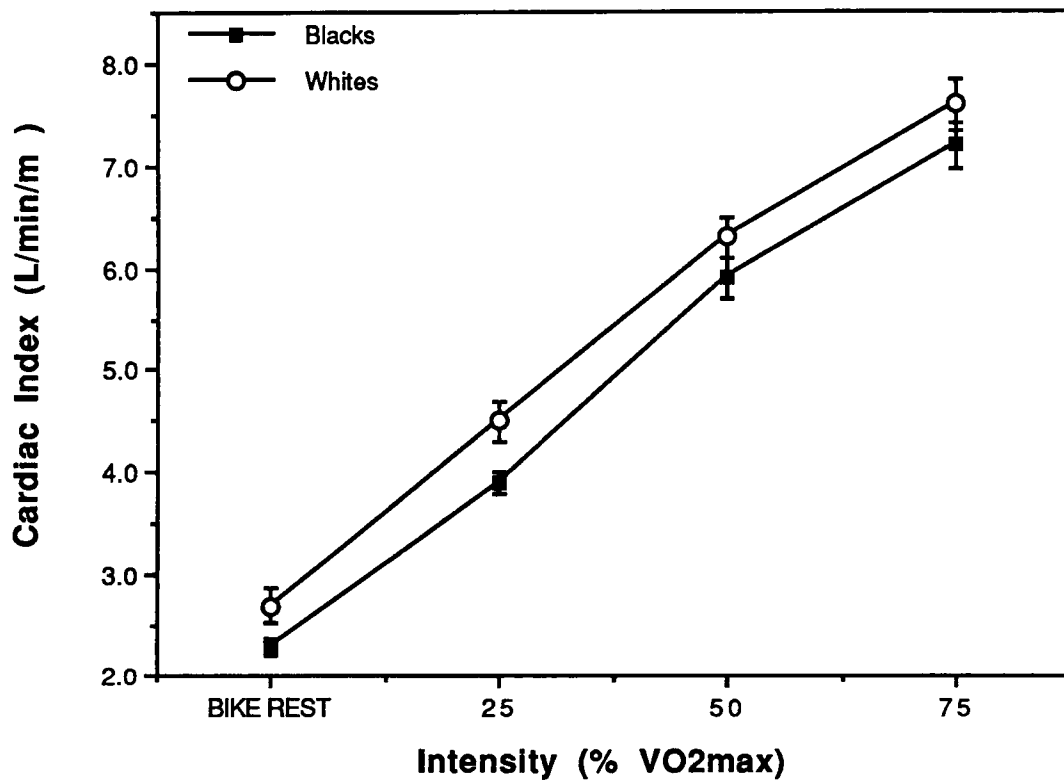


Figure A-3. Cardiac index related to dynamic exercise. A significant main effect for Race was found ($p = .02$). Values are means $\pm$ SE

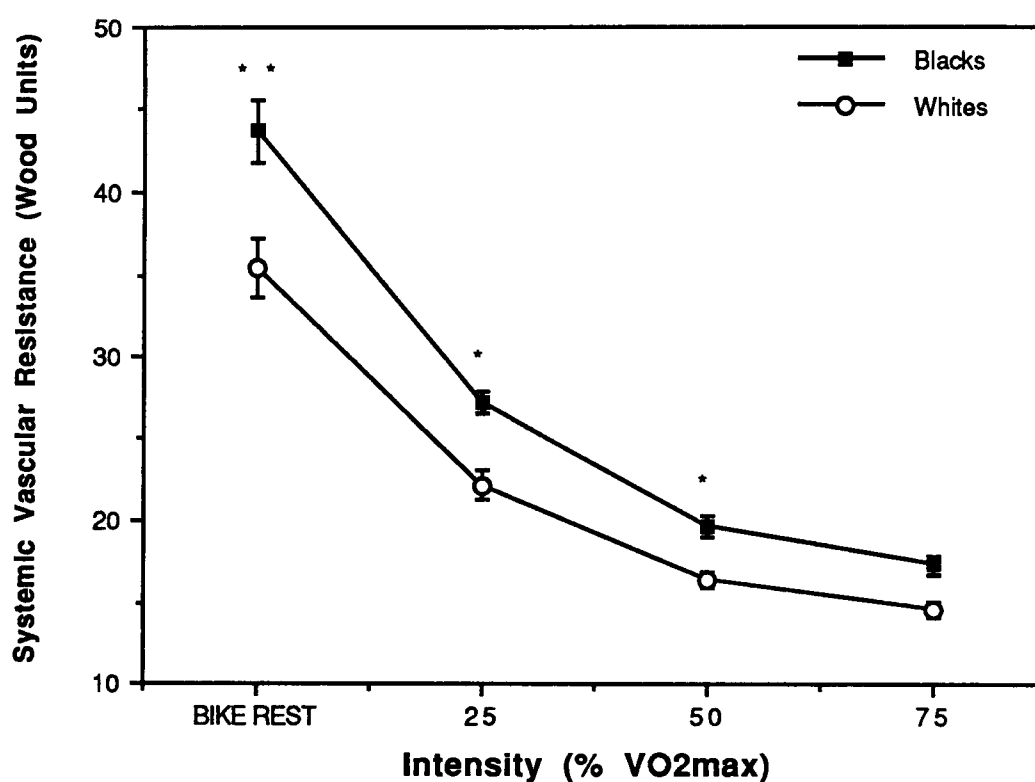


Figure A-4. Systemic vascular resistance for dynamic exercise. A significant difference was found for Race ($p < 0.001$). A significant interaction Race x Intensity was found ($p < 0.05$).

Values are means $\pm$ SE

** $p < 0.001$

* $p < 0.02$

Table A-1. Dynamic Exercise Epinephrine (E) and Norepinephrine (NE) Measures (pg/ml).

Variable	INTENSITY				
	REST		% VO _{2max}		
	Supine	Bike	25	50	75
E					
Blacks	62.2 ±5.9	81.4 ±6.9	93.7 ±9.1	121.7 ±12.4	306.9 ±42.2**
Whites	45.6 ±6.6	63.4 ±6.9	67.6 ±6.2	93.3 ±12.4	216.9 ±21.2
NE					
Blacks	199.6 ±23.3	312 ±29.8	386.6 ±34.7	483.9 ±42.4	1275.6 ±159**
Whites	241.8 ±19.6	428.1 ±27.3	526.3 ±30.6	654.9 ±67.3	1556 ±123

Values are means ± SE

** p < 0.001

Table A-2. Repeated Measures ANOVA Table for Blacks and Whites on Systolic Blood Pressure (SBP) Measures During Dynamic Exercise.

Source	SS	df	MS	F
<i>Between Subjects</i>				
Race	3257.340	1	3257.340	4.049*
Error	22524.0	28	804.429	
<i>Within Subjects</i>				
Intensity	114674.507	4	28668.627	419.06**
Intensity * Race	1740.693	4	435.173	6.361**
Error (Intensity)	7662.000	112	68.411	

* $p < 0.05$

** $p < 0.001$

Table A-3. Repeated Measures ANOVA Table for Blacks and Whites on Diastolic Blood Pressure (DBP) Measures During Dynamic Exercise.

Source	SS	df	MS	F
<i>Between Subjects</i>				
Race	1460.160	1	1460.160	7.237*
Error	5649.413	28	201.765	
<i>Within Subjects</i>				
Intensity	4753.507	4	1188.377	28.37**
Intensity * Race	769.507	4	192.377	4.593**
Error (Intensity)	4691.387	112	41.887	

* $p < 0.05$

** $p < 0.001$

Table A-4. Repeated Measures ANOVA Table for Blacks and Whites on Mean Arterial Blood Pressure (MABP) Measures During Dynamic Exercise.

Source	SS	df	MS	F
<i>Between Subjects</i>				
Race	1987.440	1	1987.440	6.5*
Error	8456.720	28	302.026	
<i>Within Subjects</i>				
Intensity	20409.960	4	5102.490	156.17**
Intensity * Race	1029.293	4	257.323	7.876**
Error (Intensity)	3659.147	112	32.671	

* $p < 0.05$

** $p < 0.001$

Table A-5. Repeated Measures ANOVA Table for Blacks and Whites on Heart Rate (HR) Measures During Dynamic Exercise.

Source	SS	df	MS	F
<i>Between Subjects</i>				
Race	426.727	1	426.727	1.751
Error	6824.747	28	243.741	
<i>Within Subjects</i>				
Intensity	216370.040	4	54092.510	894.771**
Intensity * Race	251.907	4	62.977	1.042
Error (Intensity)	6770.853	112	60.454	

* $p < 0.05$

** $p < 0.001$

Table A-6. Repeated Measures ANOVA Table for Blacks and Whites on Cardiac Index (CI) Measures During Dynamic Exercise.

Source	SS	df	MS	F
<i>Between Subjects</i>				
Race	7.542	1	7.542	6.075*
Error	34.761	28	1.241	
<i>Within Subjects</i>				
Intensity	419.533	3	139.844	514.253**
Intensity * Race	.268	3	.089	.8046
Error (Intensity)	22.843	84	.272	

* $p < 0.05$

** $p < 0.001$

Table A-7. Repeated Measures ANOVA Table for Blacks and Whites on Systemic Vascular Resistance (SVR) Measures During Dynamic Exercise.

Source	SS	df	MS	F
<i>Between Subjects</i>				
Race	710.015	1	710.015	25.424**
Error	781.952	28	27.927	
<i>Within Subjects</i>				
Intensity	10211.471	3	3403.824	227.451**
Intensity * Race	145.585	3	48.528	3.243*
Error (Intensity)	1257.068	84	14.965	

* $p < 0.05$

** $p < 0.001$

Table A-8. Repeated Measures ANOVA Table for Blacks and Whites on Plasma Epinephrine (E) Measures During Dynamic Exercise.

Source	SS	df	MS	F
<i>Between Subjects</i>				
Race	481158.209	1	48158.209	5.661*
Error	238210.236	28	8507.508	
<i>Within Subjects</i>				
Intensity	851376.586	4	212844.146	67.233
Intensity * Race	28264.644	4	7066.161	2.232
Error (Intensity)	354564.583	112	3165.755	

* $p < 0.05$

Table A-9. Repeated Measures ANOVA Table for Blacks and Whites on Norepinephrine (NE) Measures During Dynamic Exercise.

Source	SS	df	MS	F
<i>Between Subjects</i>				
Race	842400.540	1	842400.540	7.371*
Error	3200013.732	28	114286.205	
<i>Within Subjects</i>				
Intensity	26509590.725	4	6627397.681	97.342
Intensity * Race	227481.019	4	566870.255	.835
Error (Intensity)	7625394.344	112	68083.878	

* $p < 0.05$

Table A-10. Two Factor ANOVA Table for Blacks and Whites on Parental History (PH) * Race with Changes in NE Measures During Dynamic Exercise.

Source	SS	df	MS	F
<i>Between Subjects</i>				
Race	87688.835	1	87688.835	.333
PH	1067.600	1	1067.600	.004
<i>Interaction</i>				
PH * Race	284539.625	1	284539.625	1.080
Residual	6320490.692	24	263353.779	

Table A-11. Two Factor ANOVA Table for Blacks and Whites on Parental History (PH) * Race with Changes in MAP Measures During Dynamic Exercise.

Source	SS	df	MS	F
<i>Between Subjects</i>				
Race	87688.835	1	87688.835	.333
PH	1067.600	1	1067.600	.004
<i>Interaction</i>				
PH * Race	284539.625	1	284539.625	1.080
Residual	6320490.692	24	263353.779	

APPENDIX B.**American Heart Hypertension Study: Protocols****DAY 1: SCREENING**

- 1) Health History
- 2) Blood Pressure (Colin)
 - Electrodes
 - Right Arm (Standard)
 - 15 Minutes Supine Rest Position
 - Record Room Temperature
- 3) Informed Consent
 - one file copy
 - one copy to subject
- 4) Skinfolds (Harpender Calipers)
 - Right side: chest, subscapula, tricep
 - Forearm: 10 cm from olecranon process
dorsal side, vertical skinfold
- 5) Maximal voluntary hand grip
 - Dominant arm, adjust hand grip to fit subject
 - Three trials with subject seated, arm out to side
 - Record all trials, use highest measured grip
- 6) Instructions for future days
 - no caffeine or tobacco the morning before any lab work
 - do not rush to lab, take your time, we need a good resting BP
 - no food on mornings prior to blood draws

**AMERICAN HEART ASSOCIATION: HYPERTENSION STUDY
SCREENING DATA SHEET**

NAME _____ DATE _____

AGE _____ RACE _____ HEIGHT _____ WEIGHT _____

1) Health History _____

2) Resting Blood Pressure ____/____ Room Temp _____

3) Informed Consent _____

4) Skinfolds: Chest _____

Subscapula _____

Tricep _____

Sum _____ % Fat _____

Forearm (dorsal side) _____

5) Maximal voluntary hand grip

Trial 1 _____

Trial 2 _____

Trial 3 _____ MVC _____

6) Instructions for future days _____

VO₂ MAX TEST

- 1) Weigh w/out shoes
- 2) Connect BP Electrodes
- 3) Adjust cycle height, towel on handle bar, record seat height
- 4) 15 minutes supine rest
 - record BP and HR
 - instruct subject to allow arm to hang freely during BP measures
- 5) Cycle Max
 - subject will maintain a pedal cadence of 50 rpm with the aid of a metronome
 - warm-up: two minutes @ 1 kp
 - work will increase .5 kp/min until subject can no longer sustain the 50 rpm pedal rate
 - BP's will be taken every minute
 - VO₂ will be calculated by the Rayfield System
 - Recovery: 3 minutes @ .5 kp

AMERICAN HEART ASSOCIATION: HYPERTENSION STUDY**VO2 Max Test Data Sheet**

Name _____ Date _____

1) Weight (w/out) shoes _____ lbs = _____ Kg

2) Electrodes _____

3) Adjust Cycle Height _____

4) 15 min sitting rest _____

Resting BP _____/_____

5) Work Blood Pressures

min 1 _____/_____ work load _____

min 2 _____/_____ _____

min 3 _____/_____ _____

min 4 _____/_____ _____

min 5 _____/_____ _____

min 6 _____/_____ _____

min 7 _____/_____ _____

min 8 _____/_____ _____

min 9 _____/_____ _____

min 10 _____/_____ _____

min 11 _____/_____ _____

min 12 _____/_____ _____

min 13 _____/_____ _____

min 14 _____/_____ _____

DAY 3: GRADED EXERCISE TEST (25%, 50%, 75% of Max)**1) Preliminary Set-up****A. Materials**

- * 2 pre-mixed tubes (anti-oxidant) properly labelled
- * 2 empty tubes
- * 3 3cc syringes
- * 1 vial heparin, or saline drip set-up
- * 1 teflon catheter w/3-way stop-cock
- * tourniquet, alcohol wipe

B. Equipment

1. Colin Automated Blood Pressure Machine
2. Calibrations (new aquasorb)
 - a) Rayfield analyzers, flow meters and computer on
 - b) CO₂ analyzer
 - c) Physiograph
set sensitivity - 300
use 2 blocks/%
2. Cardiac output
 - a) tanks on
 - b) mixer on
 - c) evac bag, check timer
3. Cycle Ergometer
 - a) set seat height, put towel on handle bar
 - b) drip cloth under bike
4. Recliner cot from BMR Lab

2) Subject

- A) Weigh w/out shoe
- B) Supine, quiet rest: 15 minutes

- Put catheter in place: non-dominant arm
 - * place syringe w/heparin on one end
- Put BP cuff, in place: non-dominant arm, electrodes on chest
- Supine, quiet rest: 15 minutes
- Record Room Temperature
- Record supine resting BP/HR
- Draw supine resting blood sample
 - * replace heparin with "waste syringe", draw 1cc
 - * draw 3cc of blood for Cat sample, replace heparin
 - * expel Cat sample into pre-mix tube labelled "Pre Ex"
 - * mix sample with anti-oxidant by gently inverting 3x
 - * place sample in ice bath

C) Exercise

- sit on bike, rest VO_2 , BP
- unloaded pedalling 1 min
- 1) First work load = 25% of Max VO_2 for 6 minutes
 - at min 3:
 - * evac bag
 - * pre-set appropriate % CO_2
 - * fill bag by timer switch
 - Blood Pressure @ min 4
 - * subject allows arm to hang
 - * use manual mode for BP, listen and observe mercury
 - * record observation
 - CO @ min 5
 - * disconnect Rayfield
 - * ready physiograph: record, paper speed
 - * give signal, subject is switched onto bag
 - * observe physiograph for approx 8 breaths, should equilibrate
 - * give signal, subject is swithed onto room air
 - Draw cat sample
- 2) Second, third and fourth work bouts repeat steps above

APPENDIX C.

FORM B

CRP # _____

Date Received in ORA _____

THE UNIVERSITY OF TENNESSEE, KNOXVILLE

Application for Review of Research Involving Human Subjects

I. IDENTIFICATION OF PROJECT

Date _____

Project Directors: Bassett, David R. Jr., Ph.D.
Vernon Bond, Jr., Ed.D.
Edward T. Howley, Ph.D.

Address and Phone number of PDs:

Dept. of Human Performance and Sport Studies
University of Tennessee-Knoxville
1914 Andy Holt Ave./ Knoxville, TN 37996
615 974-5111

Department: Human Performance and Sport Studies

Title of Project:

Mechanisms of Racial Differences in Blood Pressure
Reactivity to Stress

Estimated starting date: Upon CRP approval

Completion date: Dec. 30, 1992

External Supporting Agency and Identification Number:
American Heart Association- Tennessee Affiliate

Grant submission deadline:

Grant deadline: 11-1-89

Human Subjects Committee Approval deadline: 2-1-90

II. OBJECTIVES OF PROJECT

Blacks have increased rates of essential hypertension and hypertensive cardiovascular disease, when compared to whites. Racial differences in hypertension may be related to the finding that blacks have an exaggerated blood pressure (BP) response to stress. Studies have implicated an elevated systemic vascular resistance (SVR) as the cause of this BP response, but the mechanisms for the increased SVR are unknown. Phase I of the study will determine if blacks show an increased sympathetic reactivity to exercise. Cardiovascular responses (BP, SVR, cardiac index) to exercise will be measured in a bi-racial group of 30 males, and plasma norepinephrine will be assessed as an index of sympathetic activity. Phase II will examine structural

differences in the peripheral blood vessels of whites and blacks. To test whether blacks have a decreased vascular cross-sectional area, which would limit vasodilatory capacity in exercise, minimum forearm vascular resistance (mFVR) will be assessed. Phase III will examine racial differences in the sensitivity of the carotid artery baroreceptors, which attenuate the rise in BP during exercise. By comparing racial differences in the cardiovascular mechanisms responsible for BP control during exercise, we hope to be able to identify factors which contribute to the higher incidence of essential hypertension in blacks.

III. DESCRIPTION OF SUBJECTS

Healthy, normotensive males (age 18-30 years) will be recruited from the University of Tennessee-Knoxville faculty and student body, and the surrounding community. Following a blood pressure screening and medical history (see Appendix A), 30 normotensive individuals (15 whites and 15 blacks) will be invited to participate in Phase I of the study. The nature of the study will be explained, and the subjects will be asked to sign an informed consent form prior to starting the study.

In the second year of the study, an additional 30 subjects (age 18-30 years) will be invited to participate in Phase II and III. There will be some overlap with the subject population from the first year, but it would not be possible to expect all subjects to complete all phases of the study. The previous criteria and procedures will be used in recruiting subjects.

Subjects will receive \$40 for completing Phase I of the study, and \$40 for completing Phases II and III. Subjects who complete all phases of the study will receive \$80. This payment is commensurate with the time involvement for the study, and is similar to that of previous studies (eg.- \$50 for participation in a study on the energy cost of StairMaster exercise, carried out by Ed Howley, Ph.D. [1988-1989]). It is not intended as inducement for any risk involved in the study.

Criteria for exclusion of subjects:

Either of the following-

1. Casual, seated blood pressure > 145/95 mm Hg
2. Contraindications to exercise testing (see Appendix B)
3. Heart disease, renal disease, or central nervous system dysfunction (i.e.-seizures, impaired gross motor function).

IV. METHODS OF PROCEDURE

PHASE I- BP CONTROL DURING EXERCISE

This phase of the study will determine if the increased BP responsiveness of blacks during exercise is due to increased sympathetic reactivity. Neurohormonal (norepinephrine) and cardiovascular (BP, SVR, and cardiac index) responses will be

measured during static and dynamic exercise. If blacks have greater sympathetic reactivity, larger increases in norepinephrine with exercise would be expected.

Dynamic Exercise Protocol. Subjects will report to the laboratory following an overnight fast. After 30 min in the supine position, a series of resting measurements (BP, heart rate, and catecholamines) will be made. Subjects will then perform treadmill exercise at relative exercise intensities corresponding to 25, 50, 75, and 100% $\text{VO}_{2\text{max}}$ (4 min per stage). Cardiovascular measures (BP, heart rate, cardiac output, and systemic vascular resistance) will be obtained at rest and during each work load. Similar graded exercise test protocols have been previously approved by the University of Tennessee CRP (eg.- #2874 on 01/11/89).

Static Exercise Protocol. On another occasion, subjects will report to the laboratory following an overnight fast. Resting measurements will be made as previously described. Maximum grip strength (kg) will be assessed in the left arm using a hand-grip dynamometer with a force transducer connected to a recorder. The best of three trials will be considered maximal. After 10 min of recovery, subjects will perform 3 min of left-handed static exercise at 10, 20, and 30% of maximum voluntary contraction (MVC) interspersed with 2 min of rest. Cardiovascular measures and blood samples for catecholamine analysis will be obtained at each level.

Blood Pressure. Blood pressure will be measured in the right arm with the use of an automated BP monitor (Colin STBP 680). The device receives a signal from the ECG and only monitors for Korotkoff sounds after each QRS complex, thereby reducing noise artifact. Appropriate cuff sizes will be selected and systolic, diastolic, and mean BP recorded.

Blood Sampling. Following 15 min of supine rest, a 21-gauge teflon angiocath will be placed in a vein on the back of the hand. A conventional heating pad will be used to maintain flow, and patency will be maintained with a heparin-saline solution (110 U/ml). 4.0 ml samples of arterialized venous blood (five samples for dynamic exercise, four samples for static exercise) will be obtained for the catecholamine analysis. This blood sampling procedure has been previously approved by the University of Tennessee CRP (#1444 on 1/18/89).

Oxygen Uptake. Percentages of expired oxygen and carbon dioxide will be measured by having the subject breath into a computerized system connected to Applied Electrochemistry O_2 and CO_2 analyzers. Inspired ventilation will be measured with an airflow meter. Oxygen uptake and carbon dioxide production will be calculated using the Fick equation.

Cardiac Output and Systemic Vascular Resistance. Cardiac output will be measured non-invasively by the CO_2 rebreathing technique.

Arterial pCO_2 will be assessed non-invasively from the pCO_2 of expired air at end-expiration. Venous pCO_2 will be assessed non-invasively by having the subject rebreathe into a latex bag containing approximately 15% carbon dioxide and 85% oxygen for 5-10 sec. This permits a rapid equilibration with venous pCO_2 . The volume of the gas mixture will be determined by taking 150% of tidal volume. Cardiac output will be calculated by the indirect Fick equation, and these values will be corrected for body surface area (BSA) by the method of Dubois and expressed as cardiac index. Systemic vascular resistance will be calculated from the mean arterial blood pressure and cardiac index.

PHASE II- MINIMUM FOREARM VASCULAR RESISTANCE (mFVR)

Forearm blood flow (FBF) will be determined by placing a mercury-filled plastic strain gauge around the subjects left forearm. The strain gauge will be placed 10 cm distal to the olecranon process (elbow) and the left forearm will be supported at 20-30 degrees above heart level. 1 minute prior to forearm blood flow measurement, a pediatric cuff on the wrist will be inflated to suprasystolic pressure to occlude hand blood flow. A venous-congesting cuff on the upper arm will be inflated to 40-50 mm Hg for 10-15 sec and deflated for 3-5 sec over four cycles with a Hokanson rapid cuff inflator. The increase in forearm circumference will be used to calculate forearm blood flow in ml/min/100 ml of tissue.

Forearm vascular resistance (in units) will be calculated by dividing mean arterial BP by maximal forearm blood flow. Maximal forearm blood flow is achieved by 10 min of ischemic forearm exercise. A blood pressure cuff on the left upper arm will be inflated to suprasystolic pressure for 10 min and the subject will perform isometric handgrip contractions with the left hand for 5 seconds of each 30-second interval. This is identical to a procedure used by B. Egan, et al. (see Appendix C).

PHASE III- PHENYLEPHRINE INFUSION

Carotid baroreceptor sensitivity will be determined using a procedure described by Kotchen, et al. (see Appendix D). This method uses the decrease in heart rate in response to a phenylephrine-induced rise in BP to measure baroreceptor sensitivity. Subjects will report to the laboratory in the morning prior to exercise, cigarettes, or caffeine. A 3-lead ECG will be connected and a blood pressure cuff attached while the subject is supine. Heart rate and R-R interval will be computed electronically by connecting the ECG output to a microcomputer. Subjects will remain supine for 45 min before the start of the study.

Venous blood will be obtained for the measurement of resting catecholamines, and an i.v. infusion of 5% dextrose (500 ml) will be started. Once the BP is stable, bolus doses of phenylephrine will be injected starting with 20 ug (and increasing by 10-20 ug

per injection) until systolic BP is transiently increased by 30 mm Hg. Blood pressure and heart rate will be monitored continuously, and BP will be allowed to return to baseline between successive injections. This should take less than 10 min per injection (T. Kotchen, personal communication). A graph of stimulus (BP) versus response (change in heart rate) will be plotted, and the slope of the line used to define baroreceptor sensitivity.

V. SPECIFIC RISKS AND PROTECTION MEASURES

There are few ethical problems associated with the proposed research. The risks of maximal exercise testing in this population are slight. Rochmis and Blackburn (JAMA 217:1061, 1971) reported that graded exercise testing was characterized by a mortality rate of 1 per 10,000. The rate of non-fatal complications was 2.4 per 10,000. Tests conducted with apparently healthy men have an even lower risk. Gibbons et al. (J. Card. Rehab. 1:362, 1981) reported only one complication in 7057 maximal tests in healthy middle-aged men. Kaltenbach et al. (Europ. Heart J. 3:199, 1982) found no serious complications in 353,638 tests on young athletes.

Every precaution will be taken to ensure the safety of the subjects during the testing sessions. Mouthpieces will be sterilized prior to exercise testing, and blood drawing procedures will be conducted in accordance with standard practices by Dr. Bassett, Dr. Bond, or a licensed nurse.

In the phenylephrine infusion studies, there is a risk of tissue necrosis if phenylephrine is injected into the interstitium—hence, venous lines will be checked for patency to ensure that this does not occur. We will begin with very small doses of phenylephrine and increase the dosage incrementally, stopping when the systolic pressure reaches 30 mm Hg in excess of resting systolic pressure. Testing of all subjects participating in the phenylephrine infusion study will be conducted under the direct supervision of Dr. Michael Freeman, M.D., or Dr. Dan Ely, M.D (see Appendix D) at the University of Tennessee Medical Center.

Atropine will be available during the infusion study to counteract the reflex bradycardia should the patient become symptomatic, and an individual who is comfortable with the use of this medication will be present.

VI. BENEFITS VS. RISKS

The risks involved in this study are very reasonable in relationship to the anticipated benefits. Benefits to the subjects include an assessment of cardiovascular fitness, and

financial payment. The study will provide information relevant to the higher incidence of hypertension in the black population.

VII. METHODS OF OBTAINING "INFORMED CONSENT" FROM SUBJECTS

Subjects will be provided with a written consent document embodying all of the basic elements of informed consent (see attached consent forms). The consent form will be xeroxed front/back. Subjects will be instructed to read it, and to ask any questions about matters that are unclear. This form will be signed by the subject and an extra copy provided for the subject's use/information. The signed consent documents will be kept in a file cabinet in the office of the PD (Dr. Bassett) for 3 years following completion of the research.

VIII. QUALIFICATIONS OF THE INVESTIGATOR

The PD has been certified in Cardiopulmonary Resuscitation (Red Cross) and as an Exercise Specialist (American College of Sports Medicine). In addition, the PD has performed blood sampling by the method outlined in this proposal in a previous experiment approved by the University of Wisconsin Human Subjects Committee (Protocol # 88-741-051 "Effects of Endurance Training on Lactate Removal). The PD performed the catheterizations for this study.

IX. ADEQUACY OF FACILITIES TO SUPPORT RESEARCH

Not applicable.

X. RESPONSIBILITY OF PROJECT DIRECTOR

In compliance with the policies established by the University of Tennessee-Knoxville Committee on Research Participation, the project director subscribes to the principles stated in "The Belmont Report" and standards of professional ethics in all research, development, and related activities involving human subjects under the auspices of the University of Tennessee-Knoxville.

a. Approval will be obtained from the University Committee prior to instituting any change in the research project.

b. Development of any unexpected results will be reported to the University Committee.

c. A status report (Form D) will be submitted at 12 month-intervals or as requested attesting to the current status of the project.

d. Signed consent statements will be kept for the duration of the project and for at least three years thereafter.

Project Directors _____	Date _____
_____	Date _____
_____	Date _____

XI. DEPARTMENTAL REVIEW

COMMENTS/RECOMMENDATIONS:

The application described above has been subjected to departmental review and has been approved.

Department Head _____	Date _____
Chair, Departmental Review Committee _____	Date _____

MECHANISMS OF RACIAL DIFFERENCES IN
BLOOD PRESSURE REACTIVITY TO STRESS

INFORMED CONSENT FORM

Investigators: David R. Bassett, Jr. Ph.D.
Vernon Bond, Jr. Ed.D.
Edward T. Howley Ph.D.

Address:
Dept. of Human Performance and Sport Studies
School of Education
University of Tennessee-Knoxville
1914 Andy Holt Ave./ Knoxville, EN 37996

Phone: 615 974-5111

You are invited to participate in a research study, the purpose of which is to examine racial differences in the control of blood pressure. If you decide to take part, you will perform the phase(s) of the study which are checked below:

PHASE I

If you take part in this phase of the study, you will perform a maximal exercise test on a treadmill. Oxygen uptake will be measured by having you breath into a mouthpiece so that we can measure your expired air. On a second day you will return to the laboratory and perform 4 min of exercise at 25, 50, 75, and 100% of your maximal level. Blood pressure, oxygen uptake, and heart rate will be measured at each work load. In addition, we will sample 4 ml (about 1 teaspoon) of blood from a plastic line connected to a needle at rest and at each work load (20 ml or 5 teaspoons blood total). The plastic line will be flushed with a heparin solution to keep it from clotting. During the test, you will breath back-and-forth into a bag containing approximately 90% oxygen and 10% carbon dioxide for 5-10 seconds. On a third occasion these measurements will be repeated while you perform 3 min of hand-grip exercise at 10, 20, and 30% of maximum. In all, the study will require 3 testing sessions on different days (approximately 1.5 hours per day). You will receive \$40 and a report on your fitness level for your participation.

PHASES II and III

This portion of the study will involve two different visits to the laboratory. On the first visit, a device for measuring arm circumference will be placed around your arm. A blood pressure cuff on your upper arm will be inflated slightly for 10-15 sec and deflated for 3-5 sec about 4 different times. This procedure allows us to compute the blood flow to your arm on the basis of the increase in arm circumference. You will then do handgrip exercise with the left hand for 5 seconds of each 30-second

interval, with blood flow to the arm occluded. After 10 min, we will repeat these measures.

On the second visit, you will come in during the morning before exercise, cigarettes, or caffeine. You will remain lying down for 45 min before the start of the study. We will put a needle affixed to a plastic tube into an arm vein and infuse 5% dextrose (a sugar substance). Once your heart rate and blood pressure are stable, we will inject phenylephrine (an adrenaline-like substance) in stepwise fashion, until systolic BP is increased by 30 mm Hg. This will allow us to determine how sensitive your body is to changes in blood pressure. In all, phases II and III will require 2 days of your time, approximately 1.5 hours per day. You will receive \$40 for your participation in this part of the study.

The risks involved with exercise include abnormal blood pressure responses, fainting, musculoskeletal injury, disorders of the heart, and in very rare instances heart attack. In Phase III of the study there is an additional risk of muscle tissue damage if the phenylephrine does not go directly into the bloodstream. However, there is only a minor chance that serious complications will occur. In the event that physical injury occurs as a result of the study, the University of Tennessee does not provide reimbursement for medical care or other compensation. If physical injury is suffered in the course of research, or for more information, please notify the investigator in charge, Dr. David Bassett, at 974-5111. Benefits to be expected include financial payment and an assessment of your fitness.

The information in this study will be provided to you. Data from this study may be used in research reports or presentations; however, your name and other identity will not be disclosed.

You are free to decide whether or not to participate in this study and are free to withdraw from the study at any time.

Before you sign this form please ask questions about any aspects of the study which are unclear to you.

AUTHORIZATION:

I, _____, have read the above and decided to participate in the research project described above. My signature also indicates that I have received a copy of this consent form.

Participant's signature

Date

Witness

Date

PERSONAL HISTORY FORM

Name _____

Age _____

Date _____

Check X if yes

Past History

Have you ever had?

Rheumatic fever	()	()	()
Heart murmur	()	()	()
High blood pressure	()	()	()
Any heart problems	()	()	()
Varicose veins	()	()	()
Lung disease	()	()	()
Seizures	()	()	()
Irregular heart beat	()	()	()
Bronchitis	()	()	()
Emphysema	()	()	()
Diabetes	()	()	()
Asthma	()	()	()
Kidney disease	()	()	()
Liver disease	()	()	()
Hay fever	()	()	()

Present Symptoms Review:

Have you recently had?

Chest Pain	()	Frequent Urination	()
Shortness of Breath	()	Blood in Urine	()
Heart palpitations	()	Burning Sensations	()
Leg or ankle swelling	()	Severe Headache	()
Coughing of blood	()	Blurred Vision	()
Low blood sugar	()	Difficulty Walking	()
Feeling faint or dizzy	()	Weakness in Arm or Leg	()
		Numbness	()

Do you smoke?

How many per day?

Table 2-4. Contraindications to Exercise Testing

-
1. Recent acute myocardial infarction
 2. Unstable angina
 3. Uncontrolled ventricular dysrhythmia
 4. Uncontrolled atrial dysrhythmia which compromises cardiac function
 5. Congestive heart failure
 6. Severe aortic stenosis
 7. Suspected or known dissecting aneurysm
 8. Active or suspected myocarditis
 9. Thrombophlebitis or intracardiac thrombi
 10. Recent systemic or pulmonary embolus
 11. Acute infection
 12. Third degree heart block
 13. Significant emotional distress (psychosis)
 14. A recent significant change in the resting ECG
 15. Acute pericarditis
-

Table 2-5. Relative Contraindications to Exercise Testing

-
1. Resting diastolic blood pressure over 120 mm Hg or resting systolic blood pressure over 200 mm Hg
 2. Moderate valvular heart disease
 3. Digitalis or other drug effect
 4. Electrolyte abnormalities
 5. Fixed rate artificial pacemaker
 6. Frequent or complex ventricular irritability
 7. Ventricular aneurysm
 8. Cardiomyopathy including hypertrophic cardiomyopathy
 9. Uncontrolled metabolic disease (diabetes, thyrotoxicosis, myxedema, etc.)
 10. Any serious systemic disorder (mononucleosis, hepatitis, etc.)
 11. Neuromuscular, musculoskeletal, or rheumatoid disorders which would make exercise difficult
-

APPENDIX D.

CATECHOLAMINE ASSAY

WORKING PROCEDURE: Catechol-O-Methyl Transferase Purification

From: Julius Axelrod & R. Tomchick (1958) J. Biol. Chem. 232; p. 702

I. EXPERIMENTAL PRELIMINARY SET UP (Day before the Experiment)

- A) Prepare the day before about 1000 ml (1.0 liter) of Isotonic KCL. (11.63 g of KCL made up to 1.0 liter with distilled water) and placed in the refrigerator so it will be 5° C less in the morning. (5.82 g/ 500 mL)= need only 400 mL per 100 g of tissue.
- B) Also prepare a 1.0 N acetic acid solution by taking 4.0 mL of 17.5 N acetic acid (normal concentrated acetic acid and adding it slowly to 66.0 mL of distilled water.
- C) Make certain the 250 mL centrifuge tubes are clean (will need at least 4 of them).

II. EXPERIMENTAL PROCEDURE (Day of Experiment)

A) Obtain Rat Liver and General Set Up

a) On returning to the lab with the rat liver; remove the liver from the ice bucket and cut off about 100 g of liver and put it into a beaker in the ice bucket. Put the bucket in the refrigerator at 5° C or less to keep the liver cold before use. Take the remainder (most of the liver) and freeze it for future use (good for at least 3 months), wrap in plastic.

b) A large tray is then filled with crushed ice and then tap water is added. Next a quantity of Table Salt is added (50-100 g) and stirred into solution. This will be used to keep the samples cooled between steps in procedure if they are over a couple of minutes long.

III. EXTRACTION:

A) Remove the liver from the beaker in the ice bucket and cut up the 100 g of liver with scissors. Then place it in the blender.

B) Add 400 mL of the cold isotonic KCL and homogenize until there is a homogeneous mixture.

C) Centrifuge:

1) Using the 250 mL centrifuge tubes and the JA-14 head pour the 500 mL of total homogenate equally into two tubes. Fill a third tube with H₂O to balance the rotor and place all three tube in the JA-14 head.

2) Then spin the tubes first at 12,000 RPM for 30 minutes at 2° C.

3) Then pour the supernatant into two new tubes; equalize the volumes by eye, bring the water tube down to the proper level and recentrifuge for one (1) hour at 12,000 RPM and 2° C.

D) Decant the supernate into a 500 mL beaker, add a stirring bar and using 1.0 N (10-15 mL) acetic acid previously prepared, adjust the pH to 5.0. Let stand for 10 minutes in refrigerator.

E) Centrifuge in 250 mL tubes again at 12,000 RPM and 2° C.

F) Decant the supernatant into a 500 mL graduate (to get the volume) then into the same 500 mL beaker (wash out with deionized and or distilled water) as used above. Put in a magnetic stirring bar and place on the stirrer in the refrigerator.

IV. Ammonium Sulfate $(\text{NH}_4)_2\text{SO}_4$ Fractionation

A) into the 500 mL beaker containing the supernatant add 0.173 grams $(\text{NH}_4)_2\text{SO}_4$ / mL of supernatant, while stirring with the magnetic stirrer. Stir for one (1) hour.

EXAMPLE: If 350 ml of supernatant-then one would need $350 \times 0.173 = 60.55$ g of $(\text{NH}_4)_2\text{SO}_4$

B) Then pour the beaker's contents equally into two (2) 250 mL centrifuge tubes and spin at 12,000 RPM for 30 minutes at 2° C.

C) Again decant the supernatant from the tubes into the 500 mL graduate again (to get the volume) and then into the same beaker; put in the stirring bar and place it on the stirrer in the refrigerator.

D) While stirring add 0.127 grams of $(\text{NH}_4)_2\text{SO}_4$ / mL of supernatant. Stir in the cold for 1.0 hour (you lose ~ 4-5% activity here).

EXAMPLE: $368 \text{ mL} \times 0.127 = 46.74$ grams of $(\text{NH}_4)_2\text{SO}_4$.

E) Pour the beaker's contents equally into two (2) 250 mL centrifuge tubes and spin at 12,000 RPM for 30 minutes at 2° C.

F) The supernatant is discarded and about 10.0 mL of distilled water is added to each tube to dissolve the precipitate. More distilled water is added from a squeeze bottle just enough to dissolve the precipitate.

G) After the precipitate is dissolved, add it to a graduated cylinder to measure its volume (so that proper length of dialysis tubing can be used).

V. DIALYSIS

A) A 1 mM potassium phosphate buffer is prepared from a 0.5 M KPO_4 dibasic stock and titrated to pH 7.0 with 0.5 M monobasic KPO_4

$228.23 \text{ g/L} = 1 \text{ M}$ $0.22823 \text{ g/L} = 1 \text{ mM}$ $4 \text{ mL} \times 500 \text{ mM} = 2000 \text{ mL}$

B) Using Fisher Scientific Dialyzer Tubing, Cat. No. 8-667A, Size .22" diameter inflated and .39" flat width, calculate the necessary length of tube to hold the volume of solution obtained in step (IV-G above). $\text{Volume} = \frac{1}{2} \text{ diameter} \times \pi^2 \times \text{length}$. Cut off the necessary length giving several inches extra.

C) Prepare the sac by placing it into a beaker containing about 10 grams of sodium bicarbonate/ liter (amount is not critical). This solution is then brought to a boil. When the tubing opens, remove it from the beaker (being careful not to let it dry) and rinsing it with deionized water and then distilled water. The sac will float to top when boiling, push back down.

D) One end of the tubing is folded several times, length-wise, and a knot tied.

E) The sac is then filled from the other end with the dissolved precipitate from IV-G above. Tie end.

F) The dialysis tubing is placed in the 1.0 mM KPO_4 buffer for 19 hours. in the refrigerator at 5° C with 4 changes of solution (~ every 4 hours).

G) Centrifuge (18,000) the dialized preparation and remove supernatant.

H) Adjust supernatant to pH 8.1 with 2 m Tris HCL, pH 8.5 (Tris (2M)= 24.2 g + 50 mL H_2O , add conc. HCL slowly until pH=8.5. Then make up to 100 mL. You actually use only 2 mL to adjust pH, so you should only make 10 mL. 24.2 g/100 mL=2.42 g/ 10 mL.

PROCEDURE FOR CATECHOLAMINE ASSAY

I. Catecholamines + ^3H s-adenosyl methionine COMT ^3H methoxyderivatives

Epinephrine and norepinephrine are converted to ^3H methanephine and ^3H norepinephrine, respectively, during incubation with catechol-O-methyl-transferase (COMT) and ^3H S-adenosyl methionine (^3H -SAM). The corresponding methanephines are extracted into acetic acid and washed with a series of toluene isoamyl washes. The metanephine and normetanephine are separated by thin layer chromatography (TLC). They are eluted from the TLC silica gel and oxidized to vanillin with sodium periodate. The vanillins corresponding to epinephrine and norepinephrine are extracted into a toluene-liquifluor solvent and counted in a scintillation counter. The output is proportional to the levels of catecholamines.

II. REAGENTS

1. THAM--Fisher T395
2. EGTA ·Na₂--Sigma #E 4378
3. MgCl₂ ·6H₂O--Fisher M33
4. Glutathione--Sigma G4251
5. Boric Acid--Matheson Coleman & Bell (MCB) BX865,CB225
6. EDTA·Na₂ --Mallinckrodt 4931
7. Metanephine HCL-- Sigma M8625

8. Normetanephrine HCL-- Sigma 7127
9. Toluene--Fisher T-324
10. Isoamyl Alcohol--Fisher A-393
11. Glaceol Acetic Acid--Fisher A-38
12. Sodium Periodate--Fisher S-398
13. Glycerin--Fisher G33
14. Ammonium Hydrodide--Fisher A669
15. HCL--Fisher A144
16. Epinephrine--Fisher SO-F-148
17. Norepinephrine (100 µg/mL) BIORAD
18. ³H-SAM --New England Nuclear (5-15 µCi)
19. Ethanol--Chem Stores
20. Methanol--Chem Stores
21. Silica Gel Plates (10 x 20 cm 25µ) Analtech
22. Benzene--Fisher B-245
23. Tert Amyl Alcohol--Fisher A-730
24. Methyl Amine (40%)--Fisher M223
25. Liquifluor--New England Nuclear NEF903
26. H₂SO₄ (pH 2):Ethanol : (9:1)

III. DAILY WORKING SOLUTIONS

A) Assay Buffer: TRIS

Add 6.05 grams of THAM, 1.9 grams EGTA-Na₂, and 3.05 grams MgCl₂ ·H₂O to approximately 20 mL of water. ***(It is understood that distilled water is always used)* Adjust pH to 8.3 with concentrated HCL or NaOH. The initial pH is close to this value. Bring volume to 25.0 mL with water.

B) Glutathione Solution: 0.1 Molar (Prepare Fresh)

Add 30.7 mg of reduced glutathione to a small test tube and add 1 mL of water. Vortex lightly and keep on ice.

C) Stopping Solution: 1.0 M Boric Acid + 0.2 M EDTA·Na₂

1. Add: 1.55 grams boric acid + 0.93 grams EDTA·H₂O·Na₂ to 20 mL H₂O .
2. Adjust pH to 11.1
3. Bring to 25 mL with water.

{This solution can be prepared in advance and aliquots can be stored frozen.}
What size aliquots?

D) Metanephrine & Normetanephrine: 0.2 µmoles/50 µL of borate buffer, which is 0.935 mg/mL of Met and 0.88 mg/mL of NorMet. For every 5 mL of the stopping solution add 4.67 mg of metanephrine and 4.4 mg of normetanephrine. Prepare prior to use

****needs more

E) Toluene-isoamyl alcohol (3:2)

Combine 300 mL of toluene and 200 mL of isoamyl alcohol.

F) Acetic Acid: 0.1 N

Bring 2.95 mL of glacial acetic acid up to 500 mL with water.

G) Sodium Periodate Solution: 4% w/v (??) NaIO_4

1 gram of NaIO_4 up to 25 mL with water. Prepare Fresh.

H) Ammonium Hydroxide Solution: 0.05 M NH_4OH

1 mL of NH_4OH up to 300 mL with water.

I) HCL: 1.0 N

1. 8.3 mL of concentrated HCL up to 100 mL

2. 0.01 HCL: 1.0 mL of 1.0 N HCL to 100 mL (2.0 mL to 200 mL)

3. 0.001 HCL: 1.0 mL of 1.0 N HCL to 1000 mL

J) Epinephrine & Norepinephrine Standards: 100 pg/10 μL .

1. 100 μL of Biorad Epinephrine (100 $\mu\text{g}/\text{mL}$) and 100 μL of Biorad Norepinephrine (100 $\mu\text{g}/\text{mL}$) are brought to 50 mL with 0.01 N HCL in the same volumetric vessel.

****more needed

2. Bring 5 mL of the above (#1) to 100 mL with 0.001 N HCL in a volumetric.

3. 10 μL of (#2) will give 100 μg of EPI & NE.

K) Enzyme: COMT

Prepared according to the procedure of Axelrod & Tomchick (1958). This is the rat liver preparation.

L) H_2SO_4 -Ethanol Solution: (9:1)

Prepare a H_2SO_4 solution of pH=2. Simply add concentrated H_2SO_4 to water (250 mL) until pH=2.0. This will require only a drop or two. Use 9 mL of this for every 1 mL of Ethanol (95%).

M) Toluene-Liquifluor to 1000 mL of toluene.

IV. REACTION

A) Turn of water bath and set to 37⁰ C.

B) Prepare two sets of test tubes (13 x 100 mm). Each sample should be done in duplicate. The number of internal standards will depend on the type of experiment, but a duplicate should be considered a minimum. Label tubes with a permanent marker (not water base). *Use a different color to mark internal standards.*

C) Remove enzyme, samples, buffer, and standard plasma from freezer. Thaw and keep on ice. Thaw ^3H SAM only before use.

D) Prepare the enzyme cocktail in a round bottom 13 x 100 mm tube. Add in the following order in amounts sufficient for the assay plus 3 (see below): Buffer solution, glutathione and ^3H SAM and H_2SO_4 -Ethanol Solution: (9:1); vortex lightly; and add COMT enzyme.

ASSAY	x 1	x20	x50	x100	x200
Buffer	5 μ L	100 μ L	250 μ L	500 μ L	1000 μ L
Glutathione	1 μ L	20 μ L	50 μ L	100 μ L	200 μ L
^3H SAM	5 μ L	100 μ L	250 μ L	500 μ L	1000 μ L
H ₂ SO ₄ -Ethanol (9:1)*	5 μ L	100 μ L	250 μ L	500 μ L	1000 μ L
Enzyme	25 μ L	500 μ L	1.25 mL	2.5 mL	5 mL

*= This solution is used to dilute the isotope. It is the solvent vehicle for the ^3H SAM. If higher counts are required for the assay, this should be omitted and the quantity of ^3H SAM indicated on the chart should be doubled (i.e., 10 μ L/tube).

E) INCUBATION MIXTURE

1) BLANKS

- 50 μ L of sample vehicle (i.e., water that is mixed with EGTA-glutathione in the same concentration as that of blood samples).
- 40 μ L of enzyme cocktail (#4).
- 10 μ L of 0.001 N HCL

2) Standards (Internal)

- 50 μ L of plasma
- 40 μ L of enzyme cocktail
- 10 μ L of 100 pg standard

3) Plasma Samples

- 50 μ L of sample plasma
- 40 μ L of enzyme cocktail
- 10 μ L of 0.001 N HCL

4) The plasma samples and blanks can be pipetted with the fixed volume 50 μ L positive displacement pipette. The tip does not have to be replaced after each sample.

5) The enzyme cocktail, 0.001 NHCL and standards can be pipetted with the Eppendorf repeater pipette. Please note the correct setting and adaptor size for volume desired.

6) All tubes are then vortexed, centrifuged, and then put into the water bath.

F) INCUBATION

75 minutes at 37⁰ C in oscillating bath.

G) STOPPING THE REACTION

Place reaction tubes in ice bath and add 50 μ L of the Borate stopping solution that contains the metanephrine and normetanephrine carriers (see

section III-C & III-D).

Vortexing is not necessary-just gently shake tube rack.

V) EXTRACTION

The second set of acid washed tubes will be used here. They will each receive 100 μ L of 0.1 N acetic acid (see section III-F).

A) Add 2 mL of toluene-isoamyl alcohol (3:2) to incubation tubes. Vortex well and centrifuge for about 2 minutes(to be consistent) at a speed setting of 1.

B) QUICK FREEZE: Prepare an ice bath of methanol and dry ice. Methanol level should only be deep enough to cover the bottom of test tubes (i.e., ~ 1/2-1 inch). Best to use a 1 liter beaker filled with dry ice and methanol and place a 100 mL beaker inside of the larger beaker. Place a piece of dry ice and methanol inside of this beaker to do the actual freezing in this vessel.

1) Submerge tube to level of aqueous phase.

2) Blot outside of tube. Decant the organic phase into the corresponding labeled tube containing the 0.1 N acetic acid (see section V above)

3) Rhythm: Decant one tube while the next tube is in the ice bath. Be consistent-15 to 25 seconds should be enough time. 20 seconds for each sample works very nicely.

C) Vortex and centrifuge the acetic acid, organic solution (see section V-B-2)

D) Quick Freeze (15 seconds works well) and aspirate off organic phase without touching suction pipette to pellet.

E) Add 1 mL of toluene-isoamyl alcohol to thawed aqueous phase.

F) Vortex and centrifuge. HOW LONG WHAT SPEED?

G) Quick Freeze, aspiration, and discard organic phase as in (D) above. The assay may be stopped at this point. Cap tubes and keep at -20° C.

VI THIN LAYER CHROMATOGRAPHY

A) Add 100-150 μ L of ethanol to the thawed acetic acid from section (V-G above). The normal number of tubes in one spotting run is 14-both of the outside strips are omitted.

B) Slowly pull acetic acid-ethanol solution into syringe. Do not pull excess air into syringe. Should have small bubble on the end of plunger.

C) Turn on dryer and spotter.

D) Place dryer approximately 3 cm from plates and set temperature control between (200-300 $^{\circ}$ F).

E) Position syringes so that tips touch TLC plates. Set plunger speed to slowest possible speed.

F) Prepare 44 mL of developing solution (6:2:3) in a 50 mL graduate.

1. 24 mL of tertiary amyl alcohol
2. 8 mL benzene
3. 12 mL methylamine
4. mix gently (solution should be clear).

G) Put developing solution in developing tank immediately before use. Carefully place plates in tank. Develop until solvent runs the entire plate. (~ 40-50 minutes).

H) Dry plates and visualize under Ultraviolet light. Utilizing a scapel, carefully outline the spots. (hint: best separation is obtained while plates are still somewhat damp.)

I) Scrape spots into acid washed scintillation vials (use a square edged scapel). The metanephrines are the top zone and normetanephrines are the bottom zone.

VII. OXIDATION

A) Add 1 mL of 0.05 M NH_4OH to vials. Vortex vigorously.

B) Line up vials and add 50 μL of 4% periodate solution at timed intervals. Vortex vigorously.

C) Stop reaction by adding 50 μL of 10% glycerol solution (1.26 g/mL) , five minutes after step B. Maintain order and timing of step B.

D) Add 1 mL of .1 M acetic acid to each vial and vortex.

E) Add 10 mL of toluene-liquifluous (1000:50 v/v) to each scintillation vial. Shake by hand vigorously for about 10 seconds).

F) Count each vial for 2-10 minutes in scintillation counter.

APPENDIX E.

CARDIAC OUTPUT PROCEDURES - CO₂ REBREATHING METHOD

Model 2870 three way sliding valve assembly: (see diagram p.4)

1. Attach part B of large 2-way NRVB 2700 series valve to Part A at black metal ring being careful not to strip the threads.
Mouthpiece attaches to front tube on Part A.
2. Attach clean black latex rebreathing bag to the down tube - Part A (directly below the mouthpiece)
3. For room air operation, the sliding valve handle (black handle on left) is in the out position.
4. Attach 10 inch clear tube to bag fill/evac toggle switch control.

ANALYZERS

5. Calibrate gas analyzers by standard procedure. Attach sensor sensitivity enhancement device to second CO₂ analyzer (if using computer instead of physiograph to record instantaneous CO₂).
6. Prepare necessary aquasorb tubes.

COMPUTER

7. Obtain appropriate Rayfield computer program from disc organizer in Pam's office. (Exercise Physiology for Apple II Plus - Rick Rayfield)
 8. Boot up the program. When message "PUSH ANY KEY TO BEGIN" appears push RESET. At prompt] type CATALOG [return] Type Load CO₂ 9/85 [return] Type RUN [return]
- Answer the questions that appear on the screen.
- SECS IN TEST ?
?
- CO₂ RANGE - MINIMUM ? TYPE 0 [RETURN]
MAX.? TYPE 15 [RETURN]
- HIT ANY KEY TO START (Wait until you are ready to begin test)

EXTERNAL CO₂ ANALYZER/PHYSIOGRAPH SETUP

CALIBRATE THE CO₂ ANALYZER FOLLOWING STANDARD PROCEDURE
ATTACH BECKMAN MEDICAL GAS ANALYZER LB-2 CO₂ SENSOR.
(CABLE FROM SENSOR ATTACHES TO REAR OF CO₂ ANALYZER.)

CONNECT SENSOR TUBE TO CO₂ SENSOR SITE LOCATED NEAR THE MOUTHEPIECE. ** THE SENSOR MUST BE LOCATED ON THE TOP SIDE OF THE MOUTHEPIECE NOT ON THE BOTTOM SIDE)

A. CALIBRATE PHYSIOGRAPH

1. TURN MAIN POWER ON
2. TURN SERVO CHANNEL ON, RECORD SWITCH ON
3. AMPLITUDE - 300 MV/CM
4. SENSITIVITY - BETWEEN 1000 AND 300 MV/CM
5. CALIBRATE SUCH THAT 1% CO₂ = TWO LARGE BOXES(10 SMALL BOXES)

USE POSITION KNOB TO SET THE PEN NEAR THE BOTTOM OF THE PAPER IN ORDER TO ACCOMMODATE FULL % RANGE NECESSARY FOR THE TEST(4 LARGE BOXES BELOW FIRST BOLD LINE PHYSIOGRAPH PAPER).

6. SET PAPER SPEED AT .1

GAS MIXING APPARATUS - TURN POWER ON (RED BUTTON)

A. TANKS-

1. TWO GAS TANKS ARE ATTACHED TO THE GAS MIXING APPARATUS. ONE CONTAINS 100% O₂ (WITH GREEN HOSE ATTACHED). THE SECOND TANK IS A TWO GAS MIX WITH 80% O₂ /20 % CO₂ (WITH RED HOSE ATTACHED)
2. TURN BOTH TANKS ON SO THEY READ 40 PSI. IF THE PRESSURE ISN'T EQUAL BETWEEN THEM, THEY WILL START TO "WHINE".

B. PRE-TEST PROCEDURE EVACUATE THE BAG BY:

1. SWITCH VACUUM PUMP TOGGLE ON BAG FILL /EVAC TOGGLE CONTROL TO THE "ON" POSITION (TO THE RIGHT TOWARDS THE TUBE)
2. USE VACUUM PUMP TOGGLE SWITCH ON THE GAS MIXER TO MANUALLY EVACUATE THE LATEX REBREATHING BAG. HOLD UNTIL BAG IS VISIBLY EMPTY.
3. FLIP TOGGLE SWITCH TO THE OFF POSITION.

INITIATING TEST

1. MOUTH PIECE IN PLACE
2. NOSE CLIP IN PLACE
3. RESTING MEASUREMENT

- a. allow approximately 4 minutes for subject to reach steady state
- b. Get VO_2 measurement from computer print out at minute 4
- c. Get tidal volume (VI/RR). Multiply by 1.5 to obtain calculated volume
- d. Determine appropriate gas mixture and timer settings. (See chart)

TABLE FOR ESTIMATING DIAL AND TIMER SETTING BASED ON RECENT TESTS

	VO_2	% CO_2	1.5 x TV	TIMER
REST	.25	9%	1.2	2
	.46	11%		
25%	.75	12.8%	1.7	2.3
	1.21	12.8%		
50%	1.75	13.5%	2.1	2.5
	2.29	14.5%	2.9	2.9
75%	2.54	17.0%		3.3
	3.2			
	3.23	18.5%		

4. FLIP TOGGLE ON THE BAG FILL/EVAC TOGGLE CONTROL TO THE ON POSITION (TO THE LEFT TOWARDS THE TUBE.)
5. USE TIMER RESET SWITCH TO AUTOMATICALLY FILL THE REBREATHING BAG WITH THE APPROPRIATE VOLUME AND GAS MIXTURE. (QUICKLY LIFT UP TIMER RESET TOGGLE AND RELEASE IT; DO NOT HOLD.)
TIMER AUTOMATICALLY FILLS THE REBREATHING BAG.
6. MAKE CERTAIN THAT PHYSIOGRAPH PAPER AND RECORD BUTTON ARE TURNED ON, RECORD 8-10 NORMAL BREATHS TO ESTABLISH END-TIDAL CO_2 CONCENTRATION.
7. CAREFULLY OBSERVE MOVEMENT OF VALVE DIAPHRAGMS AND PUSH HANDLE IN AT THE END OF THE SUBJECT'S 8TH TO 10TH EXPIRATION (THIS MUST BE TIMED EXACTLY).
8. INSTRUCT SUBJECT TO BREATHE IN AND OUT RAPIDLY, BUT NO DEEPER THAN NORMAL. SUBJECT SHOULD EQUILIBRATE WITHIN 10 BREATHS.
9. OBSERVE PHYSIOGRAPH RECORD TO INSURE THAT THE SUBJECT HAS EQUILIBRATED WITH THE GAS MIXTURE IN THE BAG.

(PHYSIOGRAPH RECORDING MUST LEVEL OFF)

IF RECORDING CONTINUES TO CREEP UPWARD, THE CO₂ CONCENTRATION IN THE GAS MIXTURE WAS INSUFFICIENT TO OBTAIN EQUILIBRIUM; IF THE RECORDING CREEPS DOWNWARD, THE OPTIMUM CO₂ CONCENTRATION WAS EXCEEDED.

10. AT THE END OF THE 10TH EXPIRATION, SWITCH SUBJECT BACK TO ROOM AIR (PULL OUT ON BLACK HANDLE).

REPEAT STEPS 1 THRU 10 ABOVE AT VARIOUS WORKRATES IN ORDER TO OBTAIN END-TIDAL AND EQUILIBRATION VALUES DURING EXERCISE.

CAUTION - MONITOR SUBJECTS CAREFULLY AT HIGH WORK INTENSITIES AS THEY MAY EXPERIENCE DIZZINESS DURING CO₂ REBREATHING.

VITA

Ava J. Walker was born in Nashville, Tennessee on December 28, 1953. She attended The University of Tennessee, Knoxville and in March 1977 received the degree of Bachelor of Science in Physical Education. She returned to The University of Tennessee and received her Master of Science degree in Education in August, 1978. In August of 1988 she entered The University of Tennessee, Knoxville for the last time as a student and received a Doctor of Philosophy in Education in August, 1991. (And there was great REJOICING!)

She taught Physical Education and coached volleyball, basketball, and tennis for ten years at Morristown Hamblen High School East in Morristown, Tennessee. She has served as a Chemical and Medical Services Corp officer in the Tennessee Army National Guard for seven years.