

ABSTRACT

Title of Dissertation: INFLUENCE OF THE CYP11B2 -344C/T POLYMORPHISM ON PLASMA ALDOSTERONE, SODIUM EXCRETION AND BLOOD PRESSURE RESPONSES TO LONG-TERM AEROBIC EXERCISE TRAINING IN MIDDLE-AGED TO OLDER PREHYPERTENSIVES.

Jennifer Michelle Jones, Doctor of Philosophy, 2004

Dissertation Directed By: Michael Brown, PhD, Associate Professor
Department of Kinesiology

Aldosterone influences the kidney's normal regulation of blood pressure (BP), but if consistently elevated, aldosterone may contribute to hypertension. BP is decreased with aerobic exercise training (AEX), but the extent to which plasma aldosterone (PA) levels change is unclear. The CYP11B2 -344C/T polymorphism has been associated with hypertension and may contribute to the change in BP and PA with AEX. The purpose of this study was to determine if 6 months of AEX changed PA levels, 24-hour Na⁺ excretion and BP in middle-aged to older prehypertensives, and whether the -344C/T polymorphism was associated with changes in these primary outcome variables. Forty (23 Caucasians, 14 African descents (AD), 2 Asian/Pacific Islanders and 1 of other ethnicity) disease free sedentary prehypertensives completed AEX. All participants followed the AHA Step I diet. Blood samples were collected under fasting and supine conditions and PA was measured by RIA. In the total sample, PA levels decreased after AEX (p=0.04). The reduction in PA levels in

Caucasians (-37 ± 15 pg/ml, $p=0.02$) tended to be greater ($p=0.07$) than the reduction in PA levels in AD (-2 ± 8 pg/ml, $p=0.77$). Among AD, PA levels tended to decrease when controlling for baseline PA levels ($p=0.09$). The change in systolic BP (SBP) in Caucasians (-3 ± 1 mm Hg, $p=0.05$) was significantly different ($p=0.03$) than the change in SBP in AD (4 ± 3 , $p=0.28$). In the AD, 24-hour Na^+ excretion tended to increase when controlling for baseline 24-hour Na^+ excretion ($p=0.06$). Among the Caucasians, the TC+CC genotype group tended to decrease PA levels (-39 ± 21 pg/ml, $p=0.09$) and significantly decreased SBP (-4 ± 2 mm Hg, $p=0.03$). Among AD, the TT genotype group significantly decreased PA levels when controlling for baseline PA levels (-17 ± 7 pg/ml, $p=0.04$). The AD TC+CC genotype group tended to increase SBP when controlling for the change in 24-hour Na^+ excretion (11 ± 2 mm Hg, $p=0.09$) and 24-hour Na^+ excretion significantly increased when controlling for the change in BMI (14 ± 11 mmol/d, $p=0.03$). BP and PA levels appear to be more responsive to AEX in Caucasians compared to AD. The CYP11B2 -344C/T gene appears to influence the responses of hypertensive phenotypes to AEX.

INFLUENCE OF THE CYP11B2 -344C/T POLYMORPHISM
ON PLASMA ALDOSTERONE, SODIUM EXCRETION AND BLOOD
PRESSURE RESPONSES TO LONG-TERM AEROBIC EXERCISE TRAINING
IN MIDDLE-AGED TO OLDER PREHYPERTENSIVES

By

Jennifer Michelle Jones

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Advisory Committee:
Associate Professor Dr. Michael Brown, Chair
Professor Sharon Desmond
Assistant Professor Thomas Dowling
Professor James Hagberg
Assistant Professor Stephen Roth

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Dedication

To my mother who planted and began the cultivation of the seed to my success

To my father who continued to water and cultivate me when my mother could no longer

To my grandmother who provided me with light and inspiration for growth

To my aunts who continually nurtured and tended to me as if I was their own

To all of my family and friends who provided me with more love than I could ask for

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Introduction

Essential hypertension is a chronic disease in which the etiology is considered to be multifactorial and polygenic. Due to the complex pathology and the polygenic nature of hypertension, research has necessarily focused on multiple physiological mechanisms and candidate genes to better understand the factors involved in the etiology of hypertension. The renin-angiotensin-aldosterone system (RAAS) has received significant attention because of its role in the kidney's regulation of blood pressure. Components of the RAAS, such as aldosterone, have been shown to have a substantial influence on sodium (Na^+) reabsorption within the kidney, which can lead to an increase in blood pressure (54). Specifically, aldosterone is released from the zona glomerulosa region of the adrenal cortex and acts on the collecting tubules in the kidney to increase Na^+ reabsorption (54).

Given that aldosterone affects renal Na^+ handling and consequently blood pressure, alterations in aldosterone biosynthesis may contribute to the development of hypertension. This reasoning has prompted investigation into genes that might influence aldosterone biosynthesis. Recently, the aldosterone synthase (AS) gene, CYP11B2, which is expressed in the zona glomerulosa of the adrenal cortex, has been identified as a possible contributor to the development of hypertension because it codes for the enzyme AS (P450c11AS), which synthesizes aldosterone (20; 54; 161).

Polymorphisms within the CYP11B2 gene, such as the -344C/T single nucleotide polymorphism (SNP), may be responsible for the interindividual variation in aldosterone levels and may affect an individual's susceptibility to hypertension. The -344C/T polymorphism is a promoter mutation that consists of a C to T

nucleotide substitution –344 nucleotides upstream from the start of translation (155). The SNP resides in the Ad4 sequence, a nuclear receptor half-site that is required for the development and function of the adrenal glands and the region where the transcription factor, steroidogenic transcription factor (SF-1) binds (10; 104). It has been suggested that the –344C/T polymorphism may affect SF-1 binding (155), but there is continued debate on whether this affects gene expression (10; 20).

Recently, Henderson et al. reported that the risk of hypertension was associated with the T allele of the -344 C/T polymorphism in hypertensives of African descent (66). Similarly, Brand and colleagues found that the T allele was more frequent in hypertensive Caucasians compared to normotensive Caucasians (14; 29). The T allele has also been linked to the age-associated increase in diastolic blood pressure (DBP) in Caucasian males (127). Despite these positive findings, other studies have reported no association between the -344C/T polymorphism and hypertension in individuals of African descent, Caucasians and Japanese (79; 150; 163).

In regards to ethnicity, there appears to be some differences in the –344C/T allele frequency. It has been reported that among those of African descent, the T allele has a much higher frequency than the C allele (163). In Caucasians, the frequency of both the T and C allele has been reported to be approximately 50% (163).

Based on the available evidence, the –344C/T allele association with aldosterone levels in the plasma and urine may differ between normotensive and hypertensive individuals. For example, in hypertensives, the C allele was associated

with greater plasma aldosterone levels and the CC genotype was associated with a lack of reduction in serum aldosterone levels after one week of oral Na⁺ loading (31; 114). Counter to these findings, Davies et al. and Brand et al. have reported that in normotensives, the T allele was associated with increased urinary aldosterone excretion and plasma aldosterone levels (14; 29).

Aerobic exercise training is effective in reducing blood pressure in hypertensive individuals (58; 59; 84; 87; 125). One possible mechanism for exercise training-induced reductions in blood pressure is an increase in Na⁺ excretion (16; 84), and it is possible that a reduction in plasma aldosterone levels with exercise training could contribute to the increase in Na⁺ excretion (13; 67; 86).

Despite controlling for variables known to affect the blood pressure response to aerobic exercise training, there continues to be hypertensive individuals who change and other individuals who do not change their blood pressure and other hypertension-related phenotypes with aerobic exercise training. Based on the observed interindividual variability in the blood pressure response to aerobic exercise training, studies have been conducted to determine the influence of common gene variants on the responses of hypertension-related phenotypes to aerobic exercise training.

The purpose of the current study was to determine whether 6 months of aerobic exercise training reduced plasma aldosterone levels, increased 24-hour Na⁺ excretion and reduced blood pressure in middle-aged to older prehypertensives. Additionally, because the CYP11B2 -344C/T polymorphism has been related to plasma aldosterone, Na⁺ handling and blood pressure it was important to investigate

whether the -344C/T polymorphism influenced the responses of these variables to aerobic exercise training.

Methods

Screening

Participants between the ages of 50-75 years were recruited from the College Park, Maryland and the District of Columbia metropolitan area, via advertisements in newspapers, radio public service announcements, direct mail and health fairs. The Institutional Review Board of the University of Maryland, College Park approved the study. Participants underwent an initial telephone screening and were excluded from the study if they had a body mass index (BMI) greater than 37 kg/m^2 , any form of cardiovascular disease (CVD), liver disease, diabetes, pulmonary disease, or orthopedic conditions that would impair their ability to exercise. Females were excluded if they were not post-menopausal for more than 2 years. For who that were post-menopausal, they were asked to maintain their hormone replacement status for the entirety of the study. Potential participants on more than 2 antihypertensive medications were excluded. Participants in the study were required to be sedentary, therefore those exercising more than 2 times per week for more than 20 minutes were considered active and were excluded.

Participants who met the initial study inclusion criteria provided written informed consent and were scheduled for two screening visits. The first screening visit involved a 2-hour post prandial glucose tolerance test and blood samples were obtained for chemistries and DNA analysis. Participants were excluded if they exhibited a fasting blood glucose $> 126 \text{ mg/dl}$, or a blood glucose greater than 200 mg/dl at 2 hours. Participants were excluded if they had a glomerular filtration rate (GFR) $< 60 \text{ ml/min/1.73 m}^2$, which was estimated by use of the Modification of Diet

in Renal Disease (MDRD) equation, and serum creatinine levels >1.5 mg/dl, both ensured that they did not have evidence of renal disease. BMI was verified and three casual blood pressure measurements were taken on each arm using standard sphygmomanometer. Participants with an average systolic blood pressure (SBP) < 120 or >159 mm Hg and/or diastolic blood pressure (DBP) <80 or >99 mm Hg were excluded from the study.

During the second screening visits, participants performed a physician supervised graded exercise test (Bruce protocol) to screen for CVD. ECG, blood pressure, and heart rate were measured before the treadmill test, at the end of each stage, and during the 6 minutes after the test. The test was terminated upon the onset of cardiovascular signs and/or symptoms, or when the participants could no longer continue. Subjects were included in the study if they did not exhibit any cardiovascular signs or symptoms and had less than 2mV ST segment depression (2).

Dietary Stabilization/Medication Tapering

Dietary and weight changes are known to independently contribute to the reduction in blood pressure (9; 42; 49). In order to control for such effects, all participants were required to be weight stable and follow a controlled diet in order to determine the independent effects of aerobic exercise training on plasma aldosterone levels, Na⁺ excretion, and blood pressure. Participants attended a 6-week dietary class taught by a Registered Dietician two times per week. The participants followed the American Heart Association (AHA) Step I diet (50-55% of calories from carbohydrates, 30-35 % from fat, 20-25% from protein, 350 mmol/day of cholesterol, and 3g/day of salt) for the entirety of the study. The participants were required to stay

within 5% of their study entry body weight throughout the study. The participants completed a 7-day food record to ensure that they adhered to the diet. Participant's diet records were analyzed by the dietician using the Nutritionist IV software.

Participants using antihypertensive medications were tapered off of their medications during the 6-week dietary period with written approval from their personal physician. Participants needed to maintain their blood pressure between 120-159/80-99 mm Hg to be included in the study. Participants with SBP < 120 or >159 mm Hg and/or DBP <80 or >99 mm Hg consistently during the 6-week dietary period were excluded from the study.

Baseline Testing

Casual Blood Pressure Measurement: Casual blood pressure was measured in all participants on three separate days according to the JNC VII guidelines (19). The average of the casual blood pressure measurements recorded on separate baseline testing days was the primary outcome variable used in the data analysis. In the event that casual blood pressure measurements were only obtained on two separate baseline testing days, these values were averaged and used as the primary outcome variable in the data analysis.

24-hour Urine Collection: Participants underwent urine collection to measure 24-hour Na⁺ and potassium (K⁺) excretion. The participants were given 2 urine collection bottles in which to collect their urine over the 24-hour period, starting the morning they picked up the containers (7am-9am) and ending after their first urination of the morning. They were also given a cooler filled with ice to keep the urine cold. Participants returned the urine containers the following morning. The

urine was processed at the University of Maryland, College Park, Hypertension and Exercise Physiology Laboratory and sent to Quest Diagnostic Laboratories for analysis of Na^+ and K^+ excretion (CLIA License 21D0218877).

Maximal Oxygen Consumption (VO_2max): To develop an exercise prescription specific for each participant and to assess their cardiovascular fitness, participants performed a physician supervised VO_2max test. Participants began exercising on a treadmill at an intensity equivalent to 70% of the peak heart rate they achieved during their screening exercise test. Participants were fitted with a mouthpiece and a nose clip in order to collect the expired air necessary to measure oxygen consumption. The grade was increased 2% every 2 minutes, and ECG, heart rate and blood pressure was measured every 2 minutes and after the test. The test was stopped when participants could no longer continue or upon onset of cardiovascular signs and/or symptoms (2). Oxygen uptake was measured using a computerized on-line VO_2 system including a gas analyzer (Mass Spectrometer MGA-1100, Marquette Electronics Inc., Milwaukee, WI) and a bi-directional turbine flow meter (Ventilation Measurement Module VMM-2, Interface Associates, Aliso Viejo, CA). VO_2max was measured continuously, and in order to ensure that a true VO_2max two of the three criteria were met ($\text{RER} > 1.1$, $\text{HR} > [220-\text{age}]$, and <150 ml/min increase in VO_2 during the last two minutes of the test).

Body composition: Changes in percent body fat may act as a confounding variable when measuring the independent effect of exercise training on blood pressure, plasma aldosterone and Na^+ excretion (9; 46-49; 78; 129). Therefore, dual energy X-ray absorptiometry (DEXA) was used to assess any body composition

changes that occurred during the 6-month aerobic exercise training period. Fat mass and fat free mass were measured. Participants were instructed to fast for 12 hours prior to the start of the test.

Measurement of Aldosterone: Blood samples for the measurement of plasma aldosterone were collected before the start of the oral glucose tolerance test (OGTT). Participants were instructed to undergo a 12-hour fast prior to blood sample collection and to exclude all ibuprofen and antihistamines 48 hours before the OGTT (17). The aldosterone samples were collected while the participant was supine for 15-20 minutes (142). Blood samples were collected in EDTA tubes and centrifuged at 3000 rpm for 20 minutes at 4°C, and the plasma was aliquoted into 1.5 ml microtubes and stored at -20°C (85). Radioimmunoassay was used to measure plasma aldosterone levels (¹²⁵I Coat-A-Count Aldosterone kit, Diagnostic Products Corporation).

Genotyping: Genotyping was performed at the Functional Genomics Laboratory in the Department of Kinesiology at the University of Maryland. DNA was extracted from whole blood samples using techniques described by Miller (101). DNA amplification was performed by polymerase chain reaction (PCR), with the use of the CYP11B2 (-344C/T) forward primer 5'-AGG-GTG-TAC CTG TGT-CAG-GGC A-3' and the CYP11B2 (344C/T) reverse primer 5'-CCT-CTC-CTT-TCT-CCA-GGG-CTG A-3'. The PCR was performed at a denaturing temperature of 95°C for 5 minutes, followed by 35 cycles at 95°C for 30 seconds, 53°C for 30 seconds, 72°C for 30 seconds and 72°C for 5 minutes. Digestion was performed with the Hae III enzyme, and TT homozygotes were detected at 186 base pairs (bp), CT

heterozygotes were detected at 186, 115, and 71 bp, and CC homozygotes were detected at 115 and 71 bp.

Exercise Training

Participants underwent a 6 month supervised aerobic exercise training intervention, which was held at the University of Maryland Wellness Research Laboratory. Participants exercised 3 times per week and were given a wrist heart rate monitor (Model 6124, Polar Electro, Canada) to monitor their prescribed heart rate. During the first week of exercise training, the participants exercised for 20 minutes at 50% of heart rate reserve (HRR). Exercise duration was increased gradually by 5 minutes per week until the participants were exercising for 40 minutes. At the 6th week, exercise intensity was increased by 5% of their HRR every week until they were exercising at 70% of HRR. Seated blood pressures were measured at the beginning and end of each exercise session. The participants recorded their blood pressure, heart rate, weight, exercise intensity and duration in a logbook provided for them. These log books were analyzed to ensure that the participants were adhering to their exercise prescription. Once the participants completed the 10th week of exercise training, they were required to add an extra day of unsupervised exercise for 45-60 minutes at <70% of HRR to their current exercise prescription.

Final Testing

Upon completion of the 6-month exercise training protocol, participants repeated all tests performed at baseline. This included casual blood pressure measurement, 24-hour urine collection, VO₂max, body composition, and aldosterone

measurement. The participants continued exercising until all final tests were completed. In addition, the final tests were performed after participants provided a 7-day food record to ensure dietary compliance, and 24-36 hours after their regular exercise session.

Data Analysis

Statistical analyses were performed using SPSS (Version 11.0). A dependent samples t-test was used to determine whether there were significant changes in subject characteristics with aerobic exercise training in the total study population. ANCOVA was performed in the total sample to determine if significant changes occurred in the primary outcome variables (plasma aldosterone levels, 24-hour Na⁺ excretion and casual blood pressure) with aerobic exercise training. Variables known to influence the primary outcome variables were used as covariates. ANCOVA was also performed when ethnicity and gender were entered separately as grouping variables. Linear regression analyses were conducted to determine if there were relationships between the change values for plasma aldosterone levels, 24-hour Na⁺ excretion, and blood pressure.

An independent samples t-test was performed to compare subject characteristics between the two ethnicities (Caucasians and individuals of African descent) and a dependent t-test was performed within each ethnic group. ANCOVA was performed within each ethnic group. Linear regression analyses were also conducted within each ethnic group to determine if there were relationships between

the change values for plasma aldosterone levels and 24-hour Na⁺ excretion, and casual blood pressure.

The literature has reported that the CYP11B2 -344C/T genotype and allele frequencies differ between Caucasians and individuals of African descent (14; 66; 147; 163). Therefore, genetic analyses were performed separately within each ethnic group. A Chi-Square test was performed within each ethnic group to determine if the genotype distribution of the CYP11B2 -344C/T polymorphism was in Hardy-Weinberg equilibrium. An independent t-test was performed to compare the subject characteristics between the TT and TC+CC genotype groups before and after aerobic exercise training. A dependent samples t-test was performed within each genotype group to determine whether there were changes in subject characteristics with aerobic exercise training. ANCOVA was performed within each genotype group to compare differences in the primary outcome variables. Linear regression analyses were also conducted within each genotype group to determine if there were relationships between the change values for plasma aldosterone levels and 24-hour Na⁺ excretion, and casual blood pressure. All values are reported as means ± SE. ANCOVA results were reported as adjusted means. A p-value of ≤ 0.05 was considered statistically significant.

The variables gender, age, BMI, Na⁺ and K⁺ excretion are known to have an influence on plasma aldosterone levels; therefore they were used as covariates when measuring the effect of exercise training on plasma aldosterone levels. The variables plasma aldosterone, Na⁺ excretion and BMI are known to influence blood pressure;

therefore they were used as covariates when measuring the effect of exercise training on blood pressure. Additionally, BMI and plasma aldosterone can influence Na⁺ excretion, therefore they were used as covariates when investigating the effect of exercise on 24-hour Na⁺ excretion

Four separate assays were completed to measure plasma aldosterone levels in all of the samples. The intra-assay coefficient was 24.9% and the inter-assay coefficient was 20.4%. The sensitivity of the assay was 11 pg/ml.

Results

Total Sample

Sixty-six participants (37 females and 29 males) with a mean age of 58 ± 1 years were enrolled in this study, in which 50% ($n=33$) were Caucasian, 42% ($n=28$) were of African descent, 3% ($n=2$) were Asian/Pacific Islander and 5% ($n=3$) were categorized as other ethnicity. The participants were prehypertensive (SBP 131 ± 1 mm Hg, DBP 85 ± 1 mm Hg), had plasma aldosterone levels (82 ± 7 pg/ml) within the normal range (20-230 pg/ml) and had 24-hour Na^+ excretion (112 ± 7 mmol/d) within the normal range (52-380 mmol/d). Serum creatinine (1.0 ± 0.0 mg/dl) and GFR (76 ± 2 ml/min/1.73 m²) were less than 1.5 mg/dl and greater than 60 ml/min/1.73 m², respectively, indicating normal renal function. Additionally, the participants were overweight (BMI 28.7 ± 0.5 kg/m²) and unfit (VO_2 max 23.9 ± 0.6 ml/kg/min).

Of the 66 participants, 40 (22 males and 18 females) completed the 6 month aerobic exercise training intervention. Of those completing the aerobic exercise training intervention, 23 were Caucasian, 14 were individuals of African descent, 2 were Asian/Pacific Islanders and 1 individual was categorized as other ethnicity. In this total group, SBP, DBP, and 24-hour Na^+ excretion did not significantly change with aerobic exercise training, but there was a significant reduction in plasma aldosterone levels with aerobic exercise training ($p=0.04$) (Table 1). There was no significant change in 24-hour K^+ excretion, but there was a significant reduction in BMI, total body fat, and body weight, and a significant increase in VO_2 max with aerobic exercise training (Table 1). The change in plasma aldosterone levels were not

independently related with the training-induced changes in SBP, DBP, or 24-hour Na⁺ excretion.

Ethnicity

Both ethnic groups (African descent and Caucasian) were prehypertensive and had normal baseline plasma aldosterone levels and 24-hour Na⁺ excretion (Table 2). The BMI and VO₂ max values for each ethnic group indicated that they were overweight and unfit (Table 2). Individuals of African descent had baseline plasma aldosterone levels that were 42% lower than Caucasians (p=0.002) and baseline 24-hour K⁺ excretion that was 28% lower (p=0.002) compared to Caucasians (Table 2).

The change in plasma aldosterone levels (final vs. baseline) with aerobic exercise training tended to be different between the two ethnic groups (p=0.07) (Table 3). Final plasma aldosterone levels after aerobic exercise training also tended to be different between the two ethnic groups (p=0.08) (Table 2). Additionally, after separately controlling for final BMI and final 24-hour K⁺ excretion after aerobic exercise training, final plasma aldosterone levels after aerobic exercise training were significantly different between the two ethnic groups. Adjusted means for final plasma aldosterone levels in the two ethnic groups after aerobic exercise training, and after controlling for final BMI were 80±7 pg/ml in the Caucasian group and 60±9 pg/ml in the African descent group (p=0.03). The adjusted means for final plasma aldosterone levels in the two ethnic groups after aerobic exercise training, and after controlling for final 24-hour K⁺ excretion were 82±7 pg/ml in the Caucasian group and 58±8 pg/ml in the African descent group (p=0.01). There was no difference in the change in 24-hour Na⁺ excretion between the two ethnic groups (Table 3). The

change in SBP was significantly different between Caucasians and individuals of African descent (Caucasians -3 ± 1 mm Hg; African descent 4 ± 3 mm Hg, $p=0.03$). After aerobic exercise training, body weight was significantly lower ($p=0.02$) among individuals of African descent compared to Caucasians (Table 2).

Caucasian Group: The Caucasians significantly increased VO_2 max and reduced total body fat, body weight, and BMI after 6 months of aerobic exercise training (Table 4). Aerobic exercise training significantly reduced plasma aldosterone levels and SBP (Table 4). Baseline plasma aldosterone levels were a strong predictor of the change in plasma aldosterone levels with aerobic exercise training ($r= -0.90$, $p<0.001$), with those who had the highest baseline plasma aldosterone levels experienced the greatest reduction in plasma aldosterone levels (Figure 1). After accounting for baseline plasma aldosterone levels, the reduction in plasma aldosterone levels with aerobic exercise training remained significant. The adjusted mean for the change in plasma aldosterone levels when controlling for baseline plasma aldosterone levels was -39 ± 8 pg/ml ($p<0.001$).

Baseline SBP was a strong predictor of the change in SBP with aerobic exercise training ($r= -0.60$, $p=0.01$). Those with the greatest SBP at baseline had the greatest reduction in SBP with aerobic exercise training. There was no significant change in 24-hour Na^+ and K^+ excretion with aerobic exercise training (Table 4). The change in plasma aldosterone levels with aerobic exercise training were not independently related with the change in SBP, DBP, or 24-hour Na^+ excretion in Caucasians.

African Descent Group: The individuals of African descent significantly increased their VO₂ max with aerobic exercise training (Table 5). There was no significant change in total body fat, and BMI, and body weight tended to decrease after aerobic exercise training in individuals of African descent (Table 5). There were no significant changes in SBP, DBP and plasma aldosterone levels with aerobic exercise training (Table 5). After accounting for baseline plasma aldosterone levels, there was a tendency for plasma aldosterone levels to decrease with aerobic exercise training. The adjusted mean for the change in plasma aldosterone levels when controlling for baseline plasma aldosterone levels was -2 ± 7 pg/ml ($p=0.09$). There was also a tendency for baseline plasma aldosterone levels to be negatively related to the change in plasma aldosterone levels with aerobic exercise training ($r=-0.50$, $p=0.09$) (Figure 2). There was a tendency for 24-hour Na⁺ excretion to increase after aerobic exercise training when accounting for baseline 24-hour Na⁺ excretion ($p=0.06$). There was a tendency for a negative relationship between baseline 24-hour Na⁺ excretion and the change in 24-hour Na⁺ excretion with aerobic exercise training ($r=-0.53$, $p=0.06$). Additionally, the change in DBP was statistically significant after accounting for the change in 24-hour Na⁺ excretion ($p=0.05$). This did not occur with SBP. It was found that the change in plasma aldosterone levels with aerobic exercise training was not independently related to the change in SBP, DBP or 24-hour Na⁺ excretion.

Genotype Results

CYP11B2 -344C/T genotype and allele frequencies differ between Caucasians and individuals of African descent (14; 66; 147; 163), therefore the frequencies were determined separately in each ethnic group. Due to the small number of individuals who were either Asian/Pacific Islander or categorized as other ethnicity, genotype frequency was not determined in these two groups.

Caucasian Group: The CYP11B2 -344C/T genotype frequencies for Caucasians were 0.41, 0.39 and 0.20 for the TT, TC and CC genotypes, respectively (Table 6). The T and C allele frequencies were 0.61 and 0.39, similar to what has been found in other Caucasian populations (14; 163). The distribution of the CYP11B2 -344C/T genotype frequencies were in Hardy-Weinberg equilibrium ($\chi^2=1.05$, $p=0.61$). The TT genotype group tended ($p=0.06$) to be younger than the TC+CC genotype group (Table 7), but there were no other differences in baseline subject characteristics between the two genotype groups.

There were 9 participants in the TT genotype group and 13 in the TC+CC genotype group who completed the study. The change in plasma aldosterone levels with aerobic exercise training was significantly different between the two genotype groups after accounting for baseline plasma aldosterone levels. The adjusted means for change in plasma aldosterone levels in the two genotype groups when controlling for baseline plasma aldosterone levels were -23 ± 10 pg/ml for the TT genotype group and -47 ± 8 pg/ml for the TC+CC genotype group ($p<0.001$). Other variables known to affect plasma aldosterone levels did not affect the change in plasma aldosterone levels

between the two genotype groups when used as covariates. Final values for plasma aldosterone levels after aerobic exercise training were significantly lower among the TC+CC genotype group compared to the TT genotype group (Table 8). The change in SBP was significantly different between the two genotype groups when accounting for baseline SBP. The adjusted means for the change in SBP in the two genotype groups when controlling for baseline SBP were -2 ± 2 mm Hg for the TT genotype group and -4 ± 1 mm Hg for the TC+CC genotype group ($p=0.02$). The change in DBP was significantly different between the two genotype groups ($p=0.04$) (Table 8).

In the TC+CC genotype group there was a significant reduction in SBP, BMI, total body fat, and body weight and a trend toward a reduction in plasma aldosterone levels (Table 9). The change in plasma aldosterone levels with aerobic exercise training was not independently related to the change in SBP and 24-hour Na^+ excretion within any genotype group. There were no significant changes in the plasma aldosterone, 24-hour Na^+ excretion and blood pressure in the TT genotype group (Table 10).

African Descent Group: The CYP11B2 -344 T and C allele frequencies for individuals of African descent were 0.75 and 0.25, respectively (Table 11). These frequencies are similar to previous studies that have included individuals of African descent (66; 163). The genotype frequencies were 0.54, 0.42, and 0.04 for the TT, TC and CC genotype groups, respectively (Table 11). The distribution of the genotype frequencies was in Hardy-Weinberg equilibrium ($\chi^2=0.427$, $p=0.75$). At baseline, there was a tendency for the TC+CC genotype group to have a greater percent body fat ($p=0.07$) compared to the TT genotype group (Table 12). There were

no other differences in baseline subject characteristics between the two genotype groups.

There were 6 participants in the TT genotype group and 8 in the TC+CC genotype group who completed the study. After controlling for baseline plasma aldosterone levels, there was a difference in the change in plasma aldosterone levels with aerobic exercise training between the two genotype groups. The adjusted means for the change in plasma aldosterone levels in the two genotype groups when controlling for baseline plasma aldosterone levels were -22 ± 9 pg/ml in the TT genotype group and 12 ± 8 pg/ml in the TC+CC genotype group ($p=0.01$). Final plasma aldosterone levels after aerobic exercise training were significantly greater ($p=0.01$) among the TC+CC genotype group compared to the TT genotype group (Table 13). The change in SBP was also significantly ($p=0.05$) different between the two genotype groups (Table 13), with the TT genotype having a non-significant reduction in SBP (Table 14) and the TC+CC genotype group tending to increase in SBP with aerobic exercise training (Table 15). There was no significant difference between the two genotype groups in the changes in DBP or 24-hour Na^+ excretion with aerobic exercise training.

After accounting for baseline plasma aldosterone levels, the change in plasma aldosterone levels was significant in the TT genotype group. The adjusted mean for the change in plasma aldosterone levels in the TT genotype group when controlling for baseline plasma aldosterone levels was -17 ± 7 pg/ml ($p=0.04$). Adjusting for baseline plasma aldosterone levels, the change in plasma aldosterone levels was not significant in the TC+CC genotype group. Among the TC+CC genotype group, there

was a tendency ($p=0.10$) to increase SBP with aerobic exercise training (Table 15). SBP tended to increase with aerobic exercise training when accounting for the change in 24-hour Na^+ excretion in the TC+CC genotype group. The adjusted mean for the change in SBP in the TC+CC genotype group when accounting for the change in 24-hour Na^+ excretion was 11 ± 2 mm Hg ($p=0.09$). Adjusting for the change in 24-hour Na^+ excretion, the change in SBP was not significant in the TT genotype group. There was a significant increase in 24-hour Na^+ excretion after accounting for the change in BMI among the TC+CC genotype group. The adjusted mean for the change in 24-hour Na^+ excretion in the TC+CC genotype group when controlling for the change in BMI was 14 ± 11 mmol/d ($p=0.03$). Adjusting for the change in BMI, the change in 24-hour Na^+ was significant in the TT genotype group. In both genotype groups, the change in plasma aldosterone levels with aerobic exercise training was not independently related to the change in SBP, DBP and 24-hour Na^+ excretion.

Table 1. Subject characteristics before and after aerobic exercise training in the total sample.

Variable	N	Before	After	P-value
BMI (kg/m ²)	39	28.3±0.6	27.8±0.6	0.002
Weight (kg)	39	84.2±2.2	82.4±2.1	0.001
Total Body Fat (%)	29	37±2	35±2	0.001
VO ₂ max (ml/kg/min)	38	24.6±0.8	28.1±0.9	<0.001
Na ⁺ excretion (mmol/d)	37	119±9	124±10	0.43
K ⁺ excretion (mmol/d)	37	66±4	68±4	0.56
Total Urine Volume (ml/d)	37	2104±143	2197±144	0.40
Systolic BP (mm Hg)	34	132±2	131±2	0.54
Diastolic BP (mm Hg)	33	84±1	84±1	0.86
Plasma Aldosterone (pg/ml)	37	95±10	75±5	0.04

Values are unadjusted means ± SE.

Samples sizes vary because of missing data.

Table 2. Comparison of subject characteristics between the African descent and Caucasian groups before and after aerobic exercise training.

Variable	African descent		Caucasians	
	Baseline	Final	Baseline	Final
Age (years)	57±1 (n=28)		58±1 (n=33)	
Height (cm)	167±2 (n=28)		173±2 (n=33)	
Weight (kg)	81.7±2.3 (n=25)	76.6±3.0 (n=13)**	85.9±2.8 (n=31)	86.9±2.8 (n=23)
BMI (kg/m ²)	29.2±0.7 (n=25)	26.9±0.8 (n=13)	28.6±0.7 (n=31)	28.7±0.7 (n=23)
SCr (mg/dl)	1.1±0.0 (n=26)		1.0±0.0 (n=31)	
GFR (ml/min/1.73m ²)	77±3 (n=26)		75±2 (n=31)	
Total Body Fat (%)	40±2 (n=20)	40±3 (n=11)	36±2 (n=32)	34±2 (n=16)
VO ₂ max (ml/kg/min)	22.2±0.6 (n=24)*	25.9±1.6 (n=13)**	24.8±0.9 (n=30)	29.2±1.1 (n=22)
Na ⁺ excretion (mmol/d)	109±11 (n=25)	118±11 (n=13)	114±10 (n=31)	132±15 (n=22)
K ⁺ excretion (mmol/d)	51±4 (n=25)*	69±8 (n=13)	70±4 (n=31)	69±4 (n=22)
Total Urine Volume (ml/d)	1828±151 (n=25)	1957±206 (n=13)	2154±150 (n=31)	2395±191 (n=22)
Systolic BP (mm Hg)	129±2 (n=21)	134±4 (n=10)	132±2 (n=31)	131±2 (n=21)
Diastolic BP (mm Hg)	86±1 (n=20)	84±2 (n=9)	85±1 (n=28)	84±2 (n=21)
Plasma Aldosterone (pg/ml)	62±7 (n=28)*	61±9 (n=14)	107±12 (n=28)	81±7 (n=20)

SCr, serum creatinine; GFR, glomerular filtration rate. Values are unadjusted means ± SE. * p≤0.05 Baseline ethnic differences; ** p≤0.05 Final ethnic differences.

These are not within ethnic group comparison sample sizes. Within ethnic group comparisons are shown in Tables 4 & 5.

Table 3. Change values for primary outcome variables and 24-hour K⁺ excretion for individuals of African descent and Caucasians.

Variable	African Descent	Caucasians
Change Na ⁺ excretion (mmol/d)	4±12 (n=13)	9±8 (n=22)
Change K ⁺ excretion (mmol/d)	12±8 (n=13)	-2 ±5 (n=22)
Change Systolic BP (mmHg)	4±3 (n=10)	-3±1 (n=21)*
Change Diastolic BP (mm Hg)	-1±2 (n=9)	0±1 (n=21)
Change plasma aldosterone (pg/ml)	-2±8 (n=14)	-37±15 (n=20)

Values are unadjusted means ± SE. * Significant difference at p≤0.05.

Table 4. Subject characteristics before and after aerobic exercise training in Caucasians.

Variable	N	Before	After	P-value
BMI (kg/m ²)	23	29.1±0.8	28.7±0.7	0.01
Weight (kg)	23	88.2±2.9	86.9±2.8	0.01
Total Body Fat (%)	16	36±2	34±2	0.001
VO ₂ max (ml/kg/min)	22	25.4±1.1	29.2±1.1	<0.001
Na ⁺ excretion (mmol/d)	22	123±14	132±15	0.29
K ⁺ excretion (mmol/d)	22	71±5	69±4	0.67
Total Urine Volume (ml/d)	22	2227±201	2395±191	0.36
Systolic BP (mm Hg)	21	133±2	131±2	0.05
Diastolic BP (mm Hg)	21	84±2	84±2	0.83
Plasma Aldosterone (pg/ml)	20	119±16	81±7	0.02

Values are unadjusted means ± SE.

Table 5. Subject characteristics before and after aerobic exercise training in individuals of African descent.

Variable	N	Before	After	P-value
BMI (kg/m ²)	13	28.0±1.0	26.9±0.8	0.06
Weight (kg)	13	79.5±3.4	76.6 ±3.0	0.06
Total Body Fat (%)	11	40±3	40±3	0.56
VO ₂ max (ml/kg/min)	13	22.3±1.0	25.9±1.6	<0.001
Na ⁺ excretion (mmol/d)	13	114±10	118±11	0.77
K ⁺ excretion (mmol/d)	13	57±8	69±8	0.16
Total Urine Volume (ml/d)	13	2002±215	1957±206	0.79
Systolic BP (mm Hg)	10	130±3	134±4	0.28
Diastolic BP (mm Hg)	9	85±2	84±2	0.50
Plasma Aldosterone (pg/ml)	14	64±9	61±9	0.77

Values are unadjusted means ± SE.

Table 6. Genotype and Allele frequencies for Caucasians.

Genotype Frequencies	TT	TC	CC
	13(0.41)	12(0.39)	6(0.20)
Allele Frequencies	T	C	
	38(0.61)	24(0.39)	

n(%)

Table 7. Baseline subject characteristics for TT and TC+CC genotype groups in Caucasians.

Variable	TT	TC+CC
Age (years)	56±1 (n=13)	60±2 (n=18)
SCr (mg/dl)	1.0±0.1 (n=12)	1.0±0.4 (n=17)
GFR (ml/min/1.73m ²)	75±4 (n=12)	75±2 (n=17)
Weight (kg)	86.3±5.0 (n=13)	87.5±3.4 (n=16)
BMI (kg/m ²)	28.9±1.3 (n=13)	28.4±1.0 (n=16)
Total Body Fat (%)	37±3 (n=12)	34±2 (n=18)
VO ₂ max (ml/kg/min)	24.4±1.3 (n=13)	25.7±1.4 (n=15)
Na ⁺ excretion (mmol/d)	123±19 (n=12)	108±14 (n=17)
K ⁺ excretion (mmol/d)	75±6 (n=12)	66±6 (n=17)
Total Urine Volume (ml/d)	2371±228 (n=12)	1984±215 (n=17)
Systolic BP (mm Hg)	130±3 (n=12)	134±3 (n=17)
Diastolic BP (mm Hg)	83±3 (n=11)	86±1 (n=16)
Plasma Aldosterone (pg/ml)	119±19 (n=11)	99±17 (n=16)

SCr, serum creatinine; GFR, glomerular filtration rate. Values are unadjusted means ± SE.

There were no significant differences.

Table 8. Subject characteristics in the Caucasian TT and TC+CC genotype groups after aerobic exercise training.

Variable	TT	TC+CC
BMI (kg/m ²)	29.1±0.8 (n=9)	28.4±1.2 (n=13)
Weight (kg)	89.7±4.2 (n=9)	86.4±3.7 (n=13)
Total Body Fat (%)	35±2 (n=7)	30±3 (n=8)
VO ₂ max (ml/kg/min)	29.1±1.6 (n=9)	29.7±1.6 (n=12)
Na ⁺ excretion (mmol/d)	157±31 (n=8)	123±15 (n=13)
K ⁺ excretion (mmol/d)	74±8 (n=8)	68±5 (n=13)
Total Urine Volume (ml/d)	2401±149 (n=8)	2369±314 (n=13)
Systolic BP (mm Hg)	130±4 (n=8)	130±2 (n=12)
Diastolic BP (mm Hg)	86±3 (n=8)	83±2 (n=12)
Plasma Aldosterone (pg/ml)	98±8 (n=8)	70±9 (n=12)*
Change plasma aldosterone (pg/ml)	-36±20 (n=8)	-39±21 (n=12)
Change Na ⁺ excretion (mmol/d)	20±16 (n=8)	6±9 (n=13)
Change K ⁺ excretion (mmol/d)	-8±10 (n=8)	4±5 (n=13)
Change Systolic BP (mm Hg)	-2±2 (n=8)	-4±2 (n=12)
Change Diastolic BP (mm Hg)	3±2 (n=8)	-2±1 (n=12)*

Values are unadjusted means ± SE. * Significant difference at p≤0.05.

Table 9. Subject characteristics before and after aerobic exercise training in the Caucasian TC+CC genotype group.

Variable	N	Before	After	P-value
BMI (kg/m ²)	13	28.8±1.2	28.4±1.2	0.01
Weight (kg)	13	87.6±3.7	86.4±3.7	0.01
Total Body Fat (%)	8	33±3	30±3	0.004
VO ₂ max (ml/kg/min)	12	25.2±1.7	29.7±1.6	<0.001
Na ⁺ excretion (mmol/d)	13	116±17	123±15	0.47
K ⁺ excretion (mmol/d)	13	64±7	68±5	0.35
Total Urine Volume (ml/d)	13	1966±278	2369±314	0.03
Systolic BP (mm Hg)	12	135±3	130±2	0.03
Diastolic BP (mm Hg)	12	85±2	83±2	0.15
Plasma Aldosterone (pg/ml)	12	109±21	70±9	0.09

Values are unadjusted means ± S.E.

Table 10. Subject characteristics before and after aerobic exercise training in the Caucasian TT genotype group.

Variable	N	Before	After	P-value
BMI (kg/m ²)	9	29.6±0.9	29.1±0.8	0.17
Weight (kg)	9	91.1±4.7	89.7±4.3	0.16
Total Body Fat (%)	7	37±3	35±2	0.15
VO ₂ max (ml/kg/min)	9	26.1±1.4	29.1±1.6	0.02
Na ⁺ excretion (mmol/d)	8	137±26	157±31	0.25
K ⁺ excretion (mmol/d)	8	82±6	74±8	0.46
Total Urine Volume (ml/d)	8	2680±271	2401±149	0.31
Systolic BP (mm Hg)	8	132±5	130±4	0.49
Diastolic BP (mm Hg)	8	83±4	86±3	0.18
Plasma Aldosterone (pg/ml)	8	134±24	98±8	0.12

Values are unadjusted means ± S.E.

Table 11. Genotype and allele frequencies for individuals of African descent.

Genotype Frequencies	TT	TC	CC
	14(0.54)	11(0.42)	1(0.04)
Allele Frequencies	T	C	
	39(0.75)	13(0.25)	

n(%)

Table 12. Baseline subject characteristics for TT and TC+CC genotype groups in individuals of African descent.

Variable	TT	TC+CC
Age (years)	58±1 (n=14)	57±2 (n=12)
SCr (mg/dl)	1.1±0.0 (n=13)	1.0±0.1 (n=11)
GFR (ml/min/1.73m ²)	74±3 (n=13)	82±7 (n=11)
Weight (kg)	78.1±2.5 (n=12)	83.6±3.6 (n=12)
BMI (kg/m ²)	28.1±0.9 (n=12)	30.0±1.1 (n=12)
Total Body Fat (%)	37±2 (n=11)	44±3 (n=8)
VO ₂ max (ml/kg/min)	22.1±0.8 (n=12)	22.4±1.0 (n=12)
Na ⁺ excretion (mmol/d)	126±18 (n=13)	89±13 (n=11)
K ⁺ excretion (mmol/d)	47±3 (n=13)	55±9 (n=11)
Total Urine Volume (ml/d)	1823±168 (n=13)	1903±279 (n=11)
Systolic BP (mm Hg)	129±3 (n=12)	130±4 (n=8)
Diastolic BP (mm Hg)	85±2 (n=11)	87±2 (n=8)
Plasma Aldosterone (pg/ml)	64±11 (n=14)	59±10 (n=12)

SCr, serum creatinine; GFR, glomerular filtration rate. There were no significant differences between the two genotype groups.

Table 13. Subject characteristics for TT and TC+CC genotype groups in individuals of African descent after aerobic exercise training.

Variable	TT	TC+CC
BMI (kg/m ²)	25.6±0.8 (n=6)	28.1±1.3 (n=7)
Weight (kg)	71.5±2.8 (n=6)	80.9±4.6 (n=7)
Total Body Fat (%)	35±4 (n=6)	46±5 (n=5)
VO ₂ max (ml/kg/min)	25.6±1.7 (n=6)	26.1±2.7 (n=7)
Na ⁺ excretion (mmol/d)	110±21 (n=6)	124±12 (n=7)
K ⁺ excretion (mmol/d)	52±5 (n=6)	83±13 (n=7)
Total Urine Volume (ml/d)	1622±223 (n=6)	2245±305 (n=7)
Systolic BP (mm Hg)	128±5 (n=6)	143±6 (n=4)
Diastolic BP (mm Hg)	81±2 (n=5)	87±3 (n=4)
Plasma Aldosterone (pg/ml)	37±8 (n=6)	79±11 (n=8)*
Change Plasma aldosterone (pg/ml)	-17±12 (n=6)	8±10 (n=8)
Change Na ⁺ excretion (mmol/d)	-9±17 (n=6)	14±17 (n=7)
Change K ⁺ excretion (mmol/d)	5±8 (n=6)	18±13 (n=7)
Change Systolic BP (mm Hg)	-1±3 (n=6)	11±5 (n=4)*
Change Diastolic BP (mm Hg)	-3±2 (n=5)	1±3 (n=4)

Values are unadjusted means ± SE. * Significant difference at p≤0.05.

Table 14. Subject characteristics before and after aerobic exercise training in the African descent TT genotype group.

Variable	N	Before	After	P-value
BMI (kg/m ²)	6	25.9±0.9	25.6±0.8	0.32
Weight (kg)	6	72.5±3.1	71.5±2.7	0.31
Total Body Fat (%)	6	35±3	35±4	0.91
VO ₂ max (ml/kg/min)	6	22.1±0.9	25.6±1.7	0.02
Na ⁺ excretion (mmol/d)	6	119±18	110±21	0.62
K ⁺ excretion (mmol/d)	6	48±6.1	52±5.2	0.55
Total Urine Volume (ml/d)	6	1669±157	1622±223	0.86
Systolic BP (mm Hg)	6	129±4	128±5	0.70
Diastolic BP (mm Hg)	5	85±2	81±2	0.22
Plasma Aldosterone (pg/ml)	6	54±15	37±8	0.24

Values are unadjusted means ± S.E.

Table 15. Subject characteristics before and after aerobic exercise training in the African descent TC+CC genotype group.

Variable	N	Before	After	P-value
BMI (kg/m ²)	7	29.7±1.4	28.1±1.3	0.11
Weight (kg)	7	85.5±4.7	80.9±4.6	0.11
Total Body Fat (%)	5	46±5	46±5	0.28
VO ₂ max (ml/kg/min)	7	22.5±1.7	26.1±2.7	0.02
Na ⁺ excretion (mmol/d)	7	109±13	124±12	0.44
K ⁺ excretion (mmol/d)	7	66±13	83±13	0.23
Total Urine Volume (ml/d)	7	2288±352	2245±305	0.86
Systolic BP (mm Hg)	4	132±6	143±6	0.10
Diastolic BP (mm Hg)	4	86±3	87±3	0.66
Plasma Aldosterone (pg/ml)	8	71±12	79±11	0.43

Values are means ± S.E. Significant difference at p≤0.05.

Figure 1. Relationship between baseline plasma aldosterone levels and the change in plasma aldosterone with aerobic exercise training in Caucasians.

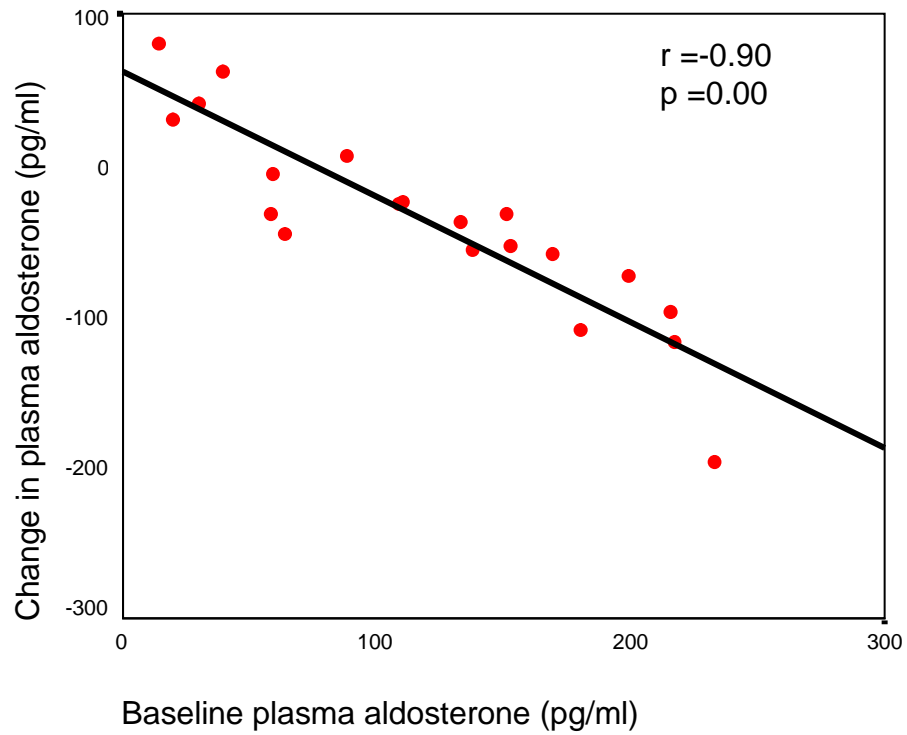
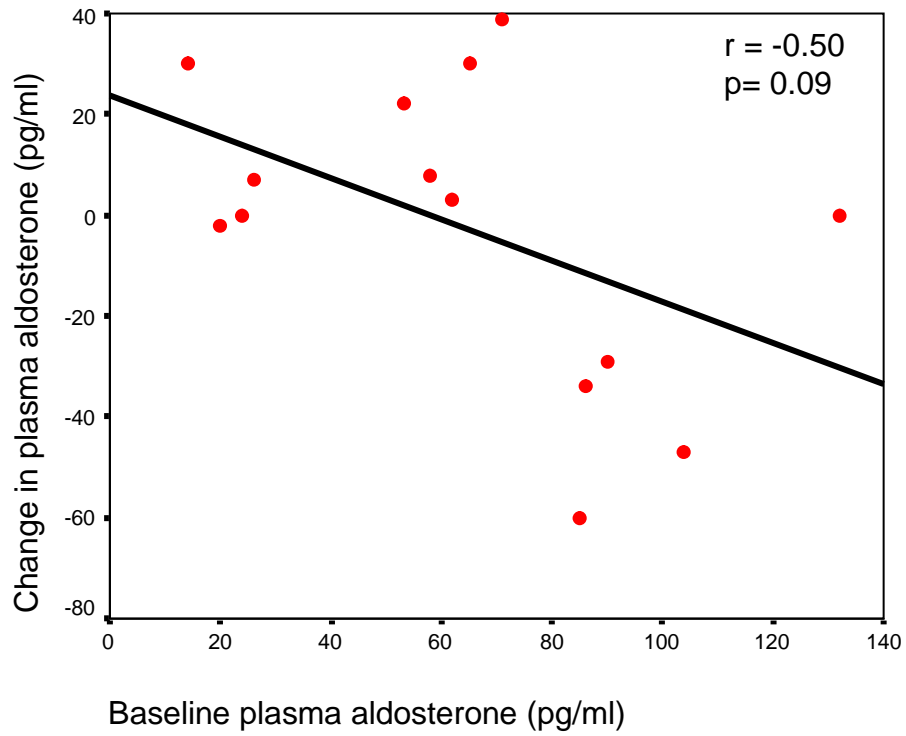


Figure 2. Relationship between baseline plasma aldosterone levels and the change in plasma aldosterone levels with aerobic exercise training in individuals of African descent.



Discussion

The purpose of the current study was to determine whether 6 months of aerobic exercise training reduced plasma aldosterone levels, increased 24-hour Na^+ excretion and reduced blood pressure in middle-aged to older prehypertensives. Additionally, the study determined whether the CYP11B2 -344C/T polymorphism influenced the change in these primary outcome variables with aerobic exercise training. This was the first study to investigate the effect of aerobic exercise training on plasma aldosterone levels in a prehypertensive population in both Caucasians and individuals of African descent, as well as the first to determine whether the -344C/T polymorphism identified prehypertensives who changed plasma aldosterone levels and blood pressure with aerobic exercise training.

The current study was not designed to investigate ethnic differences in the responses of the primary outcome variables to aerobic exercise training, but it was found that the Caucasians and individuals of African descent responded differently to the aerobic exercise training. In light of these ethnicity-dependent responses, presenting the results without accounting for these ethnic differences would provide an inaccurate interpretation of how aerobic exercise training affected the primary outcome variables.

Aerobic exercise training reduced plasma aldosterone levels in the prehypertensive Caucasian population. There are no studies that have reported a reduction in plasma aldosterone levels with aerobic exercise training in a prehypertensive population, and very few that have done so in a normotensive or hypertensive population (13; 67). Hespel et al. were one of the first to report that in a

normotensive population, greater reductions in plasma aldosterone levels were associated with an increase in physical work capacity with aerobic exercise training (67). Braith et al. demonstrated that among heart failure patients, plasma aldosterone levels significantly decreased by 50 pg/ml after 16 weeks of aerobic exercise training (13). The reduction in plasma aldosterone levels among the Caucasians in the current study were approximately 12-13 pg/ml smaller than the plasma aldosterone reduction in the Braith et al. study. The larger plasma aldosterone reduction among the Braith et al. participants may have been because their participants were less healthy and had greater baseline plasma aldosterone levels than the Caucasian group in the current study.

In the current study, baseline plasma aldosterone levels did contribute to the change in plasma aldosterone levels with aerobic exercise training in the Caucasian group, but after controlling for its small influence, the change in plasma aldosterone levels remained significant. This finding highlights the effectiveness of aerobic exercise training in reducing plasma aldosterone levels in Caucasian prehypertensives. Several mechanisms may explain the exercise training-induced reduction in plasma aldosterone levels. Aerobic exercise training has been shown to suppress plasma renin activity (PRA) in normotensive and hypertensive individuals (34; 67; 86; 98). The reduction in PRA can result in the reduction in angiotensin II (ang II) leading to a decrease in aldosterone biosynthesis (13; 54; 67). Subsequently, Braith et al. also reported a reduction in ang II levels in heart failure patients with aerobic exercise training (13). Sympathetic stimulation, which is known to activate renin release leading to the release of ang II (33; 54), has been shown to decrease

with aerobic exercise training in hypertensive individuals (34; 86). Therefore, it can be suggested that the reduction in sympathetic stimulation with aerobic exercise training may be a mechanism by which aldosterone biosynthesis decreases (67). Though the evidence is limited, there is also the possibility of an exercise-induced reduction in adrenal stimulation contributing to the reduction in plasma aldosterone levels (67; 146).

After 6 months of aerobic exercise training, the Caucasian participants also experienced a small, but significant reduction in SBP and no reduction in DBP. Larger reductions in SBP and DBP have been reported with similar aerobic exercise training programs (58; 59; 82; 135). In the current study, the prehypertensive status of the participants may have contributed to the small reduction in SBP. In previous aerobic exercise training studies that included hypertensive participants, baseline blood pressures were at hypertensive levels (58; 59; 82; 135). The baseline blood pressures for the Caucasians in the current study were below hypertensive levels, possibly allowing for less of a reduction in SBP with aerobic exercise training. There have been previous aerobic exercise training studies in which the population had blood pressures at prehypertensive levels (22; 45). In these previous studies, there was either a small reduction or no significant reduction in blood pressure (22; 45). Therefore, there is evidence to support that prehypertensives may reduce their blood pressure with aerobic exercise training, but the magnitude of the reductions may be less than in hypertensives.

It was found that there was no change in 24-hour Na⁺ excretion among the Caucasians. Very few studies have investigated to what extent 24-hour Na⁺ excretion

changes with aerobic exercise training in Caucasians, and of those studies that have, there were no significant changes (34; 58; 136). The significant increases in 24-hour Na^+ excretion that have been reported mainly occurred with aerobic exercise training in hypertensive Japanese (84) or hypertensives of African descent (16). In both of these populations, it has been suggested that Na^+ retention may contribute to their hypertension. Therefore, it is possible that in Caucasian populations, there may not be a change in Na^+ excretion with aerobic exercise training.

The change in plasma aldosterone levels with exercise training were not related to the change in blood pressure or 24-hour Na^+ excretion in the Caucasian participants. This does not necessarily mean that the reduction in plasma aldosterone levels did not contribute to the reduction in SBP or that plasma aldosterone's reduction is not by itself beneficial. Garcia et al., reported that the normal relationship between plasma aldosterone and Na^+ excretion is not observed among hypertensives (73). It is very possible that those that are prehypertensive also experience the same phenomenon, and because blood pressure is indirectly affected by aldosterone, a correlation between plasma aldosterone and blood pressure may not be observed. There is also the possibility that aldosterone's influence on the development of hypertension in Caucasians is through a pathway other than renal Na^+ handling. Recently, a review by Oberleithner reported that vascular endothelial cells can synthesize aldosterone, express mineralocorticoid receptors and contain epithelial Na^+ channels (108). Therefore, the action of aldosterone on the vascular endothelial cell is similar to what occurs in the renal collecting duct (108). It has been suggested that when aldosterone is present, there is an influx of water leading to the swelling of the

endothelial cells (108). This swelling is thought to lead to endothelial cell dysfunction (108). There is also evidence to suggest that aldosterone can alter renal and peripheral vascular vasoconstrictor and vasodilator dysfunction, which could ultimately contribute to the development of hypertension (7; 8; 37; 134). Therefore, aldosterone appears to have different pathways by which it can contribute to the development and maintenance of hypertension. In the present study, aerobic exercise training reduced plasma aldosterone levels in prehypertensive Caucasians, possibly slowing the further progression of hypertension.

The individuals of African descent who were included in the present study were also prehypertensive and had baseline plasma aldosterone levels that were within the normal range. After aerobic exercise training, plasma aldosterone levels did not significantly change for the individuals of African descent, but after accounting for baseline plasma aldosterone levels, there was a tendency for plasma aldosterone levels to be reduced with aerobic exercise training. Those with baseline plasma aldosterone levels less than 75 pg/ml were more likely to increase plasma aldosterone levels with aerobic exercise training and those who had baseline plasma aldosterone levels greater than 75 pg/ml, decreased plasma aldosterone levels with aerobic exercise training. Therefore, the lack of a significant average change in plasma aldosterone levels in the individuals of African descent may have been a result of these differential changes based on the baseline plasma aldosterone levels.

The differential change in plasma aldosterone levels that occurred in the African descent group may have revealed a larger and more important finding in that the directional change in plasma aldosterone levels with aerobic exercise training is

dependent on whether or not individuals of African descent have suppressed plasma aldosterone levels at baseline. The low plasma aldosterone levels at baseline may support findings that Na^+ retention in some individuals of African descent suppresses plasma aldosterone levels and other components of the RAAS (40; 64; 96; 97; 128). Na^+ retention was not measured in the current study, but based on the findings it appears that some of the individuals of African descent may have experienced some Na^+ retention. For example, 24-hour Na^+ excretion tended to increase when removing the effect of baseline 24-hour Na^+ excretion. Additionally, baseline 24-hour Na^+ excretion tended to contribute to the change in 24-hour Na^+ excretion with exercise training, and exhibited a similar relationship pattern that occurred between baseline plasma aldosterone levels and the change in plasma aldosterone levels. This suggests that the individuals that suppressed baseline Na^+ excretion were more likely to increase 24-hour Na^+ excretion after aerobic exercise training. On a larger scale, the similarity in plasma aldosterone and 24-hour Na^+ excretion relationship patterns may further support evidence that among individuals of African descent, Na^+ handling influences their plasma aldosterone levels and the response of plasma aldosterone to aerobic exercise training is influenced by the response of Na^+ excretion to aerobic exercise training.

Among the individuals of African descent, 24-hour Na^+ excretion after aerobic exercise training was negatively related to DBP with aerobic exercise training. In which as 24-hour Na^+ excretion increased there was reduction in DBP. Even though 24-hour Na^+ excretion did not change significantly with aerobic exercise training, it is possible that the slight increase in 24-hour Na^+ excretion contributed to

the reduction in blood pressure. Guyton clearly states that due to the sensitivity of the pressure natriuresis mechanism in the kidney, only slight changes in the level of Na^+ output are necessary to cause shifts in the level of blood pressure (55).

There was no association between plasma aldosterone and 24-hour Na^+ excretion, SBP and DBP among the individuals of African descent. This lack of relationship may have been due to Na^+ retention-related suppression of the RAAS that has been suggested to occur in some individuals of African descent (40; 64; 96; 97; 128). If this is true, then it is unlikely that aldosterone is driving Na^+ reabsorption and blood pressure. Rather, it is the Na^+ retention that is driving plasma aldosterone suppression and therefore blood pressure is probably not likely to be completely influenced by plasma aldosterone following exercise training. Unfortunately, the present study did not find a relationship between the change in 24-hour Na^+ excretion and the change in plasma aldosterone following exercise training. This may have been due to the lack of uniform changes in plasma aldosterone and 24-hour Na^+ excretion. The individuals of African descent appeared to have substantial within-group heterogeneity in the change in plasma aldosterone levels and 24-hour Na^+ excretion with exercise training. A larger sample size may have allowed for a clearer separation between those individuals who had low levels of Na^+ excretion and suppressed plasma aldosterone levels, and those that did not. This may have made it easier to determine to what extent aerobic exercise training affected these variables in individuals of African descent.

There were no significant changes in SBP or DBP with aerobic exercise training among the individuals of African descent. These findings differ from studies

that have reported blood pressure reductions in individuals of African descent (34; 87) and differ from the findings in the Caucasian participants in the current study. Again, a difference between the current study and other studies in individuals of African descent is that the baseline blood pressures were much lower than the studies that reported reductions in blood pressure (34; 87). The ethnic differences in blood pressure response to aerobic exercise training may have resulted from pathophysiological differences in the mechanism of hypertension development that are reported between individuals of African descent and Caucasians (40; 64; 96).

The results of the present study indicated that there were distinct differences between the individuals of African descent and Caucasians before and after aerobic exercise training. The baseline plasma aldosterone levels of the individuals of African descent were 42% lower than Caucasians. Additionally, plasma aldosterone levels after aerobic exercise training were significantly lower in individuals of African descent than Caucasians after accounting for BMI and 24-hour K^+ excretion after aerobic exercise training. The prevalence of lower plasma aldosterone levels among individuals of African descent compared to Caucasians has been a topic of much debate (40; 64; 96; 115; 116). Some studies have not found basal plasma aldosterone levels to be different between individuals of African descent and Caucasians (35; 40; 41; 64; 96), whereas other studies have found that in individuals of African descent basal plasma aldosterone levels are suppressed (115; 116) or have an attenuated response to RAAS stimulation (40; 41; 64). Therefore, even though the evidence is mixed, there is some support that plasma aldosterone levels can be suppressed in both normotensive and hypertensive individuals of African descent.

K^+ is known to be an important stimulus of aldosterone biosynthesis (20; 54) and lower potassium intake among individuals of African descent has been found to be one factor that accounts for the differences in plasma aldosterone levels between the two ethnic groups (91; 115). Langford et al. reported that ethnic differences in plasma aldosterone levels were eliminated when normotensives of African descent and normotensive Caucasians were placed on a 10 week homogenous K^+ diet (91). A limitation of the present study was that K^+ intake was not controlled. This may have accounted for significantly lower baseline 24-hour K^+ excretion among individuals of African descent. Contrary to this, when controlling for 24-hour K^+ excretion before and after aerobic exercise training, plasma aldosterone levels continued to be significantly lower among the individuals of African descent. These findings are supportive of a study by Pratt et al. who showed that ethnic differences in K^+ intake did not solely account for the ethnic differences in plasma aldosterone levels (115).

The individuals in the current study were not obese, but were considered to be overweight. BMI was slightly lower and body weight was significantly lower among individuals of African descent compared to Caucasians. Obese individuals have been shown to have greater plasma aldosterone levels than non-obese individuals (46-48; 124). When controlling for BMI after aerobic exercise training, plasma aldosterone levels after aerobic exercise training were still lower in individuals of African descent. This is the first study to demonstrate that the plasma aldosterone response to aerobic exercise training is different between these two ethnic groups. These differences in exercise training induced-responses do not appear to be attributed to differences in K^+ intake or body composition. Therefore, the ethnic differences in

plasma aldosterone levels may be due to differences in RAAS regulation that have been suggested by previous research (40; 64; 128).

Among the Caucasians, for the primary outcome variables plasma aldosterone and SBP the sample sizes were not sufficient to meet 80% power. Regardless, after 6-months of aerobic exercise training the TC+CC genotype group was more responsive in that they significantly reduced SBP and tended to reduce plasma aldosterone levels, while the TT homozygotes did not. Rankinen et al. reported strong linkage between the 8q21 region, the location of CYP11B2, and the change in SBP during cycle ergometry at 50 W following 20 weeks of aerobic exercise training among Caucasians (118). The findings of Rankinen et al. combined with the findings from the current study indicate that the CYP11B2 -344C/T gene polymorphism or a polymorphism that is in linkage with the -344C/T polymorphism, may influence the change in plasma aldosterone levels and SBP with aerobic exercise training.

The phenomenon of the genotypes with the dominate allele being associated with the change in hypertension phenotypes has been demonstrated in gene-exercise studies involving other RAAS gene polymorphisms, such as the ACE I/D and the AGT M235T (57; 119; 120; 161). Hagberg et al. and Zhang et al. reported that the ACE I allele was associated with a reduction in blood pressure with aerobic exercise training (57; 161). Rankinen et al. and Rauramaa et al. found that the AGT 235M allele was associated with a reduction in blood pressure with aerobic exercise training (119; 120). The results of the aforementioned studies combined with the results of the present study suggest that RAAS genes may have a significant influence on the blood

pressure response to aerobic exercise training in prehypertensive and hypertensive Caucasians.

The previous gene-exercise studies did not measure RAAS intermediate phenotypes and this has prevented them from making a stronger physiological link between the gene polymorphism and the blood pressure reduction with aerobic exercise training (59; 119; 120). In the current study, the measurement of plasma aldosterone enhanced the study's ability to determine a potential physiological link between the gene and the endpoint phenotype, blood pressure. There was not a significant relationship between blood pressure and plasma aldosterone levels in any of the genotype or ethnic groups.

Final plasma aldosterone levels were lower after aerobic exercise training among the Caucasian TC+CC genotype group compared to the Caucasian TT genotype group. Additionally, after controlling for baseline plasma aldosterone levels, the TC+CC genotype group had a greater reduction in plasma aldosterone levels compared to the TT genotype group with exercise training. The genotype-associated differences in plasma aldosterone levels after aerobic exercise training may have been the result of molecular differences between the T and C alleles. Compared to the -344T allele, the -344C allele has been associated with tighter binding of the transcription factor SF-1 to the non-essential regulatory sequence Ad4 (10; 20; 155). It has been suggested that the tighter binding of SF-1 to Ad 4 makes SF-1 unavailable to bind to other functional CYP11B2 gene regulatory sites (14; 93; 147). Hence, it is suggested that the -344C allele is associated with less CYP11B2 gene transcription

and aldosterone biosynthesis (14; 94; 147). The lower aldosterone biosynthesis associated with the -344C allele possibly enabled those in the current study with the TC+CC genotype to be more responsive to the exercise training-induced mechanisms that contribute to the reduction in plasma aldosterone levels. Alternatively, the -344T allele has been associated with less binding of SF-1 to Ad 4, making SF-1 more available to bind to other functional regulatory sites resulting in increased CYP11B2 gene transcription and aldosterone biosynthesis (14; 94; 147). The greater aldosterone biosynthesis associated with the -344T allele may have caused those in the TT genotype group to experience more resistance to the exercise training-induced mechanisms that contribute to the reduction in plasma aldosterone levels.

When controlling for baseline SBP, the Caucasian TC+CC genotype group had a significantly greater reduction in SBP compared to the Caucasian TT genotype group. Additionally, the reduction in DBP among the TC+CC genotype group was significantly different compared to the TT genotype group. The reduction in blood pressure among the TC+CC genotype group may have been partly due to their reduction in plasma aldosterone levels. As discussed earlier, aldosterone can cause peripheral vasoconstriction, affecting resting vascular tone (7; 8; 134). The reduction in plasma aldosterone levels with aerobic exercise training may have lead to greater exercise training-induced resting vasodilation among the TC+CC genotype group leading to their reduction in blood pressure (93; 118). However, there was no significant association between the change in plasma aldosterone levels and the changes in SBP and DBP to support this. In future research, it may be necessary to

measure vascular endothelial function to determine whether the -344C/T polymorphism is associated with the change in resting vascular tone.

Among the Caucasians, for the primary outcome variable 24-hour Na^+ excretion the sample size was sufficient to meet the requirements of 80% power. The lack of a genotype-associated reduction in 24-hour Na^+ excretion and the lack of a relationship between the change in plasma aldosterone levels and the change in 24-hour Na^+ excretion may indicate that among Caucasians, the contribution of aldosterone to elevated blood pressure is more associated with endothelial function than renal Na^+ handling.

Among the individuals of African descent, for all of the primary outcome variables the sample size was not sufficient to meet 80% power. Despite this lack of statistical power, after controlling for baseline plasma aldosterone levels, the change in plasma aldosterone levels was significantly different between the two genotype groups. Also, the TT genotype group tended to have lower final plasma aldosterone levels after aerobic exercise training compared to the TC+CC genotype group. The genotype associations with the change in plasma aldosterone levels among the individuals of African descent were opposite to what was found among the Caucasians. Therefore, it is difficult to determine whether the molecular differences between the T and C allele, which were discussed above, provide a sufficient explanation for the genotype associations with the change in plasma aldosterone levels for both ethnic groups. Among the individuals of African descent, both genotype groups had similar baseline plasma aldosterone levels, and as stated earlier, these levels were suppressed compared to the Caucasians. Therefore, it was

interesting that the TT genotype group experienced a -17 pg/ml reduction in plasma aldosterone levels with aerobic exercise training when controlling for baseline plasma aldosterone levels. Their plasma aldosterone concentration after aerobic exercise training was 37 pg/ml, which is suggestive of further aldosterone suppression after aerobic exercise training. It appears that the TT genotype may be less advantageous for individuals of African descent, a population known to experience RAAS suppression. The TC+CC genotype group experienced a non-significant increase in plasma aldosterone levels with aerobic exercise training. This may suggest that the TC or the CC genotype may be more advantageous among individuals of African descent because it is less likely to lead to further suppression of plasma aldosterone levels with aerobic exercise training.

After aerobic exercise training, the African descent TT genotype group experienced a non-significant reduction in 24-hour Na^+ excretion and the TC+CC genotype group experienced a significant increase in 24-hour Na^+ excretion, after controlling for the change in BMI. These findings may indicate that in individuals of African descent with the TT genotype, aerobic exercise training does not increase Na^+ excretion and this may contribute to the further suppression of plasma aldosterone levels with aerobic exercise training. The exercise training-induced increase in 24-hour Na^+ excretion that was observed in the TC+CC genotype group may prevent the further suppression of plasma aldosterone levels with aerobic exercise training. A larger sample size will be necessary to confirm these suggestions.

The African descent TC+CC genotype group tended to increase SBP with aerobic exercise training. For SBP, there were only 4 individuals of African descent

with the TC+CC genotype, and all four individuals increased their SBP with aerobic exercise training. Therefore, it is difficult to discern whether this finding is due to chance or if it provides some initial evidence that the TC+CC genotype is associated with an adverse SBP response to aerobic exercise training.

In conclusion, the results from the current study indicated that Caucasians and individuals of African descent differ in their responses of plasma aldosterone levels, 24-hour Na⁺ excretion and blood pressure to aerobic exercise training. Ethnic differences in the regulation of the RAAS may have contributed to the differences in the responses of these primary outcome variables. The findings also revealed that the CYP11B2 -344C/T polymorphism may influence the responses of plasma aldosterone, 24-hour Na⁺ excretion and blood pressure to aerobic exercise training, but the allele-associated responses differed between individuals of African descent and Caucasians. It is unlikely that the -344C/T polymorphism alone influenced the responses of these primary outcome variables, therefore further investigation is needed to determine whether other CYP11B2 polymorphisms that are in linkage disequilibrium with the -344C/T polymorphism, may in conjunction with the -344C/T polymorphism contribute to the exercise-training induced responses.

Appendix A — Statement of Research Purpose

Purpose of the Study

The purpose of the current study was to determine whether 6 months of aerobic exercise training reduced plasma aldosterone levels, increased 24-hour Na⁺ excretion and reduced blood pressure in middle-aged to older prehypertensives. Additionally, whether the CYP11B2 –344C/T polymorphism influenced the responses of these primary outcome variables to aerobic exercise training.

Significance of Study

Hypertension continues to be an indiscriminate chronic disease that places older individuals at increased risk for the development of potentially fatal conditions such as cardiovascular and renal disease (19). Aerobic exercise training is considered a life style modification that can aid in the reduction and control of blood pressure in hypertensive individuals (1; 19) and has proven to effectively reduce blood pressure in hypertensive individuals (58; 59; 82; 84; 87). There is an ongoing effort to elucidate the physiological mechanisms involved in the reduction of blood pressure with aerobic exercise training, and one system that may have great influence but has not been adequately researched, is the RAAS. Therefore, the current study determined if plasma aldosterone levels changed with aerobic exercise training, and if the change in plasma aldosterone influenced Na⁺ handling and blood pressure response in a prehypertensive population. Thus, the current study is contributing to the

understanding of the mechanisms involved in the reduction of blood pressure with aerobic exercise training.

In recent years, there have been a few gene-exercise research studies that found that the ACE I/D and AGT M235T polymorphisms were associated with blood pressure changes with aerobic exercise training (57; 119; 120; 162). Because these RAAS genes appear to influence blood pressure responses to aerobic exercise training, it is important to investigate whether other important RAAS genes also influence blood pressure responses to aerobic exercise training. The CYP11B2 -344C/T polymorphism may be a likely candidate due to the evidence demonstrating that it is related to blood pressure, Na⁺ handling and aldosterone levels (29; 31; 66; 114). Therefore, the second objective of this study was to determine whether the -344C/T polymorphism was associated with changes in plasma aldosterone levels, 24-hour Na⁺ excretion and blood pressure response to aerobic exercise training. The current study was also important because the aforementioned gene-exercise studies lacked the measurement of the intermediate phenotype associated with the genes that were investigated. This has prevented the previous studies from making a strong physiological link between the gene polymorphism and the reduction in blood pressure with aerobic exercise training. Therefore, by measuring plasma aldosterone, the current study had the potential to better understand the role that the -344C/T polymorphism had in the reduction of blood pressure with aerobic exercise training.

Research Hypotheses

1. Due to limited research on the response of plasma aldosterone and Na⁺ excretion to aerobic exercise training, long-term aerobic exercise training will reduce plasma aldosterone levels, increase 24-hour Na⁺ excretion, and reduce blood pressure in middle-aged to older prehypertensives.
2. The change in plasma aldosterone levels will be associated with the change in 24-hour Na⁺ excretion and the change in blood pressure.
3. The majority of CYP11B2 -344C/T association studies have reported that the presence of the mutant T allele was associated with elevated blood pressure (14; 29; 66; 141; 147). Traditionally, in gene-exercise training studies that have included RAAS gene polymorphisms, such as the ACE I/D and the M235T, the presence of the mutant allele is associated with a lack of exercise-induced changes in blood pressure (57; 119; 120; 161). Therefore, in the present study it was hypothesized that participants with the TC+CC genotype would have a greater reduction in the intermediate phenotype, plasma aldosterone levels, a greater increase in the intermediate phenotype, 24-hour Na⁺ excretion, and a greater reduction in the endpoint phenotype, blood pressure, after aerobic exercise training compared to those with the TT genotype. The changes in phenotypes associated with the TT genotype group would not be different from zero.

Delimitations

The following were delimitations of the study:

1. The study population consisted of prehypertensive men and women between the ages of 50-75 years on 2 or less antihypertensive medications. They were void of diabetes, kidney, liver and cardiovascular disease.
2. The study focused on plasma aldosterone levels, and therefore was not able to assess urinary aldosterone changes with aerobic exercise training.
3. The women in the study were post-menopausal and were required to maintain their hormone replacement status. This was necessary because changes in hormone replacement could affect plasma aldosterone levels.
4. A gene-exercise association was exclusively studied in the -344C/T polymorphism. Therefore, it was not possible to determine if there were interactions between -344C/T and other polymorphisms of the CYP11B2 gene or other genes of the RAAS.

Limitations

The following factors were limitations of the study:

1. During the initial screening, participants were asked to report any health problems, their current fitness level, and any medications being taken. It was possible that there was inaccurate reporting by participants, which lead to the inclusion of participants with unreported health problems. This could have negatively impacted the data.
2. Participants were asked to self-report dietary intake throughout the study to ensure they were meeting AHA Step I guidelines. There was the possibility

that inaccurate reporting of dietary intake affected plasma aldosterone levels, 24-hour Na⁺ excretion and blood pressure measurements.

3. A total of 40 participants completed the study, of which 14 were of African descent and 23 were Caucasian. The small sample size within each ethnic group, reduced the power to detect genotype-dependent aerobic exercise training-induced changes in plasma aldosterone levels, 24-hour Na⁺ excretion and blood pressure.
4. There was no control group included in the current study.

Definition of Terms

Adrenal Gland: Endocrine and neuroendocrine gland that lies superior to the kidneys. It has two distinct parts, the adrenal medulla is the inner portion that produces catecholamines and adrenal cortex is the outer portion that produces steroid hormones.

CYP11B2 Cis-regulatory Element: responsible for the transcriptional regulation of the CYP11B2 gene.

Cytochrome P450: monooxygenases which catalyze many reactions involved in drug metabolism, synthesis of cholesterol, steroid hormones and other lipids.

Exon: the sequences that are represented in the mature RNA.

G-protein Coupled Receptor: cell surface receptor that associates with an intracellular trimeric GTP-binding protein (G protein) after receptor activation by an extracellular ligand.

Intron: intervening sequences that are removed when the primary transcript is processed to give the mature RNA.

Locus: is the position on a chromosome at which the gene for a particular trait resides.

Phosphatidylinositol 4,5- Bisphosphate: an inositol phospholipid which is present in small amounts in the inner half of the plasma membrane lipid bilayer. G-protein coupled receptors mainly act through this pathway.

Polymorphism: refers to the simultaneous occurrence in the population of genomes showing allelic variations.

Pressure natriuresis: an increase in arterial pressure of only a few mm Hg that doubles the output of salt.

Regulatory sequence: DNA sequence to which a gene regulatory protein binds to control the rate of assembly of the transcriptional complex at the promoter.

Reporter Gene: a coding unit whose product is easily assayed. It may be connected to any promoter of interest so that expression of the gene can be used to assay promoter function.

Transcriptional Factors: proteins that help to position RNA polymerase correctly on promoter, aid in pulling apart the two strands of DNA to allow for transcription to begin, and release RNA polymerase from the promoter into the elongation mode once transcription has begun.

Appendix B – Review of Literature

Hypertension is considered to be a primary risk factor for the development of CVD, and it has been reported that the relationship between blood pressure and CVD risk is continuous, consistent and independent of other risk factors (19). The influence of hypertension goes beyond the spectrum of CVD since it is also responsible for contributing to the incidence of stroke, heart failure and kidney disease (19). Vasan et al. recently reported on data from the Framingham Heart Study in which it was found that the residual lifetime risk for developing hypertension in middle-aged and elderly individuals was 90% (153). It has been reported that for individuals between the ages of 40-70 years with blood pressure in the ranges of 115/75 to 185/115 mm Hg, each 20 mm Hg or 10 mm Hg incremental increase in SBP or DBP, respectively, was associated with a doubling of the risk of developing CVD (19; 92). Thus, hypertension continues to be a disease associated with aging and poses a great public health burden (153).

Many physiological systems are involved in the regulation of blood pressure and therefore there are potentially many avenues for disregulation of blood pressure. For example, the RAAS has generated great interest because it is intimately related to the function of the kidney, the long-term regulator of blood pressure (54; 55; 60). Thus, the primary focus of this literature review is to discuss the involvement of the RAAS, specifically aldosterone, in the regulation of blood pressure and its possible contributions to the etiology of hypertension. Recently, hypertension has been shown to have a substantial genetic component. Therefore, this review will also focus on the –344C/T single nucleotide polymorphism (SNP) of the CYP11B2 gene in light of

preliminary evidence suggesting that the variant is associated with hypertension as well as with variability in aldosterone levels. Furthermore, aerobic exercise has been recognized as a lifestyle modification that can be prescribed alone or in conjunction with other antihypertensive therapies as an effective overall strategy for the prevention and treatment of hypertension (1; 19). Therefore, there will be a discussion of the effect of aerobic exercise training on the hypertensive phenotypes blood pressure, Na⁺ excretion, and aldosterone levels, and also on the interaction between genetics and exercise in the treatment of hypertension.

Renin-Angiotensin-Aldosterone-System

The RAAS is an essential and intimate partner in the kidney's regulation of fluid homeostasis and blood pressure. The RAAS should not only be thought of as a system that exerts its influence on the kidney, but also as a system that is born within the kidney. The kidney is considered to be one of the most complex and necessary organs within the body, and its ability to regulate fluid homeostasis and blood pressure comes from the millions of functional units, called nephrons, that lie within it (138). Each nephron contains key vasculature, such as the afferent and efferent arterioles, and tubular elements, such as the loop of Henle, proximal, distal, and collecting tubules, that can be influenced by variables of the RAAS to elicit change in fluid homeostasis and eventually blood pressure (138).

The RAAS includes renin, angiotensinogen, angiotensin I (ang I), ang II, and aldosterone (54). Renin, an enzyme, is responsible for the activation of the RAAS. It is stored in its inactive form, prorenin, in the juxtaglomerulosa (JG) cells of the afferent and efferent arterioles that feed into the network of capillaries called the

glomerulus (54; 138). Reductions in blood pressure and blood volume stimulate the conversion of prorenin to renin (54).

Numerous factors can contribute to the renin release; however three mechanisms are thought to have the greatest influence: intrarenal baroreceptors, sympathetic nervous system, and macula densa receptors (60). The intrarenal baroreceptors, located in the JG cells, influence renin release by responding to changes in vascular wall tension and transmural pressure (30; 60). A second mechanism of renin release is through activation of the sympathetic nervous system (SNS) (54; 60). The kidney is highly innervated by the SNS; specifically, there is exclusive adrenergic innervation of the afferent and efferent arterioles leading to the glomerulus (33). The SNS can directly influence renin release by stimulating beta₂ adrenergic receptors, located on the JG cells, when there is a reduction in blood pressure, or an acute change in blood volume (33; 54). Renin release can also be indirectly stimulated via activation of alpha-adrenergic receptors, which leads to an increase in Na⁺ reabsorption in the proximal tubules of the kidney (60). The increase in Na⁺ reabsorption results in a reduction of Na⁺ chloride (Na⁺Cl⁻) delivery to the macula densa leading to renin release (32). The third mechanism involves the macula densa, which is a cluster of specialized cells located in the early portion of the distal tubule and in very close proximity to the JG cells of the afferent and efferent arterioles (60). The macula densa stimulates the release of renin by sensing changes in Na⁺Cl⁻ concentration to the distal tubule (54). A decrease in arterial pressure can cause a decrease in glomerular filtration rate (GFR), resulting in a reduction in flow rate through the loop of Henle and an increase in Na⁺Cl⁻ reabsorption in the ascending

limb of the loop (54). The consequent increase in Na^+ reabsorption by the ascending limb reduces the Na^+Cl^- concentration that passes the macula densa and thereby initiates a signal from the macula densa to the afferent and efferent arteriole stimulating the release of renin from the JG cells (54; 60). The release of renin leads to ang II release, which causes efferent arteriole vasoconstriction (54). Combined with a simultaneous reduction in afferent arteriole resistance, GFR and arterial pressure increases (54).

Once released, renin is not responsible for bringing about the physiological effects that aid in the regulation of blood pressure. Rather, renin initiates the steps needed to produce ang II, one of the vasoactive substances of the RAAS (54). Renin splits the substrate angiotensinogen (a plasma protein produced in the liver) to ang I, a 10 amino acid peptide (54). Once released, ang I circulates in the blood, however it has a very weak vasoconstrictive effect and therefore causes no physiological change in the vasculature (54). Despite this weak effect, ang I is important because it is the precursor peptide that is converted to the 8 amino acid peptide ang II, by angiotensin converting enzyme (ACE), which is produced in the endothelium of the lung blood vessels (54).

There are two physiological pathways by which ang II can affect blood pressure. The first pathway is via the vasculature, in which the presence of ang II in the blood leads to the vasoconstriction of arterioles, increased total peripheral resistance and eventually increased arterial pressure (54). Ang II also acts on the veins causing mild vasoconstriction to increase venous return (54). The first pathway of action by ang II is considered an acute method of increasing blood pressure (54).

The second pathway by which ang II affects blood pressure is through its influence on the kidney and is considered to be a slower, but more powerful method of increasing blood pressure (54). Ang II acts on the renal vasculature and renal tubules to increase Na^+ and water reabsorption, which over hours to days will lead to an increase in blood pressure (54). Ang II is also responsible for stimulating the release of aldosterone, the final component of the RAAS, and is also known to aid in the kidney's regulation of blood pressure (54).

Renal-Fluid-Feedback-System and Pressure Natriuresis

Before introducing aldosterone and its influence on blood pressure regulation, it is important to have a general understanding of how aldosterone and ang II function within the kidney to aid in the long-term regulation of blood pressure. The maintenance of mean blood pressure is reliant on the maintenance of fluid homeostasis, in which equilibrium is achieved between fluid input and fluid output (54; 55; 60). The kidney is the organ solely responsible for fluid homeostasis and because of this it is referred to as the long-term regulator of blood pressure (54). The kidney regulates blood pressure over the long-term via the renal fluid-feedback system (RFFS) (60). In the event of an increase or decrease in blood pressure due to changes in fluid volume, it is through the RFFS, that the kidney rectifies these changes and returns blood pressure to normal levels (54; 60).

The primary component of the RFFS that allows the kidneys to regulate fluid volume and blood pressure is the pressure-natriuresis mechanism (53; 54; 60; 61). This mechanism demonstrates the effect that blood pressure has on urinary Na^+ and water excretion (55). In healthy individuals mean blood pressure is approximately

100 mm Hg, this is the single precise pressure considered to be the equilibrium pressure point (55). At the equilibrium pressure point, net fluid intake and renal fluid output are in equilibrium (55). The kidney continuously functions to maintain the equilibrium pressure point (55). Due to this fine regulation of fluid homeostasis, even a slight increase in blood pressure above the equilibrium pressure point causes the kidney to increase Na^+ and water excretion to decrease fluid volume (54; 60). When fluid loss exceeds fluid intake, extracellular fluid volume will decrease and blood pressure will return to the equilibrium pressure point (55; 60). In the case of a reduction in blood pressure below the equilibrium pressure point, the kidneys increase water and Na^+ reabsorption to increase extracellular fluid volume, which returns blood pressure to the equilibrium pressure point (55; 60). The RFFS is not responsible for regulating the moment-to-moment changes in blood pressure, but rather it is responsible for regulating blood pressure over the long-term (53; 60).

The kidney's ability to make such fine adjustments to fluid volume and arterial pressure is reliant on variables of the RAAS such as aldosterone and ang II (60). Therefore, aldosterone and ang II are considered to be integral components of the RFFS that increase renal Na^+ and water reabsorption (60).

Aldosterone and the Kidney

Similar to ang II, aldosterone is another component of the RAAS that can elicit physiological changes in the kidney that aid in the regulation of arterial pressure. Unlike ang II, aldosterone is not an amino acid peptide; it is a mineralocorticoid that is released from the zona glomerulosa, a region within the adrenal gland (54). The renal tubules are one of the areas targeted by aldosterone

(54). The initial precursor of aldosterone is cholesterol, and with the aid of several enzymes, cholesterol is converted to several different steroid hormones, one of them being aldosterone (54). The steps in the biosynthesis of aldosterone from cholesterol occur mainly in the mitochondria and the endoplasmic reticulum of the zona glomerulosa cells (54). In one of the final steps of aldosterone biosynthesis, corticosterone is converted to aldosterone by the enzyme aldosterone synthase (P450c11AS) (54).

There are several mechanisms, such as changes in osmolarity in the extracellular fluid, that regulate aldosterone biosynthesis, but the most important regulators are K^+ ion concentration, and ang II levels in the blood (54; 138). An increase in K^+ concentration in the extracellular fluid is known to stimulate aldosterone biosynthesis (138). When K^+ levels are elevated, aldosterone biosynthesis increases leading to K^+ secretion into the renal tubules, and an elevation in K^+ excretion (138). Additionally, with the increase in K^+ secretion into the renal tubules comes an increase in Na^+ reabsorption by the renal tubules (138). The second important regulator of aldosterone biosynthesis is ang II (20). Located on the zona glomerulosa are ang II type I (AT_1) receptors, and in times of reduced blood pressure and blood volume ang II levels will be elevated in the blood and will act on these G-protein coupled receptors and stimulate the biosynthesis and release of aldosterone (20; 77; 137; 140). The net result is an increase in Na^+ reabsorption and K^+ secretion. The specific cellular mechanisms by which K^+ and ang II regulate aldosterone biosynthesis will be discussed later in the section on the CYP11B2 (aldosterone synthase) gene. There is another adrenal stimulus that must be mentioned. Plasma

aldosterone levels can change based on a person's posture (17). When an individual is supine, plasma aldosterone levels are lower and range from 20-230 pg/ml, and when an individual is upright, plasma aldosterone levels are higher ranging from 40-600 pg/ml (114).

Once aldosterone is released it acts on the renal tubules; specifically the collecting tubules and to a lesser extent the distal tubules and collecting ducts (54). Aldosterone increases Na^+ reabsorption in these specific regions of the renal tubules by acting on the epithelial cells, and to a greater extent, the principal cells (54; 138). Aldosterone easily diffuses across the tubular epithelia and principal cells, and binds to a cytoplasmic receptor (138). The aldosterone-receptor complex moves into the nucleus of the cell stimulating two pathways of action, one that elicits an acute response and one that is a slower response (138). The acute mechanism of action involves aldosterone stimulation of Na^+ channels located on the apical end of the cell, to open (138). This leads to an influx of Na^+ into the cells increasing the intracellular Na^+ concentration (138). On the basolateral side of the cell there is increased activity of the Na^+ - K^+ ATPase channels, which are Na^+ and K^+ antiport ion channels that pump Na^+ out of the cell and K^+ inside the cell (138). The result is an increase in Na^+ reabsorption into the blood and secretion of K^+ into the lumen of the renal tubules (138). In the slower mechanism of action, when the aldosterone-receptor complex enters the nucleus, DNA transcription is initiated and specific portions of the DNA are induced to form several types of messenger RNA (mRNA) related to the process of Na^+ and K^+ transport (54; 138). The mRNA enters the cytoplasm and undergoes translation leading to protein synthesis (54). The proteins formed become membrane

transport proteins that are required for Na^+ K^+ and hydrogen transport across the cell membrane (54). In addition, some of the proteins are designated as specific enzymes, such as ATPase, needed for Na^+ - K^+ ATPase activity, and luminal membrane proteins, a channel protein inserted into the apical end of the cell (54). The insertion of the luminal protein channels aids in the rapid diffusion of Na^+ from the lumen of the renal tubule into the cell (54).

The end result of both mechanisms is that there is an increase in Na^+ reabsorption into the blood and an increase in K^+ secretion into the urine. The increased Na^+ reabsorption in the extracellular fluid does not necessarily cause a very large increase in Na^+ concentration in the extracellular fluid (54). The reason is that with the rise in Na^+ concentration is a concomitant increase in water reabsorption, but the net result is an increase in extracellular fluid volume (54).

Based on aldosterone's ability to create extracellular fluid volume adjustments that affect blood pressure, aldosterone like ang II, is an integral component of the RFFS. Therefore, aldosterone and ang II are factors that function with the kidney to ensure that extracellular fluid volume remains relatively constant despite disturbances in body fluid homeostasis (60).

Aldosterone and Essential Hypertension

Despite aldosterone's necessary role in the kidney's long-term regulation of blood pressure, if consistently elevated over a few days aldosterone can cause an individual to become hypertensive (54). A mechanism for aldosterone to cause hypertension is that when aldosterone levels are elevated over a few days there is an increase in extracellular fluid volume and blood pressure (54). An increase in

extracellular fluid volume of 5-15% can cause a 15-25 mm Hg increase in blood pressure (54). Based on the pressure-natriuresis mechanism, the elevation in blood pressure is necessary to increase Na^+ and water excretion, reducing extracellular fluid volume (54). The result is the restoration of balance between fluid intake and fluid output in which the rate of gain of salt and water will be zero, despite the excess of aldosterone. However, the elevation in blood pressure necessary to restore fluid balance will cause the individual to become hypertensive (54).

High levels of aldosterone secretion are known to cause a secondary form of hypertension called primary aldosteronism, which is usually due to a small tumor of the zona glomerulosa cells (54). Even though the etiology of essential hypertension is considered to be polygenic, in some individuals it is possible that a slight dysfunction in certain physiological systems, such as the RAAS, can influence the development of hypertension. Due to aldosterone's Na^+ retaining effect, there is the potential for consistent elevations in aldosterone levels, within a normal level (20-600 pg/ml) (112), to contribute to the development of hypertension.

Guyton, one of the developers of the RFFS and the pressure-natriuresis theory, explained that only slight elevations in extracellular fluid volume are needed to cause shifts in the renal-function curve (i.e. the pressure natriuresis mechanism) that can lead to hypertension (55). The example given by Guyton involved hypertensive patients being treated with spironolactone, a medication given to block the influence of steroid hormones, such as aldosterone (132). The patients stopped taking spironolactone for 1-2 weeks, which resulted in a 20-40% increase in plasma volume and a rise in arterial pressure to their initial untreated hypertensive levels

(132). The patients remained off of their spironolactone, and over the following weeks plasma volume reduced to normal levels, but surprisingly there was no reduction in arterial pressure (132). Guyton explained that to compensate for the small amount of fluid retention that was occurring due to aldosterone, the equilibrium pressure point was shifted and reset to a higher mean arterial pressure (55). This was necessary so that equilibrium between fluid intake and output could be re-established in light of increased fluid retention.

The main point of Guyton's example was to illustrate that hypertension in some individuals could be due to an upward shift in the equilibrium pressure point that results from small but consistent elevations in Na^+ retention (55). The Na^+ retention experienced by some hypertensives may be the result of slight alterations in aldosterone biosynthesis and/or other mechanisms that impact renal Na^+ handling (55).

There is evidence of elevated plasma aldosterone levels among hypertensive individuals. In studies that have compared mild and severe hypertensives to matched normotensives, it was found that plasma aldosterone levels were significantly higher among the hypertensives compared to the normotensives (23; 44; 73; 74; 95). It is important to note that in these studies plasma aldosterone levels of both the hypertensives and normotensives were well within the normal range, but the hypertensives displayed significantly greater levels. For example, Garcia et al. reported average plasma aldosterone levels of 198 pg/ml among hypertensives and 147 pg/ml among normotensives (73). Cortes et al. found that average plasma aldosterone levels were 116 pg/ml among hypertensives and 99 pg/ml among

normotensives, again demonstrating the same phenomenon as in the Garcia study (23). Additionally, hypertensives have been reported to show a significant decrease in the metabolic clearance rate of aldosterone, and a positive correlation between resting mean arterial pressure and plasma aldosterone levels (44; 95). Therefore, it is possible that the elevated plasma aldosterone levels displayed by the hypertensives contribute to the maintenance of a hypertensive state (73).

Normally, plasma aldosterone is negatively correlated with urinary Na^+ excretion. This has been confirmed among normotensives but not among hypertensives (73). Garcia et al. suggested that the lack of correlation between plasma aldosterone and urinary Na^+ excretion among the hypertensives may represent some type of disturbance in aldosterone biosynthesis that may or may not be directly linked to changes in Na^+ regulation (27; 69; 149).

Aldosterone's response to an adrenal stimulus, such as salt loading and ang II infusion has been shown to differ between hypertensives and normotensives. Ishii et al. reported that after 4 days of salt loading, the reduction in plasma aldosterone levels that occurred among hypertensives was significantly less than normotensives (71). This was indicative of blunted suppression of aldosterone biosynthesis among the hypertensives. Ishii et al. suggested that the blunted aldosterone response displayed by the hypertensives may be the result of altered adrenal cortex sensitivity to circulating ang II (71). In normotensives, Na^+ loading reduces the sensitivity of the adrenal cortex to ang II (109), this results in the suppression of aldosterone biosynthesis. Some hypertensives may experience dysfunction in adrenal responsiveness, in which the sensitivity of the adrenal cortex to ang II is not

suppressed under Na⁺ loaded conditions (71). There are studies that have demonstrated that hypertensives may display alterations in adrenal responsiveness to ang II infusion. Kisch et al. reported that hypertensives had a significant increase in plasma aldosterone levels at lower ang II infusion rates compared to normotensives (83). It was concluded that hypertensives demonstrated enhanced adrenal responsiveness to ang II infusion (83). Schlaich et al. also reported that normotensives with a family history of hypertension displayed a hyper-responsiveness to ang II infusion (133). Williams et al. also found that hypertensive patients placed on a 200 mEq Na⁺ diet had a greater increase in plasma aldosterone in response to infused ang II compared to normotensives (157).

There is evidence to support that among some hypertensives, plasma aldosterone levels are significantly elevated and have a distribution range that is wider than that of normotensives (73). From the available data, it appears that consistent elevation in plasma aldosterone within a normal range may contribute to the development and maintenance of hypertension.

Ethnicity and Age Effects on Aldosterone Levels

Ethnic Differences: It has been determined that there are some ethnic differences in PRA, and plasma levels of ang II and aldosterone. Specifically, this has been observed when comparing individuals of African descent and Caucasians. It has been reported that under basal and stimulated conditions, individuals of African descent have suppressed PRA, and plasma ang II and aldosterone levels (40; 64; 128). A review by Sagnella et al. explains that the reports of differences in RAAS function among those of African descent and Caucasians first appeared in the 1960s

when a study by Helmer and Hudson reported lower PRA among hypertensives of African descent compared to hypertensive Caucasians (65). In reviewing studies that compared PRA between hypertensive and normotensives of African descent and hypertensive and normotensive Caucasians, Sagnella et al. reported that on average hypertensives and normotensives of African descent had PRA that was 50% lower than Caucasian hypertensives and normotensives (128). However, Sagnella reported that there appeared to be a wide range of variability in PRA among hypertensives and normotensives of African descent, and therefore a large proportion of these individuals may have had PRA that was in the same range as Caucasians (128). Therefore, lower PRA among those of African descent continues to be a topic of debate.

Ethnic differences in plasma aldosterone levels have also been investigated in normotensive and hypertensives of African descent and Caucasians. Studies have demonstrated that there appears to be some suppression of plasma and urinary aldosterone levels in normotensive and hypertensives of African descent when compared to Caucasians (40; 64; 115; 116). One study by Pratt et al. measured urinary and plasma aldosterone levels in children of African descent and Caucasian children (115). Urinary aldosterone levels were lower in the children of African descent compared to Caucasian children (115). In addition, there were no differences in Na^+ excretion between the two groups, but the children of African descent did have lower K^+ levels, which was indicative of lower K^+ intake (115). Since K^+ levels can affect aldosterone biosynthesis (20), lower K^+ intake may have contributed to the lower aldosterone biosynthesis, leading to less aldosterone excreted in the urine. Pratt

et al. suggested that the children of African descent could have had a lower degree of responsiveness to K^+ , and this could have accounted for the lower urinary aldosterone excretion (115). Regardless of these suggestions, when controlling for K^+ intake, aldosterone excretion was still lower in the children of African descent (115). Not only were urinary aldosterone levels different between the two groups, but plasma aldosterone levels were also lower among the children of African descent (115). The study by Pratt et al. indicates that there are possible ethnic differences in plasma and urinary aldosterone levels among children of African descent children. Due to the use of healthy children and lack of control of Na^+ and K^+ intake, it is difficult to make a definitive conclusion that the same ethnic differences in aldosterone levels observed in the children also occur in adulthood and contribute to the development hypertension (115).

There have been studies performed in adult normotensives and hypertensives investigating ethnic differences in plasma aldosterone levels. Fisher et al. conducted a study in which hypertensives and normotensives of African descent and Caucasian hypertensives and normotensives were placed on a standardized Na^+ and K^+ intake of 10 mmol/d and 100 mmol/d, respectively (40). Under basal conditions, plasma aldosterone levels were the same between normotensives of African descent and Caucasian normotensives and between hypertensives of African descent and Caucasian hypertensives, but plasma aldosterone levels were lower among all hypertensives compared to normotensives (40). The responsiveness of plasma aldosterone to a change in posture differed between the two hypertensive ethnic groups in which the increase in plasma aldosterone levels when going from the supine

to the upright position were smaller in hypertensives of African descent compared to Caucasian hypertensives (40). In addition, upon infusion of ang II, which stimulates aldosterone secretion, hypertensives of African descent had lower plasma aldosterone levels compared to the Caucasians hypertensives (40). Normotensives of African descent and Caucasian normotensives did not display these same differences (40).

This study by Fisher et al. suggested that among normotensives of African descent and Caucasian normotensives there were no differences in plasma aldosterone levels under basal and stimulated conditions (40). Additionally, the differences in plasma aldosterone levels that occurred between normotensives and hypertensives were independent of ethnicity (40). The ethnic differences occurred within the hypertensive group, and were not uncovered until postural changes and ang II were used to stimulate aldosterone biosynthesis (40). These findings may have demonstrated that possible ethnic differences in aldosterone biosynthesis may only be apparent among hypertensives and under conditions that stimulate the synthesis and release of aldosterone (40).

He et al. investigated the change in PRA, and plasma aldosterone and ang II levels in response to alterations in Na^+ intake in hypertensives of African descent and hypertensive Caucasians (64). Under basal conditions in which patients consumed their usual unregulated diet, plasma aldosterone and ang II levels were not different between individuals of African descent and Caucasians, but PRA was lower among individuals of African descent compared to Caucasians (64). Following their usual dietary period, the patients were placed on a 5 day high Na^+ diet (350 mmol/day) followed by a 5 day low Na^+ diet (10 mmol/day) (64). The investigators found that

the individuals of African descent had a greater reduction in blood pressure and an attenuated increase in PRA, plasma ang II, and plasma aldosterone levels compared to Caucasians (64). Normally, a large reduction in Na^+ intake, such as the one in this study, would stimulate an increase in PRA and plasma ang II and aldosterone levels to prevent a large fall in blood pressure (64). He et al. concluded that the hypertensives of African descent might have suppressed PRA, ang II and aldosterone allowing for a greater fall in blood pressure compared to Caucasians (64). Therefore, it is possible that plasma aldosterone levels are less likely to differ among hypertensives of African descent and hypertensive Caucasians under basal conditions, but under conditions in which aldosterone biosynthesis is stimulated, ethnic differences are more likely to be uncovered.

Age: Hypertension is a disease associated with aging, and this in part may be related to alterations within the systems that regulate blood pressure as individuals age. Therefore, it is conceivable that the RAAS would experience a decline in responsiveness and activity as individuals' age. In general, it has been reported that after the second decade of life there is a steady decline in PRA that becomes pronounced between 40 and 60 years of age (24; 106; 130; 154). A similar decline with age has also been shown in many studies that have investigated the effect of age on plasma and urinary aldosterone levels (24). Crane et al. investigated the change in the RAAS with each decade of life and found that when individuals were on an unrestricted Na^+ intake there was a progressive reduction in urinary aldosterone excretion rate from the 2nd to 7th decade of life (24). Additionally, urinary aldosterone excretion was 46% lower among those in the 7th decade compared to those in the 2nd

decade (24). In regards to plasma aldosterone levels, studies have reported age-related effects occurring under conditions that would stimulate aldosterone biosynthesis (130; 154). For example, plasma aldosterone levels appeared to be suppressed in older individuals, when they experienced postural changes or Na⁺ and volume depletion (130; 154).

It appears that with age there is a general decline in RAAS activity, but specifically, there is a possible decline in adrenal responsiveness and the mechanisms responsible for the secretion of aldosterone.

Heritability of Blood Pressure, and Hypertensive Phenotypes

Blood pressure and the physiological variables involved in the regulation of blood pressure have been shown to have a genetic component (90). Williams et al. combined data from 5 heritability studies (12; 38; 39; 63; 76) and reported that genetic factors have a stronger influence on the determination of an individual's resting blood pressure than shared environment (159). Williams et al. also reported that among these studies, resting blood pressure correlations were higher among related family members compared to non-related family members from a shared environment (12; 38; 39; 63; 76; 159). For example, among genetically related individuals, such as parent-offspring, the correlations were reported to be 0.18 and 0.16 for resting SBP and DBP, respectively (12; 38; 39; 63; 76; 159). Spouse pair correlations have been reported to be as low as 0.08 and 0.06 for resting SBP and DBP, respectively (12; 38; 39; 63; 76; 159). Additional family aggregation studies have investigated the heritability of resting blood pressure among parent-offspring, sibling-sibling and monozygotic (MZ) and dizygotic (DZ) twins (52; 103). The

family aggregation values for these relationships have ranged from 0.25-0.68 and 0.41-0.58 for SBP and DBP, respectively (12; 38; 39; 52; 63; 76; 103). These heritability and family aggregation studies provide evidence that the establishment and regulation of an individual's resting blood pressure is partly genetically determined.

Essential hypertension differs from other monogenic forms of hypertension, such as primary aldosteronism or Liddle's syndrome because it is polygenic, meaning that there may be a multitude of genes that contribute to its development (90; 159). Early debate about the genetics of essential hypertension occurred between two researchers, Pickering and Platt (144). Pickering suggested that essential hypertension was polygenic and was not due to a single loci mutation (90; 110). Platt believed that essential hypertension was a monogenic, single loci, autosomal mutation that followed Mendelian inheritance (111). Years of research have weakened Platt's argument and provided support for Pickering's argument that essential hypertension is indeed a multifactoral disease, involving the interaction of multiple genes, and is not due to a single loci mutation that follows Mendelian inheritance (90). The technique of simple segregation analysis has been used to help delineate whether the pattern of inheritance of hypertension fits that which would be predicted by a single-locus model, and there has been limited evidence to demonstrate that essential hypertension follows such a pattern of inheritance (90). It has been suggested, however, that there may be rare subgroups of families in which the pattern of inheritance of hypertension can be predicted by a single-loci, but this has been difficult to detect (90).

Given the complex genetic etiology of hypertension, there are not many studies that have been able to clearly demonstrate its pattern of inheritance. In general, studies have reported on the heritability of blood pressure and/or the phenotypes associated with the regulation of blood pressure. There are some studies that have reported on the family aggregation of hypertension by investigating family history (70). For example, Williams et al. reported, based on unpublished data, that 70% of individuals who developed hypertension before the age of 55 had a positive family history of hypertension, which included having siblings and/or parents with hypertension (158). It has also been reported that young individuals with a positive family history of hypertension had a four-times greater risk of developing hypertension compared to those with no family history of hypertension (70; 158).

As mentioned earlier, because hypertension is a heterogeneous trait, it has been difficult for studies to demonstrate genetic linkage (158). Therefore, researchers have focused their attention on the heritability of intermediate hypertension phenotypes in which genetic linkage is thought to be more evident (158). The Cardiovascular Genetics Research Clinic at the University of Utah studied the heritability of intermediate phenotypes that may contribute to the development of hypertension in 98 pedigrees with a family history of hypertension (156). They reported individuals with lower urinary excretion of kallikrein, a potent vasodilator (54), were two-times more likely to have a first-degree relative with hypertension (11). In addition, those with a family history of hypertension were found to have an increase in intraerythrocytic Na^+ concentration and Ouabain binding sites, which reflected the number of $\text{Na}^+ - \text{K}^+$ -ATPase pumps on the surface of red blood cells (62).

An increase in both phenotypes (intraerythrocytic Na^+ concentration and Ouabain binding sites) (11; 62), reflected a possible increase in renal tubular Na^+ reabsorption that could have contributed to the development of hypertension. Williams et al. suggested that two or three of these traits in combination with specific environmental factors could explain the occurrence of essential hypertension in some individuals (156).

Additional studies have investigated the heritability of other intermediate phenotypes that may contribute to hypertension. In family aggregation studies it has been reported that among individuals with hypertensive first-degree relatives, plasma renin levels were higher and their ability to excrete a Na^+ load was lower compared to those with normotensive first-degree relatives (50). There is also evidence of heritability with respect to aldosterone excretion and plasma aldosterone levels. Manatunga et al. reported that among young, (6-17 years), MZ and DZ twins, the interclass correlation coefficient for aldosterone excretion was 0.69 and 0.29, respectively, which was indicative of strong heritability (99). Additionally, Manatunga et al. found that among siblings of African descent, the interclass correlation for aldosterone excretion was 0.51, again indicative of strong family aggregation (99). Grim et al. reported that plasma aldosterone levels under basal conditions and in response to volume expansion were heritable among monozygotic and dizygotic twins (51). Additionally, it was found that urinary K^+ and fractional Na^+ excretion showed significant genetic variation under volume expanded conditions (51). Grim et al. concluded that the renal regulation of Na^+ and K^+ excretion, as well as homeostasis, may be partly influenced by heritability (51);

therefore, it can be suggested that dysregulation of these phenotypes can aggregate among families and contribute to the incidence of hypertension that occurs among related individuals. Kotchen et al. demonstrated that among hypertensive African descent sib-pairs, the response of indices of renal clearance to a norepinephrine stimulus and the PRA response to changes in posture were heritable (88).

Furthermore, a family aggregation study among individuals of African descent investigated the heritability of salt sensitivity and found that SBP, DBP, and mean arterial blood pressure (MAP) responses to a change in Na⁺ load were heritable, suggesting a genetic component to salt sensitivity (143).

Evidence of the heritability of blood pressure and the phenotypes that influence it establishes the precedence that the level of blood pressure is a heterogeneous trait in which its regulation may be the result of the interaction of multiple genes. The assumption can be made that the development of hypertension may be partly due to genetic variation within different pathways that regulate blood pressure. Therefore, in researching the genetics of hypertension, it is important to use these physiological pathways as a guide in determining what genes may be important in the investigation of hypertension.

CYP11B2 Gene and Aldosterone Biosynthesis

The CYP11B2 gene, also known as the aldosterone synthase (AS) gene, is responsible for aldosterone biosynthesis, and therefore attention has been focused on its possible contribution to the development of hypertension. CYP11B2 stands for cytochrome P450, family 11, subfamily B, polypeptide 2, and encodes for a member of the cytochrome P450 superfamily of enzymes, P450c11AS (4). The P450c11AS

enzyme, also called AS, is a monooxygenase that has 18-hydroxylase and 11-beta hydroxylase activity, both of which are necessary for catalyzing the synthesis of aldosterone (4; 26; 81)

The CYP11B2 gene is located on the long arm of chromosome 8q, contains 9 exons and 8 introns, and was first discovered by Kawamoto and colleagues (81). It was suspected to be a less active gene closely related to the CYP11B1 gene, which is involved in glucocorticoid production (81). Even though both the CYP11B1 and CYP11B2 genes were found to have exon sequences that were 93% identical, they differed significantly in their promoter region (81). The CYP11B2 gene was found to encode an enzyme that had both 18-hydroxylase and 11-beta-hydroxylase activity (81), whereas the CYP11B1 gene only encoded for the 11-beta-hydroxylase enzyme (81). Therefore, it was determined that the CYP11B2 and CYP11B1 genes, though closely related, were two separate genes that produced two separate enzymes (81). CYP11B2 encoded for an enzyme that catalyzed the synthesis of mineralcorticoids, while CYP11B1 encoded for an enzyme that catalyzed the synthesis of glucocorticoids (81).

Aldosterone biosynthesis occurs in two phases, an acute phase and a chronic phase (21). The acute phase occurs within minutes and involves the transfer of cholesterol (the initial precursor to aldosterone) to the mitochondria of the glomerulosa cells (21). This process is partly regulated by intracellular calcium (Ca^{2+}), calmodulin (CaM), and calcium/calmodulin-dependent kinases (CaMK) (21). CaM is a multipurpose intracellular Ca^{2+} receptor that mediates many Ca^{2+} -related intracellular processes (5). While CaMK are responsible for the phosphorylation of

proteins involved in the signaling pathways stimulated by Ca^{2+} (5). Both CaM and CaMK are also important factors in the chronic phase of aldosterone biosynthesis which involves the signaling cascade necessary for regulating CYP11B2 gene expression (21).

In the chronic phase, aldosterone biosynthesis is regulated by CYP11B2 gene expression (21). There have been recent discoveries in the last few years that have uncovered possible steps in the regulation of CYP11B2 expression. One being that there are two main physiological regulators, ang II and K^+ (20). Ang II and K^+ can directly act on the adrenal gland and lead to an increase in CYP11B2 mRNA and protein levels (21). The suggested mechanism by which ang II and K^+ regulate CYP11B2 gene expression involves the increase in intracellular Ca^{2+} levels (20). When plasma ang II levels become elevated, ang II will binds G-protein coupled AT_1 receptors located in the adrenal glomerulosa cells (137). This leads to the intracellular activation of phospholipase-C (PL-C) a plasma membrane bound enzyme, which hydrolyzes phosphatidylinositol bisphosphate (PIP_2), an inositol phospholipid, to inositol triphosphate (IP_3) (5; 117). IP_3 , is a small water soluble molecule that leaves the plasma membrane, diffuses through the cytosol and then binds to IP_3 gated Ca^{2+} channels (5). The binding of IP_3 to the Ca^{2+} channels leads to the release of Ca^{2+} and to an increase in intracellular Ca^{2+} concentration (5; 20; 117). K^+ stimulates the release of Ca^{2+} by causing the depolarization of the plasma membrane of the adrenal glomerulosa cells (117). The depolarization leads to the activation of voltage-sensitive Ca^{2+} channels and the release of Ca^{2+} , which causes an increase intracellular Ca^{2+} concentration (20). Clyne et al. found that the increase in intracellular Ca^{2+}

concentration leads to an increase in CYP11B2 gene expression, confirming that intracellular Ca^{2+} concentration has a very important role in the induction of CYP11B2 (20).

The mechanism by which Ca^{2+} influences CYP11B2 gene expression is through the CaM-CaMK cascade (21). It is suggested that both CaM and CaMK are important in the induction of CYP11B2 by ang II and K^+ (21). Condon et al., through the use of H295R adrenocortical cells transfected with luciferase reporter constructs driven by CYP11B2 5'-flanking DNA, demonstrated that when two separate inhibitors for CaM and CaMK were used, expression of CYP11B2 by ang II and K^+ was inhibited (21). The regulation of the CYP11B2 by ang II and K^+ via the Ca^{2+} -CaM-CaMK signaling pathway requires important gene regulatory elements located within the CYP11B2 gene. Within the 5' flanking region of the CYP11B2 gene is a cis-regulatory element, a region within the promoter where the transcription factor CaMKI-295 binds and influences transcription of CYP11B2 (20; 21). The cis-regulatory element is located between -64 and -347 bp, and when this region is deleted, CYP11B2 gene expression by CaMKI-295 decreases significantly (21). In addition, there is a second important regulatory region identified at position -71/-64 bp, called the cAMP response element (CRE) (21). Both Clyne and Condon identified this region as essential for regulation of CYP11B2 transcription by ang II, K^+ , and cAMP (20; 21). Mutations in this region have been found to block CaMKI and ang II stimulated gene expression (20; 21). The CYP11B2 CRE has been shown to bind transcription factors such as CRE-binding (CREB) protein and members of activating transcription factors (ATF-1 and ATF-2) (21). Condon et al. suggests that once the

Ca²⁺-CaM CaMKI cascade is activated by ang II, and K⁺, CaMKI phosphorylates CREB and ATF-1, which could increase their ability to enhance transcription of the CYP11B2 gene (21).

Once the CYP11B2 gene product, P450c11AS, has been produced it is responsible for the two terminal steps in the conversion of corticosterone to aldosterone (18-hydroxylation and 18-methyloxidation) (26). The molecular steps responsible for the expression of CYP11B2 are intricate and continue to be elucidated. Due to the fact that the CYP11B2 is essential in aldosterone biosynthesis, it is possible that mutations in the regulation of this gene may contribute to chronic elevations in aldosterone levels and Na⁺ reabsorption, which may be important in the etiology of hypertension.

CYP11B2 –344C/T Polymorphism and the Association with Hypertension

The CYP11B2 gene has recently been proposed as a hypertension candidate gene based on its involvement in aldosterone biosynthesis. Mutations in the CYP11B2 gene or abnormalities in the regulatory factors of the gene have been shown to lead to aldosterone related disorders (102) Based on this evidence, researchers have investigated whether less severe mutations in different regions of the CYP11B2 gene could possibly contribute to the development of hypertension (14).

For example, the –344C/T promoter polymorphism of the CYP11B2 gene, occurs in the core sequence of a regulatory element called Ad4, a nuclear receptor half-site consisting of a sequence of base pair repeats (10). Regulatory elements are vital in the regulation of the initiation of transcription by RNA polymerase (5). There are specific regulatory proteins, such as activator proteins that bind to the regulatory

elements called enhancer elements (5). Once bound, the activator proteins greatly enhance the initiation of transcription by attracting, modifying, and positioning RNA polymerase and other transcription factors onto the promoter (5). The specific activator protein that binds to the Ad4 element is a monomeric orphan nuclear receptor protein called steroidogenic factor-1 (SF-1) (10). An orphan nuclear receptor protein, such as SF-1, remains in an inactive state until the binding of an unknown hydrophobic signal molecule, once activated SF-1 binds to Ad4 (5). It has been reported that the Ad-4 is essential for the coordinated development and function of the adrenal gland, and that SF-1 is required for full expression of many other cytochrome P450 genes (10; 104). Therefore, it could be suggested that a mutation in the Ad4 region, such as the -344C/T SNP, could alter the binding of SF-1 and consequently gene expression.

White and Slutsker have reported that the presence of the C allele of the -344C/T polymorphism causes SF-1 to bind four times as tighter on a molar basis as compared to the presence of the T allele (155). These findings were confirmed by Bassett et al. in which it was demonstrated, using an electrophoretic mobility shift assay, that the C allele bound SF-1 tighter than the T allele (10). Despite suggestions that the binding of SF-1 to Ad4 was necessary for CYP11B2 expression, molecular studies by Bassett et al. determined that the binding of SF-1 to Ad4 was not essential for the expression of CYP11B2 (10). By cotransfecting H295R cells with reporter constructs containing the 5' flanking DNA of the CYP11B2, CYP11A1, CYP11B1 and CYP17 with an expression plasmid containing the coding region for SF-1, Bassett et al. found that the expression of the CYP11B2 gene was significantly less compared

to the other CYP genes (10). In addition, with increasing SF-1 concentration CYP11B2 gene expression continued to decrease (10). Therefore, based on the findings of Bassett et al., the binding of SF-1 to Ad4 does not appear to be necessary for the expression of CYP11B2 (10; 20). Additionally, Bassett et al. investigated whether the C or T allele associated SF-1 binding affected gene expression, but found that regardless of the allele, the presence of SF-1 did not affect CYP11B2 gene expression (10).

Together, these findings indicate that the Ad4 site is a functional regulatory element that binds SF-1 and therefore influences the expression of such CYP genes as CYP11A1, CYP11B1, and CYP17, but this is not true for CYP11B2 (10). It appears that even though SF-1 binds to the CYP11B2 Ad4 site in the same manner as the other CYP genes, SF-1 is not involved in the regulation of CYP11B2 transcription (10). Thus, there is some evidence that the -344C/T polymorphism may not be a functional mutation, which may be a surprising conclusion in light of the studies reporting an association between the -344C/T polymorphism and hypertension, and variations in both plasma and urinary aldosterone levels (14; 29; 66; 114; 147).

One recent study by Henderson et al. found that the population attributable risk of hypertension associated with the presence of the T allele was 37.4% among African descent males and 27.7% among African descent females (66). These findings were further supported by Tiago et al., in which the association between the -344C/T polymorphism and ambulatory and office blood pressure was investigated in hypertensives of African descent (147). Ambulatory and office SBP was higher among those with the T allele (147). In addition there was reduced nocturnal decline

in SBP among those with the T allele (147). The association between the -344C/T polymorphism and blood pressure has also been investigated in the Caucasian hypertensive population. Both Davies et al. and Brand et al. found a higher frequency of the T allele among hypertensives compared to controls (14; 29). Staessen et al. demonstrated that in a European Caucasian population of both hypertensives and normotensives, an increase in relative risk of hypertension was associated with the T allele, and the CC genotype was associated with the lowest SBP and DBP (141).

There is a possibility that the association between the -344C/T polymorphism and hypertension may be age-dependent. Russo et al. demonstrated that the T allele was associated with the age-associated increase in DBP in Caucasian males (127). Furthermore, in the majority of the studies that have shown an association between the T allele and hypertension the participants have had a mean age of 40 years and higher (14; 29; 89; 141; 147). It is well accepted that hypertension is a disease associated with aging, however it can also be suggested that there are some genes polymorphisms that exert a greater influence on blood pressure as one ages.

Most investigations of the -344C/T polymorphism and blood pressure have examined Caucasians, while there are only a limited number of studies that have used other populations. The T and C allele frequencies of hypertensives of African descent were reported to be .78 and .22, respectively (163). Among Japanese hypertensives, the T and C allele frequencies were similar to hypertensives of African descent, .66 and 0.34, respectively (151). By comparison, the T allele frequency is reportedly lower (.52) and the C allele higher (.47) among Caucasian hypertensives (163).

Not all studies have found that the T allele is associated with hypertension, it is possible that ethnic differences in the -344C/T allele frequencies may contribute to the inconsistent findings among studies. For example, studies in Japanese hypertensives have demonstrated that the C allele appears to be more frequent than in normotensive Japanese controls (145; 151). Tamaki et al. compared the frequency of the TT and TC+CC genotypes in Japanese hypertensives, and controls and found that the frequency of the TC+CC genotype was significantly greater in the hypertensives than normotensives, 52.2% and 43.2% respectively (145). In addition, when investigating 24 hour ambulatory blood pressure in the same Japanese hypertensives, it was found that the TC+CC genotype group was classified as nondippers, meaning that they had a blunted nocturnal decline (145). Similarly, Tsukada et al. also reported that the frequency of the C allele was higher among Japanese hypertensives compared to normotensives (151). In contrast, Matsubara et al. reported that the nocturnal decline in blood pressure was greater among the CC genotype compared to those carrying the T allele in community dwelling Japanese, suggesting that the T allele was associated with a blunted nocturnal decline in blood pressure (100). Furthermore, studies by Kato et al. and Tsujita et al. reported no association between the -344C/T polymorphism and hypertension in the Japanese hypertensive populations (79; 150). Possible explanations for the conflicting findings among these studies may stem from heterogeneity among the Japanese population used. The study by Matsubara reported using community dwelling Japanese, but did not specify whether or not these individuals were hypertensive or normotensive (100). Tsukada et al. mentioned that the differences between their findings and those of Kato et al. may actually be related

to geographic environmental differences in the Japanese that were examined in their study (151). The population in the Tsukado study was from rural and suburban districts, whereas the Japanese group studied by Kato was mostly from the city (Tokyo) (151). Such distinctions are important since the Japanese from the rural environment have been found to ingest more Na^+ than those from the urban environment; therefore, the difference in results between the two studies may have been related to gene-environment interactions, in which one specific genotype association may have developed over generations due to specific dietary habits (151).

There have been discrepancies in regards to the association between the -344C/T polymorphism and hypertension among other populations as well. Zhu et al., found that there was no association between -344C/T polymorphism and hypertension between Caucasians and individuals of African descent (163). These findings differed from the previously mentioned studies that investigated the association within Caucasian populations and populations of African descent (14; 29; 66; 141; 147). It is difficult to discern why Zhu et al. did not find a genotype association with BP, despite having a robust sample size. Possible differences in study design could have contributed to the lack of findings.

Available literature provides evidence that the -344C/T polymorphism is associated with blood pressure and hypertension. It is known that there can be substantial interindividual variability in intermediate phenotypes related to the development of hypertension, such as plasma aldosterone and renal Na^+ handling. Variation in these intermediate phenotypes may be partially attributed to genetic variation. The CYP11B2 -344C/T polymorphism has been found to be associated

with plasma and urinary aldosterone and Na⁺ excretion. Pojoga et al. investigated the association between the -344C/T polymorphism and plasma aldosterone levels in Caucasians with mild to moderate hypertension (114). Plasma aldosterone levels were measured after participants completed an overnight fast and were supine for 90 minutes (114). It was determined that the C allele was associated with greater plasma aldosterone levels. There was a C allele dosage effect in which plasma aldosterone levels were highest among the CC genotype group, intermediate among the TC genotype group and lowest among the TT genotype group (114). It is important to note that the greater plasma aldosterone levels in C allele carriers were well within normal range and were not considered to be at pathologic levels consistent with secondary forms of hypertension, such as hyperaldosteronism. Pojoga et al. reported that normal supine plasma aldosterone levels range from 20-230 pg/ml and upright values range from 40-600 pg/ml (112). Therefore, the point should be taken that the -344C/T polymorphism may account for the slight elevations in plasma aldosterone levels that are within a normal range, and due to aldosterone's Na⁺ retaining qualities, this could contribute to the development of hypertension.

There have been only a few other studies that have investigated the association between the -344C/T polymorphism and plasma or urinary aldosterone levels (14; 29; 163) . Brand et al. reported that the T allele was associated with elevated serum aldosterone levels in normotensives (14). These results may have differed from Pojoga et al. study because the hypertension status of the participants was different. Pojoga studied hypertensives and Brand et al. studied normotensives (14; 114). Zhu et al. investigated the association between the -344C/T polymorphism

and plasma aldosterone levels in hypertensives of African descent and Caucasian hypertensives (163). No association was found between the -344C/T polymorphism and plasma aldosterone levels in either population. This could have been due to the difference in the methods used to obtain plasma aldosterone. Zhu et al. obtained blood samples while participants were seated, however they did not report the amount of time that the participants were seated, whether the participants were fasted, or the time of day blood samples were obtained (163). Aldosterone is sensitive to changes in body position and diet and can differ based on the time of day the blood sample is obtained (17). Therefore, lack of control of these variables could have contributed to the lack of association between the -344C/T polymorphism and plasma aldosterone levels in the Zhu et al. study.

The association between urinary aldosterone levels, Na⁺ excretion and the -344C/T polymorphism has also been investigated. Davies et al. reported that the T allele was associated with higher urinary aldosterone excretion rates compared to those with the C allele; however, the hypertensive status of the participants was not indicated. Due to aldosterone's influence on renal Na⁺ handling, some studies have investigated the association between the -344C/T polymorphism and urinary Na⁺ excretion (31). Delles and colleagues conducted a study in young men with mild hypertension and found that after one week of an oral Na⁺ load, those with the TT genotype had a greater increase in 24-hour urinary Na⁺ excretion compared to those with the CC genotype (31). Additionally, hypertensives with the TT or TC genotype were found to decrease serum aldosterone levels to a greater extent compared to those with the CC genotype (31). The greater increase in Na⁺ excretion in the TT genotype

group in addition to the suppression of serum aldosterone levels indicated that T allele carriers demonstrated a normal response to an increase in Na⁺ load. Whereas, those with the CC genotype experienced an altered response in which the reduced Na⁺ excretion was an indication of Na⁺ retention that may have resulted from reduced suppression of aldosterone biosynthesis.

The association of the C allele with elevated plasma aldosterone levels and reduced Na⁺ excretion is somewhat perplexing in light of the fact that in many studies, it was the T allele not the C allele that was associated with hypertension. It is logical to think that because the T allele is associated with hypertension, it may also be associated with an elevation in the hypertension-related phenotype, such as plasma aldosterone, which can contribute to an elevation in blood pressure. This pattern has been demonstrated with other hypertension candidate gene polymorphisms, such as the AGT M235T polymorphism, in which in some studies the T allele was associated with both hypertension and elevated plasma angiotensinogen levels (75; 160).

Based on the relationship between aldosterone and renal Na⁺ handling, a few studies have investigated the association between the -344C/T polymorphism and salt sensitivity of blood pressure (15; 80; 113). These studies did not find an association between the -344C/T polymorphism and salt sensitivity (15; 80; 113). The reason for this could be: 1) the heterogeneity in Na⁺ loading protocols used to measure salt sensitivity, 2) the criteria for categorizing someone as salt sensitive is not consistent among studies, 3) the CYP11B2 gene may be one of many genes that influences blood pressure responsiveness to changes in a Na⁺ load, and the effect of a

single polymorphism such as the –344C/T may not be sufficient to significantly influence this phenotype.

The evidence thus far demonstrates that the –344C/T polymorphism shows an association with hypertension and plasma aldosterone levels. These results are somewhat surprising based on findings that the Ad4 regulatory site, where the –344C/T polymorphism occurs and SF-1 binds, has been found to be nonfunctional (10; 20). Consequently, there have been questions about whether the –344C/T polymorphism can cause alterations in aldosterone synthase transcription that may influence aldosterone biosynthesis and eventually blood pressure. It has been suggested that although the Ad4 site is not necessary for CYP11B2 transcription, the –344C/T polymorphism may still influence transcription based on the allele-associated differences in SF-1 binding (147). As mentioned earlier, the C allele is associated with tighter binding of SF-1 compared to the T allele (10). Therefore, when the C allele is present, the result may be tighter binding of SF-1 to the Ad4 site making SF-1 unavailable to bind to other functional regulatory sites that influence CYP11B2 transcription (14; 94; 147). The result is that the C allele is associated with reduced transcription of CYP11B2 and reduced aldosterone biosynthesis. In contrast, the presence of the T allele would cause SF-1 to bind less tightly to Ad4, freeing it up to bind to functional sites that would influence transcription of CYP11B2 and increase aldosterone biosynthesis, and over time, elevate blood pressure (14; 94; 147).

Linkage disequilibrium may be another explanation for the association between the –344C/T polymorphism and hypertension and plasma and urinary aldosterone levels. Linkage disequilibrium occurs when two alleles at different loci

occur together within an individual more often than would be predicted by random chance (123). Indeed, a few CYP11B2 polymorphisms have been found to be in linkage disequilibrium with the -344C/T polymorphism. Thus, the -344C/T polymorphism may be a marker for a functional mutation or mutations that result in phenotypic differences. The K173R and the intron 2 conversion polymorphisms have been found to be in linkage disequilibrium with -344T/C polymorphism (14; 29; 155; 163). The intron 2 conversion polymorphism results from the intron 2 of CYP11B2 being replaced by the intron 2 of CYP11B1 (14; 155). The genotypes for the intron 2 conversion are -/-, meaning both alleles have no conversion, -/+ means one allele has the conversion, and ++ means both alleles have the conversion (163). Zhu et al. found that Caucasian hypertensives had a greater frequency of the TT⁺⁺ haplotype (163). However, these same results were not found in the population of African descent. The author suggested that the two polymorphisms may not be in linkage disequilibrium in this population of African descent (163). Similarly, Davies and colleagues found that among Caucasian hypertensives and normotensives, the -344C/T and intron 2 conversion polymorphisms were in linkage disequilibrium and that the T and + allele frequencies were greater among the hypertensives compared to the normotensives (29).

In summary, there is evidence indicating that the -344C/T polymorphism is associated with hypertension. The majority of the studies report a T allele association, but there are some that report a C allele association. The reason for such discrepancies has not been clearly delineated but may be the result of differences in the study population or study design. Similarly, studies that have investigated the

-344C/T polymorphism's association with plasma and urinary aldosterone levels are few and the findings are mixed. There is some evidence that this lack of consistency could be due to the non-functional effect of the -344C/T polymorphism. That is, the binding of SF-1 to the Ad4 site where the -344C/T polymorphism occurs, may not be essential for CYP11B2 gene expression. More studies are needed to clarify the association of the -344C/T polymorphism with both hypertension and plasma aldosterone levels, not only in Caucasians, but also in other ethnic groups where available data are limited.

Effectiveness of Aerobic Exercise Training on Hypertension

As previously mentioned, hypertension is considered to be a major public health burden in which 90% of individuals over the age of 50 are at risk for developing the disease (153). As a result of hypertension being a major risk factor for the development of CVD, there is a continuous effort to increase public and practitioner awareness about nonpharmacologic treatments that will reduce and control blood pressure in those afflicted with hypertension. In 1997, the Sixth Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (1) was released with recommendations for the treatment of hypertension in individuals diagnosed as having high-normal or stages 1-3 hypertension. It was recommended that lifestyle modification, such as increasing physical activity, independent of antihypertensive therapy, be the first line of treatment for those with high-normal blood pressure (130-139/85-89) or Stage 1 hypertension (140-159/90-99) (1). In 2003, the Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood

Pressure (19) was released with an updated recommendation for the treatment of hypertension. In this report, there were some slight changes in which the category of high-normal was abolished and the new category, pre-hypertension, (comprised of individuals with blood pressures between 120-139/80-89), was created (19).

According to JNC VII, life-style modification, independent of antihypertensive medications, is recommended as first-line therapy in the treatment of hypertension for pre-hypertensive individuals (19). For those with stage 1 or 2 hypertension (stage 2 \geq 160/100), lifestyle modification in conjunction with antihypertensive medications is recommended. JNC VI and VII provide the gold standard for the treatment of hypertension, therefore the inclusion of physical activity as a therapeutic modality, speaks to its effectiveness and importance in the control of hypertension.

In 2000, Hagberg, Park and Brown reviewed all available studies that had been published on the effectiveness of aerobic exercise training on hypertension (59). Of the studies that were reviewed, there were 74 groups of patients with hypertension, 76% significantly decreased SBP with aerobic exercise training (59). Of the 73 groups of patients with diastolic hypertension, 81% significantly decreased DBP with aerobic exercise training (59). Hagberg et al. were able to demonstrate that from all available studies that investigated the effect of aerobic exercise training on hypertension, a significant proportion reported hypertensive patients reducing blood pressure with aerobic exercise training (59).

The effectiveness of aerobic exercise training to reduce blood pressure is not a haphazard science in which any given exercise intensity and duration will elicit beneficial results. Rather, the same specificity that is used when prescribing an

antihypertensive medication to a hypertensive patient is required when developing an exercise prescription for a hypertensive patient. Research has shown that an individual's exercise intensity and duration does affect the magnitude of blood pressure reduction. Hagberg et al. reported that in aerobic exercise training studies in which intensities were set below 70% of maximal oxygen consumption (VO_2 max), which is considered a low to moderate exercise intensity, hypertensive individuals had a 50% greater reduction in SBP, and a slightly greater reduction in DBP, compared to aerobic exercise training studies that used exercise intensities greater than 70% VO_2 max (59). Roman et al. reported that among females with Stage 1 or 2 Hypertension, those who exercised for 12 months at 70% of maximal heart rate had significant reductions in SBP and DBP of 20 and 18 mm Hg, respectively (125). However, when they exercised for 12 months at a high intensity over 70% of maximal heart rate, there was no significant reduction in blood pressure (125). Hagberg et al. demonstrated that among hypertensives, 9 months of aerobic exercise training at a low intensity of 50% of VO_2 max, significantly reduced in both SBP and DBP by 20 and 11-12 mm Hg, respectively. In contrast, the hypertensives that trained for 9 months at a higher intensity of 70-85% VO_2 max, only significantly reduced their DBP (58). Other studies have also supported these results (34; 72; 135; 148; 152). The findings that low to moderate intensity exercise is as effective or even more effective at reducing blood pressure in a hypertensive population compared to high intensity exercise, demonstrate that the majority of individuals can achieve adequate reductions in blood pressure without being overwhelmed by unattainable exercise expectations. Also, as stated by Hagberg et al., with lower intensity exercise,

individuals are more likely to adhere to exercise expectations without experiencing injury and/or extreme physical exhaustion (59).

The length of time it takes for reductions in blood pressure to occur when hypertensives undergo an aerobic exercise training protocol is additionally important. It is reported that average reductions in SBP and DBP of 9.8 mm Hg and 8.4 mm Hg, respectively, can occur within the first 1 -10 weeks of aerobic exercise training (59). Kohno et al. reported a 6 mm Hg and 4 mm Hg reduction in SBP and DBP, respectively, after 3 weeks of aerobic exercise training (86). Others studies such as Kiyonaga et al. reported significant reductions of 18 mm Hg and 10 mm Hg in SBP and DBP, respectively, after 10 weeks of aerobic exercise training in Japanese hypertensives (84). Urata et al. and Sasaki et al. also reported reductions in SBP and DBP of approximately 12-13 mm Hg and 4-5 mm Hg, respectively after 10 weeks of aerobic exercise training (131; 152). Therefore, within a relatively short amount of time aerobic exercise training can cause considerable reductions in both SBP and DBP among hypertensives.

Despite the significant reductions that can occur in both SBP and DBP after only a few weeks, evidence indicates that extending exercise training an extra 11-20 weeks or more can elicit further reductions in SBP, but the same has not been reported for DBP (59). Hagberg et al. found that after 3 months of aerobic exercise training, there was an 11-12 mm Hg and an 8 mm Hg reduction in SBP and DBP, respectively, and after an additional 6 months of aerobic exercise training there was a further decrease in SBP by 8-9 mm Hg and DBP by 4 mm Hg (58). Kiyonaga et al. found that an additional 10 weeks of aerobic exercise training further reduced SBP

and DBP by 3 and 4 mm Hg, respectively (84). Additionally, Motoyama et al. demonstrated that after 3 months of aerobic exercise training, SBP and DBP decreased by 15 and 9 mm Hg, respectively (105). After an additional 6 months of aerobic exercise training SBP continued to decrease, but DBP did not change any further (105).

The prevalence of hypertension increases in all aging populations independent of ethnicity. However, the prevalence and severity of hypertension and the blood pressure response to antihypertensive therapies can differ among ethnic groups. In the case of aerobic exercise training, there appears to be some differences among ethnic groups in blood pressure response. Caucasians have been one of the primary ethnicities studied in the area of aerobic exercise training and hypertension studies, and it has been demonstrated that they reduce blood pressure with aerobic exercise training (56). There have been a few studies on blood pressure response to aerobic exercise training in hypertensive Asian-Pacific Islanders, mainly Japanese (84; 86; 131; 152). This population has shown significant reductions in blood pressure in response to aerobic exercise training (84; 86; 131; 152). Additionally, it has been found that blood pressure reduction can be slightly higher among Asian-Pacific Islanders hypertensives compared to Caucasian hypertensives (56).

Individuals of African descent have an increased prevalence and severity of hypertension. Despite this, very few studies have investigated the blood pressure response to aerobic exercise training in hypertensives of African descent. Over the past few years this has slowly changed, as more studies have either included hypertensives of African descent or used hypertensives of African descent

exclusively. Brown et al, investigated the change in blood pressure in response to a short-term aerobic exercise training protocol among females of African descent with hypertension (16). There was no significant reduction in blood pressure, which may have been the result of the 7 day training protocol not being long enough to elicit a significant blood pressure response (16). Dubbert et al. included hypertensives of African descent in a 12 week aerobic exercise training study and found that they significantly reduced SBP and DBP, and that the reductions were no different from the Caucasian hypertensives in the study (34). Kokkinos et al. found that SBP and DBP decreased approximately 7 and 5 mm Hg, respectively in male hypertensives of African descent that were placed on a 16 week aerobic exercise training program (87). In the first 16 weeks of training, the men remained on medication, but after being tapered off of the medication and continuing with aerobic exercise training, the reduction in blood pressure remained the same (87). There have even been aerobic exercise training studies that included hypertensive children of African descent, and it was found that blood pressure was significantly reduced with aerobic exercise training (28; 36). More studies including hypertensives of African descent are needed to validate that aerobic exercise training is an effective mode of treatment, but from the limited available data, it appears that hypertensives of African descent may respond to training as well as hypertensive Caucasians.

In regards to age-related responses to aerobic exercise training, there have not been many studies that have clearly investigated whether one age group is more responsive to reducing blood pressure with aerobic exercise training compared to another. In the review by Hagberg et al., it was found that middle-aged hypertensives,

(41-60 years) had greater and more consistent reductions in blood pressure compared to older and younger patients (59). Hagberg et al. suggested that their findings be interpreted with caution because the number of older and younger patients, in the studies that they reviewed was much less than the middle-aged group. There has been one study by Ishikawa et al. that did compare the response of blood pressure between hypertensives that were 30-49 years and 50-69 years of age (72). After 4 and 8 weeks of aerobic exercise training, significant age related differences were associated with the blood pressure reductions, in which smaller reductions were seen within the older age group (72). Additionally, investigations that have studied hypertensive individuals over 60 years of age have also reported significant reductions in blood pressure with aerobic exercise training (58; 135). Therefore, from the limited available data it appears that most age groups reduce their blood pressure with aerobic exercise training, but possibly middle-aged hypertensives experience slightly greater and more consistent reductions in blood pressure (59).

The response of hypertensive phenotypes, such as Na^+ excretion and plasma aldosterone, to aerobic exercise training is as important as the response of blood pressure to aerobic exercise training. Since plasma aldosterone can alter renal Na^+ handling, ultimately leading to shifts in blood pressure, the investigation into whether plasma aldosterone levels and urinary Na^+ excretion change with aerobic exercise training may help identify the mechanisms that contribute to the training-induced reductions of blood pressure. Unfortunately, there have been very few studies that have investigated the change in plasma aldosterone levels and urinary Na^+ excretion with aerobic exercise training. From the few studies that are available, there is some

variability in the response of plasma aldosterone and urinary Na^+ excretion to aerobic exercise training. One study in normotensives demonstrated that after 16 weeks of training, individuals achieving the greatest work capacity experienced the greatest reduction in plasma aldosterone levels (67). Another study by Braith et al. reported a 50 pg/ml reduction in plasma aldosterone levels of heart failure patients in response to 16 weeks of aerobic exercise training (13). Not all studies have found a reduction in plasma aldosterone levels with aerobic exercise training; Kohno et al. found no reduction in plasma aldosterone levels in Japanese hypertensives after 3 weeks of aerobic exercise training (86). Urata et al. also reported a non-significant reduction in serum aldosterone concentration in Japanese hypertensives after 10 weeks of aerobic exercise training (152). Findings from Kohno et al., and Urata et al., may have been a consequence of the length of training being shorter than the other studies that reported significant reductions in plasma aldosterone levels (86; 152). The reduction in plasma aldosterone levels with aerobic exercise training can ultimately be beneficial because it can lead to a reduction in Na^+ reabsorption that may be contributing to an individual's hypertensive state.

Urinary Na^+ excretion can also change with aerobic exercise training in hypertensives. Kiyonaga et al. reported a significant 44 mEq/d increase in Na^+ excretion after 10 weeks of aerobic exercise training in Japanese hypertensives (84). Brown et al. also reported a significant 37 mEq/d increase in urinary Na^+ excretion after short-term aerobic exercise training in hypertensive females of African descent (16). Similar to plasma aldosterone, the response of urinary Na^+ excretion to aerobic exercise training is variable. Several studies that measured urinary Na^+ excretion

before and after aerobic exercise training found no significant change (34; 58; 86; 152).

Overall, aerobic exercise training has proven to be an effective mode of treatment for hypertension. Individuals with hypertension can expect to experience reductions in blood pressure within the first few weeks of aerobic exercise training, and at an intensity that is realistic and manageable for those hypertensives that are transitioning from a sedentary to active lifestyle

Heritability of Blood Pressure Response to Aerobic Exercise Training

Blood pressure and heart rate (HR) response to stimuli such as aerobic exercise training can vary among individuals. There has been a modicum of research in regards to whether these phenotypic responses may be heritable. Most of the available literature has focused on the heritability of cardiovascular phenotypic responses to acute bouts of exercise (18; 139). In the past few years, the HERITAGE Family Study has produced the only body of research to investigate the influence of genetics on the response of cardiovascular phenotypes to aerobic exercise training (6; 121). A HERITAGE study by Rice et al. reported that among 98 Caucasian families placed on a 20-week aerobic exercise training program, maximal heritabilities were 18% and 24% for SBP and HR, respectively (121). When the sample was divided into individuals with high blood pressure and normotensives, among those with high blood pressure, maximal heritabilities were 20% and 36% for SBP and HR responses to aerobic exercise training, respectively (121). In the normotensives, the heritabilities for SBP and HR responses to aerobic exercise training were not significant (121). Additionally, for the entire sample the DBP response to aerobic exercise training was

not heritable (121). An et al. reported on the heritability of exercise blood pressure and HR among 99 sedentary normotensive Caucasian families that were placed on the same aerobic exercise training protocol as the participants in the Rice et al. study (6; 121). An et al. found significant heritabilities of 34% and 29% for HR at 50 W and 60% of VO_2 max, respectively. In regards to blood pressure, a significant heritability of 22% was found for SBP at 50 W (6). There were no significant findings in regards to exercise DBP. The limited data indicates that among family aggregation studies, there appears to be heritability in the SBP response to aerobic exercise training (6; 121). Unfortunately, the family aggregation studies have not been able to demonstrate heritability of DBP. Even though there is a strong likelihood that there is genetic involvement in the blood pressure response to aerobic exercise training in both normotensive and hypertensive populations, being that there is limited data, more research in the area of heritability of such cardiovascular phenotypes as blood pressure and their response to aerobic exercise training is warranted.

Gene-Exercise Interactions and Hypertension

Numerous studies have demonstrated that aerobic exercise training is an effective mode of reducing blood pressure in hypertensive individuals. Despite these positive results, there continue to be some hypertensives that may experience no change or an increase in blood pressure with aerobic exercise training. This variability in blood pressure response continues to occur regardless of the standardization of aerobic exercise training protocol and controlling for factors such as dietary differences, weight, age, and ethnicity; all of which can contribute to variability in blood pressure response to aerobic exercise training. As a consequence,

a few researchers have begun to investigate whether genetic differences can account for the unexplained interindividual variability in the blood pressure response to aerobic exercise training.

The current body of literature that has investigated the influence of genetics and blood pressure response to aerobic exercise training, has mainly involved the ACE I/D and AGT M235T polymorphisms (57; 119; 120; 161). Gene polymorphisms of the RAAS have been researched thoroughly in regards to whether they contribute to hypertension development (25; 68; 75; 107; 126). The ACE I/D polymorphism has shown a weak association with blood pressure, but the D allele has been related to higher plasma ACE levels, compared to the I allele, which could impact blood pressure (43; 122). The AGT 235T allele has been associated with increased blood pressure and plasma AGT levels (75; 160). The in depth investigation into whether these specific gene polymorphism are associated with hypertension has greatly influenced the area of gene-exercise research as it relates to hypertension. From the limited number of studies, it appears that both the ACE D and the AGT 235T alleles are associated with a non-significant reduction in blood pressure with aerobic exercise training compared to those with the ACE I and AGT M235 alleles (57; 119; 120; 161)

Hagberg et al. were one of the first to investigate the association between the ACE I/D polymorphism and blood pressure response to aerobic exercise training in a hypertensive population (57). The genotype groups were divided into II+ID and DD, and after 9 months of aerobic exercise training it was found that both the II +ID and DD genotype groups had significantly lowered their systolic blood pressure (-10 mm

Hg, -5 mm Hg, respectively), but the reduction in the II+ID genotype group was significantly greater than the DD (57). The II+ID genotype group had significantly lowered their DBP (-10 mmHg), whereas the DD genotype group did not (57). The II+ID group's reduction in DBP was significantly greater than the DD genotype group (57).

These findings were further confirmed in a study by Zhang et al. in which Japanese hypertensives were placed on an aerobic exercise training protocol. All participants exercised for 10 weeks (161). The I allele carriers experienced a significant -9.5 mm Hg change in SBP and a -4.8 mm Hg change in DBP, respectively after 4 weeks of exercise training (161). The DD genotype group experienced a significant -5.5 mm Hg change in SBP after 4 weeks of exercise training and no significant change in DBP (161). Following 10 weeks of aerobic exercise training both SBP and DBP were significantly reduced among the I allele carriers but not among the DD genotype groups (161).

Both Hagberg et al. and Zhang et al. found that the I allele carriers experienced a reduction in SBP and DBP, whereas the D allele carriers were associated with less of a reduction in blood pressure or no reduction at all (57; 161). Possibly the elevated ACE levels that have been associated with the D allele could contribute to a lack of blood pressure response to aerobic exercise training (43; 122). Because ACE levels were not measured in these two studies it is difficult to speculate whether this could be true.

The AGT M235T polymorphism has been studied in association with blood pressure response to aerobic exercise training. Rankinen et al. investigated the

association between the M235T polymorphism and resting blood pressure and submaximal exercise blood pressure in Caucasian normotensives (119). Following 20 weeks of aerobic exercise training, significant genotype associations were found only with submaximal DBP and exclusively in men (119). The MM and MT genotype groups significantly reduced their submaximal DBP by 3.7 and 3.5 mm Hg, respectively; whereas the TT genotype group experienced a 0.4 mm Hg reduction in submaximal DBP (119). There was an interactive effect between the ACE I/D and the M235T polymorphisms, in which individuals with the TT and the II genotype experienced a reduction in submaximal DBP, but individuals with the TT genotype and the ID or DD genotype experienced no change in submaximal DBP (119).

From these findings it is apparent that the TT genotype, which is normally associated with a higher blood pressure, is also associated with a lack of reduction in exercise blood pressure, whereas the MM and MT genotypes are associated with a reduction in submaximal exercise blood pressure. Rankinen et al. suggested that the lack of reduction in submaximal blood pressure in the TT genotype group could have been due to elevated AGT levels that have been associated with the TT genotype (119). Rankinen et al. further suggested that elevated AGT levels might have indirectly counter-acted the exercise induced-vasodilation that occurs with training (119). Additionally, the lack of reduction in submaximal exercise blood pressure that occurred among those with both the AGT 235 TT and ACE DD genotype may have been the result of elevated AGT and ACE levels that could have caused physiological changes that prevented a reduction in blood pressure with aerobic exercise training (119).

A recent study by Rauramaa et al. investigated the association of the AGT M235T polymorphism with change in blood pressure over a six year period in individuals that performed unsupervised exercise during this period and those that did not (120). The study was composed of older normotensive and hypertensive men. Those that exercised during the six years and had the MM genotype, experienced an increase in seated SBP of 1 mm Hg, whereas those that had the MM genotype, but did not exercise, increased their seated SBP by 14 mm Hg (120). In regards to seated DBP, those that exercised and had the MM genotype reduced their DBP by 6.2 mm Hg (120). Among the men that had the MM genotype, but did not exercise, DBP increased by 2.8 mm Hg after 6 years (120). Additionally, among those that exercised and had the MM genotype, supine DBP decreased, but this was not observed in those with the MM genotype that did not exercise (120). No reductions in blood pressure were reported among those with the TT genotype (120).

Rauramaa et al. suggested that the MM genotype, among those that exercised, was associated with the attenuation of the age-related increase in SBP and DBP (120). It was further suggested that the lack of blood pressure reduction among those with the TT genotype could be due to the elevated AGT levels that have been associated with the T allele, a possible indication of over stimulation of RAAS (120). This over-stimulation of the RAAS could have accounted for the lack of blood pressure response among those with the TT genotype (120). Whereas, because the MM genotype has been associated with lower AGT levels, a possible indicator elevated extracellular fluid volume, exercise may be more effective in reducing blood pressure by increasing Na^+ and fluid excretion (120).

Both Rankinen et al. and Rauramaa et al. provide evidence that the AGT M235 allele shows an association with aerobic exercise training-induced reductions in blood pressure among normotensives and hypertensives (119; 120). Both studies did not have very stringent criteria in regards to what BP was considered to be normotensive and hypertensive. Possibly with more control in these studies, the associations may have been even stronger. The interactive effect between the AGT M235T and ACE I/D polymorphism was a very significant finding because it demonstrated that the genes of the RAAS may influence resting blood pressure and the blood pressure response to aerobic exercise training. Lastly, both studies could have benefited from the measurement of plasma ACE and AGT levels to determine if there were genetic associations between baseline and after-training levels of these two phenotypes.

In regards to the CYP11B2 -344C/T polymorphism, there are no studies to date that have investigated its association with hypertension with respect to aerobic exercise training, but a recent study may provide support for a possible gene-exercise interaction. A genome-wide linkage study conducted by Rankinen et al. recently reported significant linkage between the change in SBP at 50% VO_{2max} after 20 weeks of aerobic exercise training and chromosomal region 8q21 in Caucasians (118). In individuals of African descent there was suggestive linkage between baseline DBP at 80% VO_{2max} and the same 8q21 region (118). What makes these findings noteworthy is that the 8q21 region is the location of CYP11B2 (118). Such findings help to establish the precedence for gene-exercise interactions involving the

-344C/T polymorphism and blood pressure along with other hypertension-related phenotypes.

Appendix C – Detailed Methods

This current study was a part of the larger Gene Exercise Research Study (GERS) conducted at the University of Maryland, College Park. GERS is a National Institutes of Health funded study, in which the main objective is to investigate genetic and exercise interactions on hypertension.

Recruitment

Participants were recruited from the College Park, Maryland and the District of Columbia metropolitan areas, via advertisements in newspapers, radio public service announcements, direct mail and health fairs. Potential participants who were interested in the study were instructed to call the GERS office at the University of Maryland in order to receive additional information about the study and to undergo an initial telephone screening.

Telephone Screening

The telephone screening began with an overview of the study to provide potential participants information about the study obligations and the time commitment required. Potential participants were excluded if they were not between the ages of 50-75 years, had a body mass index (BMI) greater than 37 kg/m², and had any form of cardiovascular disease (CVD), liver disease and pulmonary disease, diabetes, or orthopedic conditions that would impair their ability to exercise. Females were excluded if they were not post-menopausal for more than 2 years. For those that were post-menopausal, they were asked to maintain their hormone replacement status for the entirety of the study. Potential participants were asked whether or not they had hypertension and to estimate their resting blood pressure. Those using more than 2

antihypertensive medications were excluded. Potential participants were asked about their current physical activity level, those exercising more than 2 times per week for more than 20 minutes were considered to be physically active, and were excluded. Once an individual met the initial study inclusion criteria, they were scheduled for an orientation visit, and a consent form, health history and physical activity questionnaire were mailed to the individual.

Orientation and Informed Consent

The orientation meeting was held at the GERS facilities. The orientation was directed by one of the Principal Investigators, and during this visit all participants provided their written informed consent. In addition, participants toured the facilities and had their casual blood pressure measured. Screening Visit 1 was then scheduled for all participants who signed consent forms.

Screening Visit 1

Participants arrived at the Physiology of Exercise Laboratory having fasted for 12 hours in order to undergo a 2-hour post prandial glucose tolerance test. The post-prandial glucose tolerance test required participants to drink a 10 oz, 75 g dextrose load (Fisherbrand). Blood samples were obtained for blood chemistries and to conduct DNA analyses. Participants were excluded if they exhibited a fasting blood glucose > 126 mg/dl or blood glucose greater than 200 mg/dl at 2 hours. Participants were excluded if they had a GFR < 60 ml/min/1.73 m², which was estimated using the Modification of Diet in Renal Disease (MDRD) equation, and serum creatinine levels >1.5 mg/dl, both ensured that they did not have evidence of renal disease. During the 2-hour visit, height and weight were measured to verify BMI. In addition, three casual

blood pressure measurements were obtained on each arm. Participants with an average SBP < 120 or >159 mm Hg and/or DBP <80 or >99 mm Hg were excluded from the study. During this visit, participants were scheduled for their second screening visit.

Screening Visit 2

During the second screening visit, participants underwent a physical examination by a physician and performed a physician supervised graded exercise test (Bruce protocol) to screen for CVD. During the treadmill test, ECG, blood pressure, and heart (HR) were measured before the test, at the end of each stage, and every two minutes for 6 minutes after the test. The test was terminated upon the onset of cardiovascular signs and/or symptoms, or when the participants could no longer continue. Subjects were included in the study if they did not exhibit any cardiovascular signs or symptoms and had less than a 2mV ST segment depression (2).

Dietary Stabilization/Medication Tapering

Dietary and weight changes are known to independently contribute to changes in blood pressure (9; 42; 49). In order to control for such effects, all participants were required to be weight stable and follow a standardized diet to determine the independent effects of aerobic exercise training on plasma aldosterone levels, sodium excretion, and blood pressure. Participants attended a 6-week dietary class two times per week, which was taught by a Registered Dietician. The participants followed the American Heart Association (AHA) Step I diet (50-55% of calories from carbohydrates, 30-35 % from fat, 20-25% from protein, 350 mmol/day of cholesterol,

and 3g/day of salt) for the entirety of the study. The participants were weighed once per week at the beginning of each dietary session, and were required to stay within 5% of their study entry body weight throughout the study. The participants completed a 7-day food record to ensure they were adhering to the diet. Participant's diet records were analyzed using the Nutritionist IV software.

All participants using antihypertensive medications were tapered off of their medications during the 6-week dietary period with written approval from their physician. During each dietary session, blood pressure was measured on each participant to ensure they stayed within the required range of 120-159/ 80-99 mm Hg. Participants were instructed to measure their blood pressure several times per week outside of the dietary classes and to maintain a log of their blood pressure values. Weekly telephone calls were made to the participants to ensure that their blood pressure remained within the required range. Participants with SBP < 120 or >159 mm Hg and/or DBP <80 or >99 mm Hg consistently during the 6-week dietary period were excluded from the study.

Baseline Testing

Casual Blood Pressure

Casual blood pressures were determined in all participants on three separate baseline testing days. Participants were instructed to sit for 15 minutes with their feet flat on the floor. Three blood pressure measurements were recorded on each arm within two minutes of each other. The average of the casual blood pressure measurements taken on three separate baseline testing days were used as the primary

outcome variable in the data analysis. In the event that casual blood pressure measurements were only taken on two separate baseline testing days, these values were averaged and used as the primary outcome variable in the data analysis.

24-hour Urine Collection

Participants underwent a 24-hour urine collection to measure Na^+ and K^+ excretion. The participants were given 2 urine collection bottles in which to collect their urine over the 24-hour period, starting the morning they picked up the containers (7am-9am) and ending after their first urination of the morning. They were also given a cooler filled with ice to keep the urine cold. Participants returned the urine containers the following morning. The urine was processed at the Hypertension and Exercise Physiology Laboratory and sent to Quest Diagnostics Laboratories for analysis (CLIA license 21D0218877).

Maximal Oxygen Consumption (VO_2max)

To develop an exercise prescription specific for each participant and to assess their cardiovascular fitness, participants performed a physician supervised VO_2max test. Participants began exercising on a treadmill at an intensity equivalent to 70% of the peak HR they achieved during their screening exercise test. Participants were fitted with a mouthpiece and a nose clip to collect the expired air necessary to measure oxygen consumption. The grade was increased 2% every 2 minutes, and ECG, HR and blood pressure were measured every 2 minutes during the test and every 2 minutes for 6 minutes after the test. The test was stopped when participants could no longer continue or upon onset of cardiovascular signs and/or symptoms (2). Oxygen uptake was measured using a computerized on-line VO_2 system including a

gas analyzer (Mass Spectrometer MGA-1100, Marquette Electronics Inc., Milwaukee, WI) and a bi-directional turbine flow meter (Ventilation Measurement Module VMM-2, Interface Associates, Aliso Viejo, CA). VO_2max was measured continuously, and to ensure that a true VO_2max was achieved two of the three criteria, ($\text{RER} > 1.1$, $\text{HR} > [220-\text{age}]$, and <150 ml/min increase in VO_2 during the last two minutes of the test), were met.

Body composition

Changes in percent body fat may act as a confounding variable when measuring the independent effect of exercise training on blood pressure and sodium excretion (9; 46-49; 78; 129). Therefore, participants were transported to the Baltimore VA Medical Center to undergo dual energy X-ray absorptiometry (DEXA) to assess any body composition changes that occurred during the 6-month exercise training period. Participants were instructed to fast for the 12 hours prior to the start of the test.

Measurement of Aldosterone

Blood samples for the measurement of plasma aldosterone were collected before the start of the oral glucose tolerance test (OGTT). Participants were instructed to undergo a 12-hour fast prior to blood sample collection, and because cold and pain medications such as antihistamines, ibuprofen, and acetaminophen, can alter aldosterone levels, participants were instructed to exclude these items for the 48 hours before the OGTT (17). Aldosterone levels also vary by body position, therefore the blood samples were collected while the participants were supine for approximately 20 minutes (142). Blood samples were collected in EDTA (ethylene diamine tetra-acetic

acid) tubes and centrifuged at 3000 rpm for 20 minutes at 4°C, and the plasma was aliquoted into 1.5 ml microtubes and stored at -20°C (85).

Plasma aldosterone levels were measured by a competitive binding radioimmunoassay (RIA). The RIA is based on competition between aldosterone in the sample, and a fixed amount of the aldosterone labeled with the radioisotope ¹²⁵I to bind to a limited quantity of the antibody specific for aldosterone (3). Once the system has reached equilibrium, free aldosterone (both labeled and unlabeled) is separated from the aldosterone that is bound to the antibody (3). The number of counts of ¹²⁵I labeled aldosterone is measured with the use of a gamma counter (3). The greater the number of counts of ¹²⁵I, the lower the aldosterone concentration is in the sample, whereas the lower the number of counts of ¹²⁵I labeled, the greater the aldosterone concentration is in the sample.

The ¹²⁵I kit used to measure plasma aldosterone levels in each sample was a 100 tube Coat-A-Count Aldosterone kit manufactured by Diagnostic Products Corporation. The Coat-A-Count procedure is a solid phase radioimmunoassay, in which the antibodies specific for aldosterone are immobilized to the wall of a polypropylene tube (DPC). Four plain polypropylene tubes, in which there were no antibodies attached to the wall of the tubes, were labeled NSB (non-specific binding) in duplicate and T (total activity) in duplicate. The NSB tubes measured non-specific non-immunological binding of ¹²⁵I and the T tubes measured total activity of the ¹²⁵I. Fourteen coated tubes were designated for the calibrators (standards) in which there were 7 known aldosterone concentrations (A-G) that were run in duplicate. The concentration (pg/ml) for calibrator A was 0, B was 25, C was 50, D was 100, E was

200, F was 600 and G was 1200. The remaining coated tubes were designated for participants' samples and controls, which were all run in duplicate.

The first step of the RIA required the reconstitution of the 7 calibrators, which required adding 6.0 ml of deionized water to the A calibrator. This was followed by adding 3.0 ml of deionized water to calibrators B through G. These steps were done approximately 30 minutes prior to use of the calibrators. During this same time period, 6.0 ml of deionized water was added to three controls. While the calibrators and the controls sat for 30 minutes, the samples were brought from the freezer to thaw. Once the calibrators had been reconstituted, 200 μ l of the zero calibrator A was pipeted into each NSB and A tube. This was followed by pipeting 200 μ l of the calibrators B through G into their respective tubes. Once all calibrators had been pipeted into their corresponding tubes, 200 μ l of each sample and control were also pipeted into their respective tubes. Approximately 10 minutes prior to all samples and controls being pipeted into their respective tubes, the ^{125}I was reconstituted by adding 110 ml of deionized water. Once the deionized water was added, the reconstituted ^{125}I was set aside for approximately 10 minutes. Prior to adding the ^{125}I , the vial was gently inverted to ensure thorough mixing.

After all the samples and controls had been added, 1.0 ml of the ^{125}I was added to every tube. It was required that this process be done within a 10 minute period. After the addition of the ^{125}I , each tube was capped and all tubes were vortexed. The samples then incubated for 18-20 hours at room temperature.

Following the incubation period, the caps were removed from the tubes and the liquid from each tube, except for the T tubes, was poured into the radioactive

waste container. This was followed by striking each tube against an absorbent pad, several times, to remove excess moisture. The tubes were then taken to the gamma counter (Beckman 5500) in which the number of counts in each tube was detected. Calculations were used to convert counts to concentrations. Plasma aldosterone levels were expected to be between 20-230 pg/ml (112).

Genotyping

Genotyping was performed at the Physiological Genomics Laboratory in the Department of Kinesiology at the University of Maryland, College Park. DNA was extracted from whole blood samples using techniques described by Miller (101). DNA amplification was performed by polymerase chain reaction (PCR), with the use of the CYP11B2 (-344T/C) forward primer 5'-AGG-GTG-TAC CTG TGT-CAG-GGC A-3' and the CYP11B2 (344T/C) reverse primer 5'-CCT-CTC-CTT-TCT-CCA-GGG-CTG A-3'. The PCR was performed at a denaturing temperature of 95°C for 5 minutes, followed by 35 cycles at 95°C for 30 seconds, 53°C for 30 seconds, 72°C for 30 seconds and 72°C for 5 minutes. Digestion was performed with the use of the Hae III enzyme and TT homozygotes were detected at 186 base pairs (bp), CT heterozygotes were detected at 186, 115, and 71 bp, and CC homozygotes were detected at 115 and 71 bp.

Exercise Training

Participants underwent a 6 month supervised aerobic exercise training intervention, which was held at the Wellness Research Laboratory. An exercise

prescription was developed for each participant by using the Karvonen formula, target $HR = [(max\ HR - resting\ HR) \times desired\ \%] + resting\ HR$. The participants were given a wrist heart rate monitor (Model 6124, Polar Electro, Canada), which allowed them to monitor their prescribed heart rate. Participants were expected to exercise 3 days a week throughout the 6 month period. Intensity and duration were increased gradually during the first few weeks of aerobic exercise training. During the first week of exercise training, the participants exercised for 20 minutes at 50% of heart rate reserve (HRR). Exercise duration was increased gradually by 5 minutes per week until the participants were exercising for 40 minutes. At the 6th week, exercise intensity was increased by 5% of their HRR every week until they were exercising at 70% of HRR. The increases in intensity and duration occurred only when participants completed their current intensity for three consecutive exercise sessions without any cardiovascular signs or symptoms. The gradual increase in exercise intensity and duration was used to prevent excessive fatigue and injury, which could reduce participant adherence (59). Seated blood pressures were measured at the beginning and end of each exercise training session. Blood pressure was measured once while participants were exercising. The participants recorded their blood pressure, heart rate, weight and exercise duration in a logbook provided for them. These log books were analyzed to ensure that the participants were adhering to their exercise prescription. Once the participants completed their 10th week of exercise training, they were asked to add 45-60 minutes of unsupervised exercise at <70% of HRR to their current exercise prescription.

Final Testing

Upon completion of the 6-month aerobic exercise training protocol, participants repeated all tests performed at baseline. This included casual blood pressure measurement, 24-hour urine collection, VO₂max, body composition, and plasma aldosterone measurement. The participants continued exercising until all final tests were completed. The final tests were performed after participants provided a 7-day food record to ensure dietary compliance, and 24-36 hours after their regular exercise session.

Appendix D – Aldosterone Radioimmunoassay Protocol

Materials Required

Materials	
100 polypropylene antibody coated tubes	Tubes coated with aldosterone specific antibodies. Allows for unlabeled and labeled aldosterone to bind to the side of the tube.
4 uncoated polypropylene tubes	Tubes used to measure non-specific immunological binding of aldosterone and the total counts of aldosterone.
7 Aldosterone calibrators A = 0 pg/ml E = 200 pg/ml B = 25 pg/ml F = 600 pg/ml C = 50 pg/ml G = 1200 pg/ml D = 100 pg/ml	Calibrators are 7 known concentrations of aldosterone, which are necessary to determine the standard curve that is used to identify the aldosterone concentrations of the unknown samples.
¹²⁵ I Aldosterone	Aldosterone labeled with an ¹²⁵ I tracer. It competes with an unknown amount of aldosterone in the samples to bind to antibodies located on the inside of the tubes.
Tri-level, human serum-based immunoassay controls	Three known aldosterone concentrations that are run in each assay as quality controls.
Deionized water	Used to reconstitute the calibrators and the ¹²⁵ I Aldosterone
Gamma counter	Used to measure ¹²⁵ I counts in each tube. The number of counts is used to calculate aldosterone concentration in the samples.
100 µl and 1000 µl Gilson pipettes 10 ml and 50 ml graduated cylinders	The 100 µl and 1000 µl pipettes are used for the measurement of samples, calibrators, controls and ¹²⁵ I Aldosterone. Graduated cylinders used to measure deionized water.

Reagent Preparation

Reagent	Storage	Procedures
Aldosterone calibrators	Not reconstituted: 4°C refrigerator Reconstituted: -20 °C freezer	6.0 ml of deionized water to calibrator A 3.0 ml of deionized water to calibrators B-G
Tri-level controls	Not reconstituted: 4°C refrigerator Reconstituted: -20 °C freezer	6.0 ml of deionized water to each control
¹²⁵ I Aldosterone	Not reconstituted: 4°C refrigerator Reconstituted: 4°C refrigerator	110 ml of deionized water to vial

Protocol

1. Preparation

- A.** Organize tube set up on spread sheet. A second spread sheet is needed to organize tube placement for the gamma counter.
- B.** Thaw samples.
- C.** Reconstitute calibrators 30 minutes prior to use. Add 6.0 ml of deionized water to calibrator A and 3.0 ml of deionized water to calibrators B-G.
- D.** Reconstitute tri-level controls 30 minutes prior to use. Add 3.0 ml of deionized water to controls 4, 5 and 6.
- E.** Label 2 tubes NSB and 2 tubes T. Label 7 tubes A-G in duplicate. Label the remaining tubes with the appropriate sample and controls numbers in duplicate. Place the tubes in the appropriate order in the tube rack.

2. Addition of Calibrators, Samples and Controls

- A. Pipet 200 μ l of calibrator A, which has zero concentration, into each NSB tube.
- B. Pipet 200 μ l of calibrator A into each A tube.
- C. Pipet 200 μ l of each of the remaining calibrators, B-G, into their respective tubes.
- D. Pipet 200 μ l of the samples and controls into their respective tubes.

3. ^{125}I Aldosterone Preparation and Addition

- A. Reconstitute the ^{125}I Aldosterone by adding 110 ml of deionized water to the vial. Let it stand for 10 minutes prior to use.
- B. Once calibrators, samples, and controls have been added, pipet 1000 μ l of the reconstituted ^{125}I Aldosterone into every tube. This must be done within a 10 minute period.
- C. Place tops on all tubes and vortex each tube.

4. Incubation and Decanting

- A. Incubate tubes for 18-20 hours at room temperature (15-28 $^{\circ}\text{C}$).
- B. All visible moisture must be removed from each tube **except** for the T tubes. Remove the tops from each tube one at a time and dump contents into the radioactive waste container. Vigorously strike the tubes on absorbent pads to remove all remaining moisture.

5. Reading ^{125}I Counts with the Gamma Counter

- A. The tubes must be placed into the gamma counter within 2- 3 hours after decanting.
- B. After decanting the tops of each tube must be placed back on.

C. Transfer tubes to the appropriate gamma counter racks, by referring to the gamma counter placement spreadsheet.

D. Place each rack in the gamma counter and switch the gamma counter to automated. Each tube will be counted for 1 minute.

6. Converting Counts to Concentration

A. To obtain the net counts for each tube, the average counts per minute of the duplicates must be obtained. Net counts = average counts per minute – average NSB counts.

B. To obtain the Percent ^{125}I bound to the tubes the net counts for tube A, also known as the maximum binding tube must be used.

Percent bound = (Net counts \div Net MB counts) x 100

C. Take the natural log (LN) of the known concentrations for calibrators B-G.

D. Take the logit (% bound/1-% bound) of the % bound of all tubes.

E. Run a regression with the logit of the % bound and the LN for calibrators B-G.

The logit of the % bound will be the predictor and the LN for the calibrator concentrations will be the dependent variable.

F. Obtain the slope (b) and the intercept (a) from the regression. The regression equation will be: $y = a + b(x)$, where x will be LN (% bound/1-% bound) for all of the samples and controls.

G. The sum of the regression equation will be in log form therefore the inverse log of the sum must be performed in order to obtain the aldosterone concentration into pg/ml.

Appendix E – Human Subjects Approval and Consent Form



UNIVERSITY OF
MARYLAND

INSTITUTIONAL REVIEW BOARD

2000 University
College Park, Maryland 20742-0001
301.405.2111 • 301.405.4577

Reference: IRB HSR Identification Number: 04-0105

March 8, 2004

MEMORANDUM

Notice of Results of Final Review by IRB on HSR Application

TO: Dr. Michael Brown
Ms. Jennifer James
Department of Kinesiology

FROM: Dr. Phyllis Maser-Valtion, Co-Chairperson
Dr. Marc Rogers, Co-Chairperson
Institutional Review Board

PROJECT ENTITLED:

Influence of the CYP11B2 - 344T/C polymorphism on Plasma
Aldosterone, Sodium Excretion and Blood Pressure Responses to
Long-term Aerobic Exercise Training in Middle-aged Hypertensives*

The Institutional Review Board (IRB) concurs with the departmental Human Subjects Review Committee's (HSRC's) preliminary review of the application concerning the above referenced project. The IRB has approved the application and the research involving human subjects described therein. We ask that any future communications with our office regarding this research reference the IRB HSR identification number indicated above.

We do ask that you not make any changes to the approved protocol without first notifying and obtaining the approval of the IRB. Also, please report any deviations from the approved protocol to the Chairperson of your departmental HSRC. If you have any questions or concerns, please do not hesitate to contact us at irb@dcans.umd.edu. Thank you.

ADDITIONAL INFORMATION REGARDING IRB/HSRC APPROVALS

EXPIRATION OF IRB APPROVAL. Approval of non-exempt projects expires one year after the official date of IRB approval; approval of exempt projects expires three years after that date. If you expect to be collecting or analyzing data after the expiration of IRB approval, please contact the HSRC Chairperson in your department about submitting a renewal application. **(PLEASE NOTE: If you are not collecting data from human subjects and any on-going data analysis does not increase the risk to subjects, a renewal application would not be necessary.)**

STUDENT RESEARCHERS—Unless otherwise requested, the IRB will send copies of approval paperwork to the supervising faculty researcher (or advisor) of a project. We ask that such persons pass on that paperwork or a copy to any student researchers working on that project. That paperwork may be needed by students in order to apply for graduation. **PLEASE BE ADVISED THAT THE IRB MAY NOT BE ABLE TO PROVIDE COPIES OF THAT PAPERWORK, particularly if several years have passed since the date of the original approval.**

Enclosures (where appropriate) will include stamped copy of informed consent forms included in application and, if appropriate, application submitted by the IRB; copies of this memorandum and any consent forms to be sent to the Chairperson of the Human Subjects Review Committee.

CONSENT TO ACT AS A SUBJECT IN AN EXPERIMENTAL STUDY

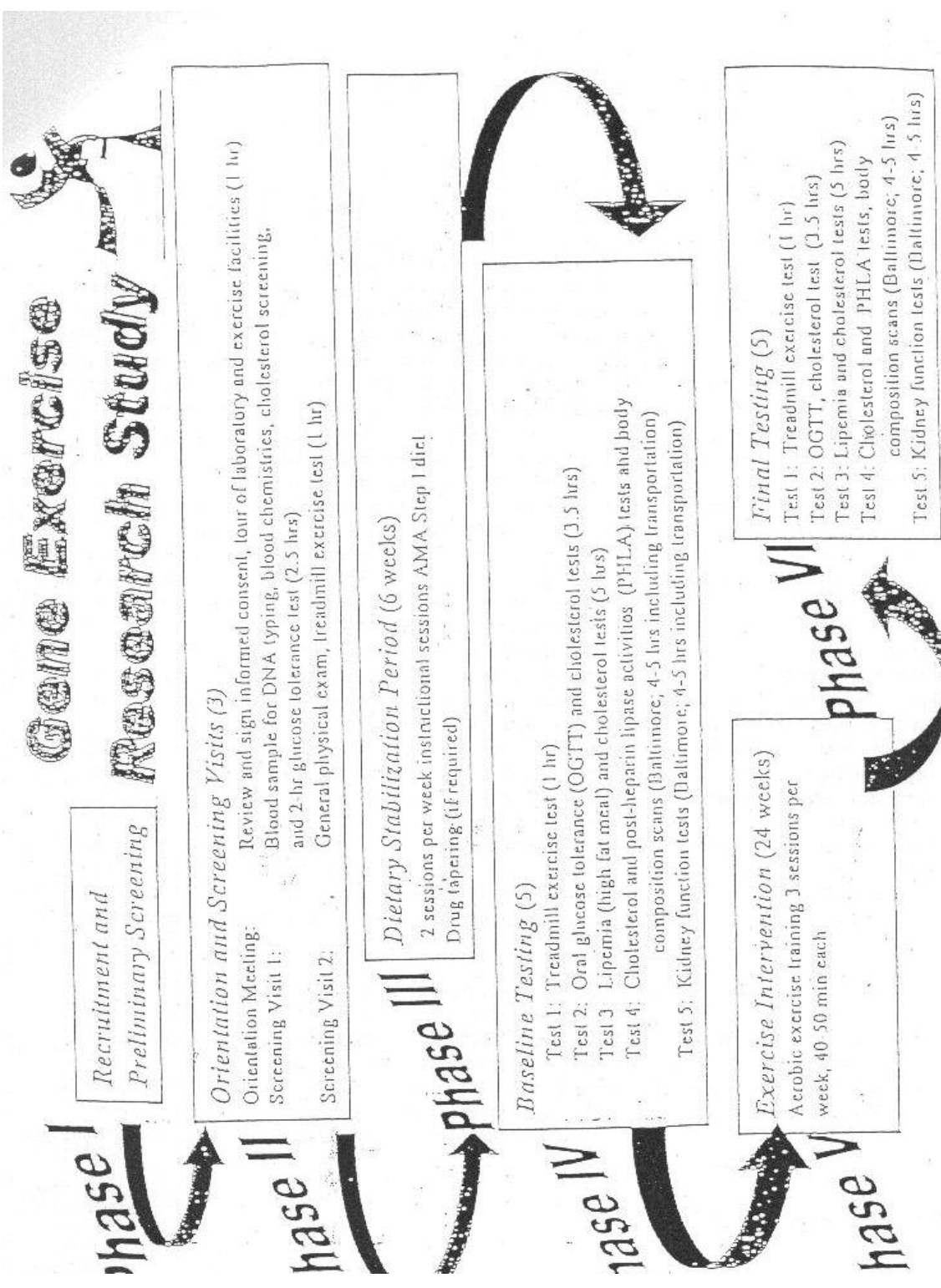
Project Title: **ACE genotype, blood pressure, and exercise training in hypertensives**

I state that I am over 18 years of age and wish to participate in a program of research being conducted by Dr. James Hagberg in the Department of Kinesiology, University of Maryland.

The purpose of this study is to determine the role that genetics may play in determining how my blood pressure changes with exercise training. This research project will require visits to University of Maryland College Park and the Baltimore VA Medical Center. The specific tests, their requirements, and time commitments are described below.

I already completed a telephone interview that determined that I am not physically active, 50 - 75 years of age, not a diabetic, have no evidence of lung disease, have an appropriate body weight for my height, and have no other medical problems that would keep me from exercising vigorously. It is also probable that I have a blood pressure that is in the Prehypertensive or Stage 1 hypertension range (Systolic blood pressure: 120 – 159; Diastolic blood pressure: 80 – 99). Furthermore, if I am a woman, I must be postmenopausal, defined as no menstrual cycles for at least the last 2 years. I understand that if I am a woman and change my hormone replacement therapy regimen during the study, my participation in the study will be terminated.

The Flow Chart on the next page indicates the different testing sessions and time required by this study. I understand that if I qualify and complete this study my total involvement will last approximately 9 months. Two of the testing visits are performed at the Baltimore VA Medical Center. I understand that I will also be asked to sign a University of Maryland Baltimore consent form for the tests conducted in Baltimore.



I understand that I will complete 1 Orientation and 2 initial screening visits. For my first visit, the study will be explained to me, my medical history will be reviewed, and I will provide my written informed consent. This visit will last about 60 minutes. On my first screening visit, I will report to the laboratory in the morning after an overnight fast and a blood sample will be drawn for blood chemistries and for isolation of my DNA. I will have my height, weight, and blood pressure measured. I understand that I may be excluded from the study if this initial blood sample shows elevated levels of glucose or creatinine in my blood. A blood sample will also be drawn 2 hours after I drink a sugar solution. This visit will last about 2 ½ hours. I understand that a total of 3 tablespoons of blood will be drawn during this visit. I understand that I will be excluded from the study at this point if I have a low red blood cell count, evidence of kidney or liver disease, evidence of diabetes, or if my blood pressure is too high or too low.

I understand that if I remain qualified to this point, I will undergo a treadmill exercise test to determine if I have heart disease. A physical examination will precede the exercise test. I will have my resting blood pressure measured. I will then complete a test on an exercise treadmill where the treadmill speed and grade will increase every 3 minutes until I cannot continue or symptoms of heart disease develop. Blood pressure, heart rate, and electrocardiogram (electrical activity of my heart) will be recorded before, during, and after the test. During this test I will have a noseclip on my nose and I will breathe through a mouthpiece so that the air that I breathe out can be analyzed. I understand that this visit will last about 1 hour and that I will be excluded from the study at this point if I have evidence of heart disease.

I understand that if I meet these requirements to enter the study and if I am taking medications to control my blood pressure, I give my permission for my private physician to be contacted to obtain their approval for me to stop taking these medications for the remainder of this study. I understand that I will be slowly withdrawn from these medications according to the plan my physician provides and that my blood pressure will be measured weekly for the remainder of the study. I also understand that if my blood pressure is too high (Systolic blood pressure: >159; Diastolic blood pressure: >99) for three consecutive weeks at any time during the study, I will be excluded from further participation in the study and referred back to my physician. If I am in the exercise training portion of the study, I understand that if this happens I will complete all Final Testing (see below) and then be referred back to my physician. I also understand that a physician from the University of Maryland School of Medicine is directly involved in this study and that he can be contacted for any medical questions, but only as they concern my involvement in this study.

I understand that if I meet these requirements to enter the study, I will undergo 6 weeks of instruction in the principles of an American Heart Association low-fat and low salt diet and must follow this diet for the remainder of this study. This program consists of two 40 minute classes each week for the 6 week duration of the program. During the final 3 weeks of this dietary program, I understand that I will have my blood pressure measured weekly for 3 weeks. I understand that my blood pressure must average in the range of 120 – 159 for systolic or 80 – 99 for diastolic blood pressure for me to continue in the study.

After this I will undergo Baseline Testing that includes the following tests that will be completed in 7 testing sessions (5 at the University of Maryland, College Park and 2 at the Baltimore VA Medical Center). I will have blood drawn on 2 occasions from a vein in my arm in the morning after an overnight fast to measure my cholesterol levels; these visits will each last about 20 minutes. I understand that a maximum of 2 tablespoons of blood will be drawn during these visits. I understand that I will also undergo a second exercise test on a treadmill to measure my cardiovascular fitness. This test will start at 70% of the highest heart rate achieved on my first exercise test and the treadmill grade will increase by 2% every 2 minutes. Blood pressure, heart rate, and electrocardiogram will be monitored before, during, and after the test. The test will be stopped when I can no longer continue. During this test I will have a noseclip on my nose and I will breathe through a mouthpiece so that the air that I breathe out can be analyzed. I understand that this visit will last about 1 hour. I also understand that my dietary habits will be measured by having me record for 7 days all of the food items that I eat. I understand that I will collect my urine for 24 hours in a container that must be refrigerated so that the amount of salt I eat in my diet can be measured; I also understand that my blood pressure will be monitored throughout this 24 hour period with a cuff around my upper arm and a “Walkman-size” controller worn at my waist. I also understand that I will undergo a 3 hour glucose tolerance test where I will come to the laboratory in the morning after an overnight fast, have a small catheter inserted in an arm vein for blood sampling, and have blood samples drawn before and for every 30 minutes after I drink a glucose solution. Additional samples will be drawn before this test to measure hormone levels in my blood that affect my blood pressure, immunological (disease-fighting), and blood clotting systems. I understand that a maximum of 7 tablespoons of blood will be drawn for this portion of the study. I understand that on another occasion after an overnight fast, I will have blood samples drawn before and every 30 minutes for 4 hours after drinking 1- 2 cups of a high-fat liquid meal. These blood samples also will be drawn through a small catheter inserted into my arm vein. The high-fat meal is made of heavy whipping cream with small amounts of chocolate, sugar, and powdered milk and tastes like a rich chocolate shake. I understand that 10 tablespoons of blood will be drawn during this test and will be used to measure how my body absorbs and uses fat from a meal and how my blood clotting, and substances that affect hunger are affected by a fat meal. Before and after I drink the high-fat meal, I understand that I will breath through a mouthpiece while my nose is closed-off with a nose clip and the air that I breath out will be collected and used to determine how much fat I use for energy while sitting at rest. I also understand that I will complete a test that takes about 1 hour to measure the blood flow in my arm at rest and immediately after stopping my arm blood flow for 5 minutes with the use of a blood pressure cuff. I understand that all of these tests listed above will be done at the University of Maryland College Park.

I understand that on a visit to Baltimore on a separate day, I will have my kidney function measured at the Clinical Research Unit, Division of Nephrology, University of Maryland at Baltimore after an overnight fast. Before the test, I will drink 17 ounces of water over a 30-minute period. A Registered Nurse will then insert a small needle into veins in both of my arms. One line will be used to give the study

medications and the other will be used to draw blood samples. Before the study drugs are given, I will provide a urine sample and a 0.7 ounce blood sample. During the test I will remain in a seated position except for when I provide urine samples.

Next, I will receive the study medications, para-aminohippurate and iothalamate, which are markers used for estimating kidney function. Para-aminohippurate (5mg/kg body weight) and iothalamate (434 mg) will be given over five minutes. Then I will receive an additional small dose of para-aminohippurate and iothalamate by a slow, continuous infusion so that I will have the necessary amounts in my blood. Four blood samples (~0.7 ounces) will be drawn over the next 2½ hours and I will be asked to collect my urine every ½ hour for the next 2½ hours. This test will take approximately 3 hours. A total of 1.4 ounces of blood will be drawn during this test. I understand that I will undergo this test twice, once before and once after 6 months of aerobic exercise training. I understand that the amount of fat I have around my waist will be measured with a CAT scan while I lie quietly on a table. Another study will be done to measure my total body fat mass and total body muscle mass while I lie quietly on a table. I also understand that if I have elevated blood cholesterol levels, I will have blood samples drawn before and after a substance that temporarily stops blood from clotting is injected into my arm vein. The blood samples will be used to measure chemicals that affect my blood cholesterol levels. A total of 4 tablespoons of blood will be drawn at this visit. I understand that I will remain in the VA Medical Center for 2 – 3 hours after this test to make sure that all bleeding is stopped. I also understand that these tests will be done at the VA Medical Center in Baltimore. I understand that each of these visits will require approximately 4 – 5 hours including travel time.

I understand that the maximum total amount of blood that will be drawn during this Screening and Baseline Testing is 28 tablespoons over 2 – 3 months. This is approximately 90% of the amount of blood that is typically drawn during a single blood donation.

I understand that after completing this testing, for 6 months I will complete 3 exercise sessions each week supervised by study personnel. I understand that I will be instructed on appropriate warmup and stretching exercises to perform prior to each exercise training session. I will be taught to measure my heart rate and to use heart rate monitors to control how hard I am exercising. The first training sessions will consist of 20 minutes of light exercise. The amount of exercise and how hard I exercise will increase gradually until I am completing 40 minutes of moderate intensity exercise every session. Exercise modes include walk/jogging, stairstepping, and cycle, cross-country ski, and rowing ergometry. I will be asked to add a 45-60 minute walk to my exercise program on weekends after the first 10 weeks of the exercise program. I understand that this is not designed as a weight loss program and that if I lose more weight than expected from the amount of exercise that I complete, I will be counseled by a dietitian against restricting how much food I eat. I will also be asked to complete food records during the exercise training program and if major dietary changes have occurred, I will also be counseled by a dietitian to resume my

original dietary habits. I understand that I may also be asked to collect my urine for 24 hours during the exercise training portion of the study.

I understand that after completing 6 months of exercise training, I will have everything reevaluated that was measured before I began the exercise program. I understand that during this 4 weeks of Final Testing a maximum of 28 tablespoons of blood will be drawn; this is approximately 90% of the amount of blood that is typically drawn during a single blood donation.

I understand that if I qualify for this study that my DNA will be isolated from my blood and analyzed at a number of sites for differences in DNA that may affect how my blood pressure changes with exercise training. I understand that some of my DNA will also be frozen for future studies. However, these studies can only analyze my DNA at sites that might affect how my blood pressure, cholesterol levels, glucose and insulin levels, bone density, body composition, immunology (disease-fighting), and cardiovascular and blood clotting systems change with exercise training.

All information collected in this study is confidential, and my name will not be identified at any time. I understand that my DNA (genetic material) will be sent to collaborating genetics laboratories that are part of this study and that a sample of my DNA will be kept in the University of Maryland Department of Kinesiology laboratories. I also understand that samples of my blood will be sent to other collaborating laboratories for other blood measurements. I understand, however, that my DNA and blood samples sent to these laboratories will be identified only by a numeric code and that only investigators at the University of Maryland College Park will know whose name is associated with each coded number. I further understand that the list of names and codes will be retained at the University of Maryland College Park for up to 25 years.

I understand the following risks are associated with my participation in this study. (1) The risk of maximal exercise testing is approximately 1 nonfatal event in 10,000 tests and 1 fatal cardiac event in 70,000 tests. Risks will be minimized by having the test administered by a physician and personnel trained in such tests and emergency procedures. I will be screened with a resting electrocardiogram and a physical examination prior to this test. An emergency cart with the necessary drugs and a cardiac defibrillator will be present at all testing sessions. (2) There is minimal risk of bruising and infection associated with blood drawing. These risks will be minimized by using sterile techniques and by having experienced personnel draw all blood samples. (3) The risk of the body composition testing is the exposure to X-rays. The amount of X-ray exposure for this test is the same as that occurring during 30 minutes of any activity outside in the sun. (4) There is some risk associated with the elevated blood pressure that I have and some risk associated with stopping the medications I take to control my blood pressure. However, a 12 month lifestyle change program including diet and exercise is part of the medical recommendations for blood pressure control for individuals with levels of blood pressure similar to

mine. In addition, I understand that my blood pressure will be monitored weekly and that this exceeds the blood pressure follow-up guidelines recommended for physicians. I also understand that if my blood pressure is too high for three consecutive weeks anytime during the study, my participation in the study will be discontinued and I will be referred back to my private physician.

I also understand that a physician associated with this project is available to deal with concerns related to my participation in this study. (5) The risk associated with the CAT scan to measure abdominal fat is the exposure to X-rays. The X-ray exposure is less than the maximum radiation dose individuals are permitted to be exposed to each year in their occupation. (6) There are no risks associated with the 24 hour urine collection. (7) The only risks associated with the measurement of the hormones in my blood that affect my blood pressure are those associated with blood drawing. (8) The risks associated with the glucose tolerance test and high fat meal are those related again to blood sampling, the possibility that my blood sugar may go too low levels at the end of the test, and the possibility of an upset stomach, primarily a stomach ache, after drinking the glucose or high-fat meal. I understand that I will be given a juice drink and small snack to minimize the chances of my blood glucose levels decreasing too much. (9) There are no

risks associated with the genetic testing because I will not be provided with these results. (10) The risk of exercise training is the possibility of a heart attack or other cardiovascular event. A large physical activity center reported that 1 nonfatal cardiovascular event occurred in 1.7 million walk/jogging miles. These risks will be minimized because I will undergo a cardiovascular evaluation before beginning exercise training. Exercise sessions will be supervised by experienced personnel trained in emergency procedures. An emergency cart with the necessary drugs and a cardiac defibrillator will be present at all supervised exercise training sessions. (11) If I have elevated blood cholesterol levels, I understand that the risk associated with the test requiring the injection into an arm vein of a substance that temporarily stops blood clotting is bleeding. This risk will be minimized by excluding people with bleeding disorders, peptic ulcers, or other blood disorders from the study. The risk is further minimized by placing a bandage on the intravenous access site after the blood sampling and observing the subject for 2 – 3 hours. (12) The risks associated with the tests to assess kidney function are low as these are routine clinical tests. The risks are the side effects of the compounds put into my blood to assess kidney function; side effects include nausea, vomiting, facial flush, a generalized feeling of warmth, and allergic reactions. The risks are also those associated with blood drawing. These risks are minimized by administering these tests in a hospital setting with nurses experienced with these methods administering the test, so that if I should experience these side effects, medical personnel and equipment are readily available to respond and treat these symptoms. (13) The risks associated with the 24 hour blood pressure recording are the possibility of sleep disturbances in about 2% of volunteers. (14) The risks associated with the measurement of the blood flow in my arm are the result of stopping blood flow to the arm for 5 minutes. This causes substantial discomfort that ceases shortly after the blood pressure cuff is removed. I understand that if I can not tolerate the discomfort, this test will be terminated immediately on my request.

I understand that this study is not designed to help me personally, but may help the investigators to determine whom exercise might benefit the most. I understand that I am free to ask questions or to withdraw from participation at any time without penalty. I understand that I will earn \$50 at the completion of Baseline Testing after the dietary stabilization period. I also understand that I will earn another \$50 after 3 months of exercise training if I complete at least 90% of my exercise training sessions. I also understand that I will earn another \$100 after completing 90% of my training sessions for 6 months and all final testing. I understand that the total amount that I earn will be paid to me at the completion of my participation in the study. I understand that if my participation in the study has to be terminated because I change my hormone replacement therapy regimen, I will only be paid for the portion of the study that I have already completed, that is, which of the stages above that I have completed.

In the event of a physical injury resulting from participation in this study, I understand that immediate medical attention is available at the Washington Adventist Hospital or the Baltimore VA Medical Center. However, I understand that the University of Maryland does not provide any medical or hospitalization coverage for participants in this research study nor will the University of Maryland provide any compensation for any injury sustained as a result of participation in this research study except as required by law.

Principal Investigator: James Hagberg, PhD, Department of Kinesiology, HLHP Building, University of Maryland, College Park, MD 20742-2611, telephone 301-

405-2487.

Subject's signature

Date

Witness

Date

Investigator

Date

Appendix F – Statistical Tables

Descriptive Statistics for the Total Population

Descriptive Statistics

	N	Minimum	Maximum	Mean		Std.
	Statistic	Statistic	Statistic	Statistic	Std. Error	Statistic
AGE	66	50	75	58.03	.68	5.488
HGT	66	149.9	191.8	170.040	1.159	9.4131
BMI_B	61	19.80	38.20	28.7492	.4945	3.86181
BMI_F	39	21.10	37.90	27.7615	.5503	3.43677
MDRD	57	54.49	132.99	76.0515	1.8817	14.20664
SCR	61	.50	1.50	1.0131	.0253	.19788
TFAT_B	57	20.50	55.50	37.0088	1.1802	8.91021
TFAT_F	29	18.60	52.80	35.4414	1.7888	9.63278
LBW_B	57	32569.00	69315.00	48826.81	1422.9041	10742.69
LBW_F	31	33934.00	76286.00	50562.65	2325.9715	12950.46
SBP_B	57	110.70	162.50	131.2526	1.3878	10.47778
SBP_F	34	111.10	155.00	130.9912	1.9787	11.53767
DBP_B	53	72.40	102.00	85.2434	.8477	6.17157
DBP_F	33	70.30	99.70	83.8758	1.2142	6.97491
NA_B	60	29.00	300.00	111.7333	7.1606	55.46594
NA_F	37	49.00	365.00	124.2703	9.6818	58.89239
K_B	60	25.00	127.60	62.1350	3.1190	24.15958
K_F	37	35.40	149.00	68.1649	3.8332	23.31658
TV_B	60	638.00	3970.00	2001.8500	102.0043	790.12157
TV_F	37	590.00	4267.00	2196.8108	144.3167	877.84391
PA_B	61	6.00	233.00	82.2951	7.0718	55.23264
PA_F	37	18.00	132.00	75.0541	5.4572	33.19467
VO2REL_B	59	17.00	35.10	23.9475	.5860	4.50132
VO2REL_F	38	18.40	38.70	28.0716	.8856	5.45938
VO2AB_B	59	.99	3.68	2.0079	.0727	.55805
VO2AB_F	38	1.41	4.07	2.3274	.1074	.66231
WGT_B	61	53.60	110.90	83.6016	1.7297	13.50946
WGT_F	39	60.00	108.20	82.3641	2.1030	13.13319
Valid N (listwise)	19					

Effects of Aerobic Exercise Training in the Total Population

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	BMI_B	28.3923	39	3.58332	.57379
	BMI_F	27.7615	39	3.43677	.55032
Pair 2	TFAT_B	37.0138	29	9.31388	1.72954
	TFAT_F	35.4414	29	9.63278	1.78876
Pair 3	LBW_B	48797.03	29	12362.11545	2295.587
	LBW_F	49137.76	29	12052.79282	2238.147
Pair 4	SBP_B	131.8441	34	10.75992	1.84531
	SBP_F	130.9912	34	11.53767	1.97869
Pair 5	DBP_B	84.0576	33	6.51426	1.13399
	DBP_F	83.8758	33	6.97491	1.21418
Pair 6	NA_B	118.6757	37	54.84780	9.01692
	NA_F	124.2703	37	58.89239	9.68185
Pair 7	K_B	65.7189	37	25.17532	4.13880
	K_F	68.1649	37	23.31658	3.83322
Pair 8	TV_B	2104.3514	37	871.95875	143.34914
	TV_F	2196.8108	37	877.84391	144.31665
Pair 9	PA_B	94.7027	37	61.55705	10.11992
	PA_F	75.0541	37	33.19467	5.45717
Pair 10	VO2REL_B	24.5684	38	4.97682	.80735
	VO2REL_F	28.0716	38	5.45938	.88563
Pair 11	VO2AB_B	2.0824	38	.59258	.09613
	VO2AB_F	2.3274	38	.66231	.10744
Pair 12	WGT_B	84.2333	39	13.54154	2.16838
	WGT_F	82.3641	39	13.13319	2.10299

Paired Samples Test

	Paired Differences						t	df	Sig. (2-tailed)
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference					
				Lower	Upper				
Pair 1	BMI_B - BMI_F	.6308	1.17050	.18743	.2513	1.0102	3.365	38	.002
Pair 2	TFAT_B - TFAT_F	1.5724	2.31746	.43034	.6909	2.4539	3.654	28	.001
Pair 3	LBW_B - LBW_F	-340.7241	1732.66609	321.74802	-999.7951	318.3468	-1.059	28	.299
Pair 4	SBP_B - SBP_F	.8529	7.99050	1.37036	-1.9351	3.6410	.622	33	.538
Pair 5	DBP_B - DBP_F	.1818	5.83376	1.01553	-1.8867	2.2504	.179	32	.859
Pair 6	NA_B - NA_F	-5.5946	42.82293	7.04005	-19.8725	8.6833	-.795	36	.432
Pair 7	K_B - K_F	-2.4459	25.39045	4.17416	-10.9115	6.0197	-.586	36	.562
Pair 8	TV_B - TV_F	-92.4595	667.46476	109.73053	-315.0033	130.0844	-.843	36	.405
Pair 9	PA_B - PA_F	19.6486	55.43576	9.11358	1.1654	38.1319	2.156	36	.038
Pair 10	VO2REL_B - VO2REL_F	-3.5032	2.54691	.41316	-4.3403	-2.6660	-8.479	37	.000
Pair 11	VO2AB_B - VO2AB_F	-.2450	.21304	.03456	-.3150	-.1750	-7.089	37	.000
Pair 12	WGT_B - WGT_F	1.8692	3.33515	.53405	.7881	2.9504	3.500	38	.001

Regression in the Total Population

Predictor: change plasma aldosterone (PA)

Dependent variable: change in SBP

Descriptive Statistics

	Mean	Std. Deviation	N
CHGSBP	-.6774	8.20958	31
CHGPA	-25.2903	58.01046	31

Correlations

		CHGSBP	CHGPA
Pearson Correlation	CHGSBP	1.000	.031
	CHGPA	.031	1.000
Sig. (1-tailed)	CHGSBP	.	.433
	CHGPA	.433	.
N	CHGSBP	31	31
	CHGPA	31	31

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.031 ^a	.001	-.033	8.34579

a. Predictors: (Constant), CHGPA

ANOVA^b

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	2.000	1	2.000	.029	.867 ^a
	Residual	2019.915	29	69.652		
	Total	2021.914	30			

a. Predictors: (Constant), CHGPA

b. Dependent Variable: CHGSBP

Predictor: change in plasma aldosterone

Dependent variable: change in DBP

Descriptive Statistics

	Mean	Std. Deviation	N
CHDBP	.1267	5.91409	30
CHGPA	-26.2333	58.75999	30

Correlations

		CHDBP	CHGPA
Pearson Correlation	CHDBP	1.000	-.241
	CHGPA	-.241	1.000
Sig. (1-tailed)	CHDBP	.	.100
	CHGPA	.100	.
N	CHDBP	30	30
	CHGPA	30	30

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.241 ^a	.058	.024	5.84211

a. Predictors: (Constant), CHGPA

ANOVA^b

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	58.673	1	58.673	1.719	.200 ^a
	Residual	955.646	28	34.130		
	Total	1014.319	29			

a. Predictors: (Constant), CHGPA

b. Dependent Variable: CHDBP

Predictor: change in plasma aldosterone
Dependent variable: change in 24-hour Na⁺ excretion

Descriptive Statistics

	Mean	Std. Deviation	N
CHGNA	5.8235	42.65895	34
CHGPA	-22.0882	56.85994	34

Correlations

		CHGNA	CHGPA
Pearson Correlation	CHGNA	1.000	-.214
	CHGPA	-.214	1.000
Sig. (1-tailed)	CHGNA	.	.112
	CHGPA	.112	.
N	CHGNA	34	34
	CHGPA	34	34

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.214 ^a	.046	.016	42.31880

a. Predictors: (Constant), CHGPA

ANOVA^b

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	2744.743	1	2744.743	1.533	.225 ^a
	Residual	57308.198	32	1790.881		
	Total	60052.941	33			

a. Predictors: (Constant), CHGPA

b. Dependent Variable: CHGNA

Ethnic Differences Independent t-test

Group Statistics

	1=cauc; 2=AD	N	Mean	Std. Deviation	Std. Error Mean
AGE	1	33	58.36	5.846	1.018
	2	28	57.36	5.215	.986
HGT	1	33	172.728	10.0371	1.7472
	2	28	166.571	8.2479	1.5587
BMI_B	1	31	28.6484	4.13738	.74310
	2	25	29.2760	3.66507	.73301
BMI_F	1	23	28.6739	3.54121	.73839
	2	13	26.9462	3.00600	.83371
MDRD	1	31	75.3135	11.79692	2.11879
	2	26	76.9313	16.84087	3.30277
SCR	1	31	.9871	.17840	.03204
	2	26	1.0577	.20430	.04007
TFAT_B	1	32	35.7063	9.15807	1.61893
	2	20	40.0300	8.50047	1.90076
TFAT_F	1	16	33.5000	7.78486	1.94621
	2	11	39.9455	10.57964	3.18988
LBW_B	1	32	50741.03	12153.69697	2148.490
	2	20	45916.30	9113.49231	2037.839
LBW_F	1	17	54263.29	13389.73938	3247.489
	2	12	45736.50	12192.49608	3519.670
SBP_B	1	31	132.1581	10.31997	1.85352
	2	21	129.4810	9.90735	2.16196
SBP_F	1	21	130.5762	9.15319	1.99739
	2	10	134.0800	13.40786	4.23994
DBP_B	1	28	84.9214	7.09002	1.33989
	2	20	86.0550	4.91630	1.09932
DBP_F	1	21	83.8095	7.86619	1.71654
	2	9	84.0222	6.08169	2.02723
NA_B	1	31	114.1290	57.70254	10.36368
	2	25	108.8400	55.96404	11.19281
NA_F	1	22	131.9091	68.97750	14.70605
	2	13	117.5385	39.81963	11.04398

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
AGE	Equal variances assumed	.130	.720	.704	59	.484	1.01	1.430	-1.855	3.868
	Equal variances not assumed			.710	58.835	.480	1.01	1.417	-1.829	3.842
HGT	Equal variances assumed	3.541	.065	2.587	59	.012	6.156	2.3796	1.3949	10.9180
	Equal variances not assumed			2.629	58.950	.011	6.156	2.3414	1.4711	10.8418
BMI_B	Equal variances assumed	.025	.875	-.593	54	.555	-.6276	1.05762	-2.74801	1.49279
	Equal variances not assumed			-.601	53.486	.550	-.6276	1.04379	-2.72075	1.46552
BMI_F	Equal variances assumed	.067	.797	1.481	34	.148	1.7278	1.16660	-.64305	4.09857
	Equal variances not assumed			1.551	28.608	.132	1.7278	1.11369	-.55135	4.00687
MDRD	Equal variances assumed	.803	.374	-.425	55	.672	-1.6178	3.80594	-9.24504	6.00950
	Equal variances not assumed			-.412	43.651	.682	-1.6178	3.92397	-9.52780	6.29225
SCR	Equal variances assumed	.002	.969	-1.393	55	.169	-.0706	.05069	-.17218	.03099
	Equal variances not assumed			-1.376	50.120	.175	-.0706	.05130	-.17364	.03244
TFAT_B	Equal variances assumed	.173	.680	-1.702	50	.095	-4.3238	2.54085	-9.42720	.77970
	Equal variances not assumed			-1.732	42.770	.091	-4.3238	2.49677	-9.35974	.71224
TFAT_F	Equal variances assumed	2.255	.146	-1.827	25	.080	-6.4455	3.52798	-13.71148	.82057
	Equal variances not assumed			-1.725	17.238	.102	-6.4455	3.73672	-14.32096	1.43005
LBW_B	Equal variances assumed	5.213	.027	1.525	50	.133	4824.7312	3163.1244	-1528.59	11178.05
	Equal variances not assumed			1.629	48.208	.110	4824.7312	2961.2156	-1128.53	10777.99
LBW_F	Equal variances assumed	.142	.710	1.751	27	.091	8526.7941	4869.5746	-1464.75	18518.34
	Equal variances not assumed			1.781	25.163	.087	8526.7941	4788.9731	-1333.04	18386.62
SBP_B	Equal variances assumed	.049	.825	.933	50	.356	2.6771	2.87061	-3.08867	8.44290
	Equal variances not assumed			.940	44.264	.352	2.6771	2.84774	-3.06116	8.41539
SBP_F	Equal variances assumed	4.925	.034	-.856	29	.399	-3.5038	4.09454	-11.87807	4.87046
	Equal variances not assumed			-.748	13.147	.468	-3.5038	4.68686	-13.61769	6.61007
DBP_B	Equal variances assumed	1.777	.189	-.616	46	.541	-1.1336	1.83977	-4.83683	2.56969
	Equal variances not assumed			-.654	45.978	.516	-1.1336	1.73315	-4.62226	2.35512
DBP_F	Equal variances assumed	.416	.524	-.072	28	.943	-.2127	2.94838	-6.25218	5.82678
	Equal variances not assumed			-.080	19.562	.937	-.2127	2.65635	-5.76171	5.33631
NA_B	Equal variances assumed	.000	.983	.346	54	.731	5.2890	15.30500	-25.39565	35.97371
	Equal variances not assumed			.347	52.136	.730	5.2890	15.25401	-25.31848	35.89655
NA_F	Equal variances assumed	.977	.330	.684	33	.499	14.3706	21.00214	-28.35855	57.09981
	Equal variances not assumed			.781	32.999	.440	14.3706	18.39123	-23.04667	51.78793

Group Statistics

	1=cauc; 2=AD	N	Mean	Std. Deviation	Std. Error Mean
K_B	1	31	70.2290	23.21049	4.16873
	2	25	50.8040	22.17180	4.43436
K_F	1	22	68.8909	19.87396	4.23714
	2	13	69.1000	30.02788	8.32823
TV_B	1	31	2154.5484	830.14400	149.09826
	2	25	1828.1600	753.87873	150.77575
TV_F	1	22	2395.3636	893.83372	190.56599
	2	13	1957.3846	744.22975	206.41219
PA_B	1	28	106.6071	64.18107	12.12908
	2	28	62.2143	36.79710	6.95400
PA_F	1	20	81.4000	31.02868	6.93822
	2	14	61.2143	33.41851	8.93147
VO2REL_B	1	30	24.8167	5.01694	.91596
	2	24	22.2417	3.04301	.62115
VO2REL_F	1	22	29.2414	5.12297	1.09222
	2	13	25.8536	5.79773	1.60800
VO2AB_B	1	30	2.1559	.66651	.12169
	2	24	1.8026	.37370	.07628
VO2AB_F	1	22	2.5360	.66099	.14092
	2	13	2.0280	.62367	.17297
CHGSBP	1	21	-2.8857	6.17003	1.34641
	2	10	3.6000	9.93714	3.14240
CHDBP	1	21	-.2857	6.04998	1.32021
	2	9	-1.2778	5.36884	1.78961
CHGNA	1	22	9.0000	39.00916	8.31678
	2	13	3.5385	43.97464	12.19637
CHGPA	1	20	-37.6000	65.02421	14.53985
	2	14	-2.3571	29.94766	8.00385

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
K_B	Equal variances assumed	2.824	.099	3.176	54	.002	19.4250	6.11666	7.16187	31.68820
	Equal variances not assumed			3.192	52.415	.002	19.4250	6.08620	7.21448	31.63558
K_F	Equal variances assumed	.745	.394	-.025	33	.980	-.2091	8.41930	-17.33828	16.92010
	Equal variances not assumed			-.022	18.315	.982	-.2091	9.34414	-19.81621	19.39803
TV_B	Equal variances assumed	.471	.496	1.523	54	.134	326.3884	214.28068	-103.219	755.99528
	Equal variances not assumed			1.539	53.194	.130	326.3884	212.04626	-98.88627	751.66304
TV_F	Equal variances assumed	.234	.632	1.486	33	.147	437.9790	294.73171	-161.657	1037.615
	Equal variances not assumed			1.559	29.096	.130	437.9790	280.92951	-136.504	1012.462
PA_B	Equal variances assumed	9.562	.003	3.175	54	.002	44.3929	13.98116	16.36233	72.42339
	Equal variances not assumed			3.175	43.019	.003	44.3929	13.98116	16.19754	72.58818
PA_F	Equal variances assumed	.004	.950	1.809	32	.080	20.1857	11.15825	-2.54290	42.91433
	Equal variances not assumed			1.785	26.757	.086	20.1857	11.30974	-3.02981	43.40123
VO2REL_B	Equal variances assumed	8.906	.004	2.208	52	.032	2.5750	1.16617	.23491	4.91509
	Equal variances not assumed			2.327	48.795	.024	2.5750	1.10672	.35073	4.79927
VO2REL_F	Equal variances assumed	.197	.660	1.801	33	.081	3.3877	1.88141	-.44001	7.21551
	Equal variances not assumed			1.743	22.848	.095	3.3877	1.94387	-.63493	7.41042
VO2AB_B	Equal variances assumed	11.165	.002	2.319	52	.024	.3533	.15236	.04762	.65908
	Equal variances not assumed			2.460	47.099	.018	.3533	.14362	.06444	.64226
VO2AB_F	Equal variances assumed	.245	.624	2.242	33	.032	.5080	.22657	.04709	.96901
	Equal variances not assumed			2.277	26.536	.031	.5080	.22311	.04988	.96621
CHGSBP	Equal variances assumed	.909	.348	-2.238	29	.033	-6.4857	2.89820	-12.41320	-.55823
	Equal variances not assumed			-1.897	12.419	.081	-6.4857	3.41870	-13.90661	.93518
CHDBP	Equal variances assumed	.045	.834	.425	28	.674	.9921	2.33605	-3.79313	5.77725
	Equal variances not assumed			.446	17.056	.661	.9921	2.22389	-3.69875	5.68288
CHGNA	Equal variances assumed	.494	.487	.382	33	.705	5.4615	14.30247	-23.63705	34.56013
	Equal variances not assumed			.370	22.922	.715	5.4615	14.76212	-25.08196	36.00504
CHGPA	Equal variances assumed	3.871	.058	-1.886	32	.068	-35.2429	18.68380	-73.30051	2.81480
	Equal variances not assumed			-2.123	28.442	.043	-35.2429	16.59726	-69.21699	-1.26873

Ethnic Differences: ANCOVA

Covariate: final BMI

Dependent Variable: final plasma aldosterone levels

Between-Subjects Factors

		N
1=cauc;	1	20
2=AD	2	13

Descriptive Statistics

Dependent Variable: PA_F

1=cauc; 2=AD	Mean	Std. Deviation	N
1	81.4000	31.02868	20
2	57.4615	31.56373	13
Total	71.9697	32.95971	33

Levene's Test of Equality of Error Variances^a

Dependent Variable: PA_F

F	df1	df2	Sig.
.242	1	31	.626

Tests the null hypothesis that the error variance of the dependent variable is equal across groups.

a. Design: Intercept+BMI_F+ETHN

Tests of Between-Subjects Effects

Dependent Variable: PA_F

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	7158.358 ^a	2	3579.179	3.890	.031	.206
Intercept	74.099	1	74.099	.081	.779	.003
BMI_F	2643.419	1	2643.419	2.873	.100	.087
ETHN	3164.050	1	3164.050	3.439	.074	.103
Error	27604.612	30	920.154			
Total	205691.000	33				
Corrected Total	34762.970	32				

a. R Squared = .206 (Adjusted R Squared = .153)

2. 1=cauc; 2=AD

Dependent Variable: PA_F

1=cauc; 2=AD	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
1	80.009 ^a	6.832	66.056	93.963
2	59.601 ^a	8.507	42.227	76.975

a. Covariates appearing in the model are evaluated at the following values: BMI_F = 27.6515.

Covariate: final 24-hour K⁺ excretion
Dependent Variable: final plasma aldosterone levels

Between-Subjects Factors

		N
1=cauc;	1	19
2=AD	2	13

Descriptive Statistics

Dependent Variable: PA_F

1=cauc; 2=AD	Mean	Std. Deviation	N
1	82.8947	31.13036	19
2	57.4615	31.56373	13
Total	72.5625	33.30789	32

Levene's Test of Equality of Error Variances^a

Dependent Variable: PA_F

F	df1	df2	Sig.
2.260	1	30	.143

Tests the null hypothesis that the error variance of the dependent variable is equal across groups.

a. Design: Intercept+K_F+ETHN

Tests of Between-Subjects Effects

Dependent Variable: PA_F

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	9340.944 ^a	2	4670.472	5.407	.010	.272
Intercept	3603.220	1	3603.220	4.171	.050	.126
K_F	4348.090	1	4348.090	5.034	.033	.148
ETHN	4591.109	1	4591.109	5.315	.028	.155
Error	25050.931	29	863.825			
Total	202882.000	32				
Corrected Total	34391.875	31				

a. R Squared = .272 (Adjusted R Squared = .221)

2. 1=cauc; 2=AD

Dependent Variable: PA_F

1=cauc; 2=AD	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
1	82.480 ^a	6.745	68.684	96.275
2	58.068 ^a	8.156	41.387	74.749

a. Covariates appearing in the model are evaluated at the following values: K_F = 70.2875.

Dependent T-test in Caucasians

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	BMI_B	29.0913	23	3.62716	.75631
	BMI_F	28.6739	23	3.54121	.73839
Pair 2	TFAT_B	35.6750	16	8.39964	2.09991
	TFAT_F	33.5000	16	7.78486	1.94621
Pair 3	LBW_B	52095.81	16	13336.25719	3334.064
	LBW_F	52886.88	16	12525.30740	3131.327
Pair 4	SBP_B	133.4619	21	11.10029	2.42228
	SBP_F	130.5762	21	9.15319	1.99739
Pair 5	DBP_B	84.0952	21	7.39618	1.61398
	DBP_F	83.8095	21	7.86619	1.71654
Pair 6	NA_B	122.9091	22	63.70984	13.58298
	NA_F	131.9091	22	68.97750	14.70605
Pair 7	K_B	71.0727	22	23.35927	4.98021
	K_F	68.8909	22	19.87396	4.23714
Pair 8	TV_B	2226.9545	22	944.06177	201.27464
	TV_F	2395.3636	22	893.83372	190.56599
Pair 9	PA_B	119.0000	20	70.33828	15.72812
	PA_F	81.4000	20	31.02868	6.93822
Pair 10	VO2REL_B	25.4136	22	5.16013	1.10014
	VO2REL_F	29.2414	22	5.12297	1.09222
Pair 11	VO2AB_B	2.2605	22	.63592	.13558
	VO2AB_F	2.5360	22	.66099	.14092
Pair 12	WGT_B	88.2391	23	13.77038	2.87132
	WGT_F	86.9391	23	13.23723	2.76015

Paired Samples Test

		Paired Differences				t	df	Sig. (2-tailed)	
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	BMI_B - BMI_F	.4174	.66309	.13826	.1307	.7041	3.019	22	.006
Pair 2	TFAT_B - TFAT_F	2.1750	2.19621	.54905	1.0047	3.3453	3.961	15	.001
Pair 3	LBW_B - LBW_F	-791.0625	1822.23140	455.55785	-1762.06	179.9361	-1.736	15	.103
Pair 4	SBP_B - SBP_F	2.8857	6.17003	1.34641	.0771	5.6943	2.143	20	.045
Pair 5	DBP_B - DBP_F	.2857	6.04998	1.32021	-2.4682	3.0396	.216	20	.831
Pair 6	NA_B - NA_F	-9.0000	39.00916	8.31678	-26.2957	8.2957	-1.082	21	.291
Pair 7	K_B - K_F	2.1818	23.65306	5.04285	-8.3054	12.6690	.433	21	.670
Pair 8	TV_B - TV_F	-168.4091	698.67437	148.95788	-478.1840	141.3658	-1.131	21	.271
Pair 9	PA_B - PA_F	37.6000	65.02421	14.53985	7.1677	68.0323	2.586	19	.018
Pair 10	VO2REL_B - VO2REL_F	-3.8277	2.54126	.54180	-4.9545	-2.7010	-7.065	21	.000
Pair 11	VO2AB_B - VO2AB_F	-.2755	.21243	.04529	-.3697	-.1814	-6.084	21	.000
Pair 12	WGT_B - WGT_F	1.3000	2.01855	.42090	.4271	2.1729	3.089	22	.005

Univariate Analysis in Caucasians:
Covariate: baseline plasma aldosterone
Dependent variable: change in plasma aldosterone

Descriptive Statistics

Dependent Variable: CHGPA

Mean	Std. Deviation	N
-37.6000	65.02421	20

Tests of Between-Subjects Effects

Dependent Variable: CHGPA

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	64758.542 ^a	1	64758.542	74.835	.000	.806
Intercept	18648.897	1	18648.897	21.551	.000	.545
PA_B	64758.542	1	64758.542	74.835	.000	.806
Error	15576.258	18	865.348			
Total	108610.000	20				
Corrected Total	80334.800	19				

a. R Squared = .806 (Adjusted R Squared = .795)

Grand Mean

Dependent Variable: CHGPA

Mean	Std. Error	95% Confidence Interval	
		Lower Bound	Upper Bound
-37.600 ^a	6.578	-51.419	-23.781

a. Covariates appearing in the model are evaluated at the following values: PA_B = 119.0000.

Regression in Caucasians
Predictor: baseline plasma aldosterone
Dependent variable: change in plasma aldosterone

Descriptive Statistics

	Mean	Std. Deviation	N
CHGPA	-37.6000	65.02421	20
PA_B	119.0000	70.33828	20

Correlations

		CHGPA	PA_B
Pearson Correlation	CHGPA	1.000	-.898
	PA_B	-.898	1.000
Sig. (1-tailed)	CHGPA	.	.000
	PA_B	.000	.
N	CHGPA	20	20
	PA_B	20	20

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.898 ^a	.806	.795	29.41679

a. Predictors: (Constant), PA_B

ANOVA^b

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	64758.542	1	64758.542	74.835	.000 ^a
	Residual	15576.258	18	865.348		
	Total	80334.800	19			

a. Predictors: (Constant), PA_B

b. Dependent Variable: CHGPA

Regression Caucasians

Predictor: baseline SBP

Dependent variable: change in SBP

Descriptive Statistics

	Mean	Std. Deviation	N
CHGSBP	-2.8857	6.17003	21
SBP_B	133.4619	11.10029	21

Correlations

		CHGSBP	SBP_B
Pearson Correlation	CHGSBP	1.000	-.566
	SBP_B	-.566	1.000
Sig. (1-tailed)	CHGSBP	.	.004
	SBP_B	.004	.
N	CHGSBP	21	21
	SBP_B	21	21

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.566 ^a	.320	.284	5.21954

a. Predictors: (Constant), SBP_B

ANOVA^b

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	243.758	1	243.758	8.947	.008 ^a
	Residual	517.628	19	27.244		
	Total	761.386	20			

a. Predictors: (Constant), SBP_B

b. Dependent Variable: CHGSBP

Regression Caucasians

Predictor: change in plasma aldosterone

Dependent variable: change in SBP

Descriptive Statistics

	Mean	Std. Deviation	N
CHGSBP	-2.8950	6.07475	20
CHGPA	-36.3500	76.94309	20

Correlations

		CHGSBP	CHGPA
Pearson Correlation	CHGSBP	1.000	-.074
	CHGPA	-.074	1.000
Sig. (1-tailed)	CHGSBP	.	.379
	CHGPA	.379	.
N	CHGSBP	20	20
	CHGPA	20	20

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.074 ^a	.005	-.050	6.22426

a. Predictors: (Constant), CHGPA

ANOVA^b

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	3.804	1	3.804	.098	.758 ^a
	Residual	697.345	18	38.741		
	Total	701.150	19			

a. Predictors: (Constant), CHGPA

b. Dependent Variable: CHGSBP

Predictor: change in plasma aldosterone

Dependent variable: change in DBP

Descriptive Statistics

	Mean	Std. Deviation	N
CHDBP	.0150	5.94123	20
CHGPA	-36.3500	76.94309	20

Correlations

		CHDBP	CHGPA
Pearson Correlation	CHDBP	1.000	-.345
	CHGPA	-.345	1.000
Sig. (1-tailed)	CHDBP	.	.068
	CHGPA	.068	.
N	CHDBP	20	20
	CHGPA	20	20

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.345 ^a	.119	.070	5.72901

a. Predictors: (Constant), CHGPA

ANOVA^b

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	79.877	1	79.877	2.434	.136 ^a
	Residual	590.788	18	32.822		
	Total	670.665	19			

a. Predictors: (Constant), CHGPA

b. Dependent Variable: CHDBP

Predictor: change in plasma aldosterone

Dependent variable: change in 24-hour Na⁺ excretion

Descriptive Statistics

	Mean	Std. Deviation	N
CHGNA	9.5500	36.95584	20
CHGPA	-35.7500	77.23844	20

Correlations

		CHGNA	CHGPA
Pearson Correlation	CHGNA	1.000	-.199
	CHGPA	-.199	1.000
Sig. (1-tailed)	CHGNA	.	.200
	CHGPA	.200	.
N	CHGNA	20	20
	CHGPA	20	20

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.199 ^a	.040	-.014	37.20924

a. Predictors: (Constant), CHGPA

ANOVA^b

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	1027.456	1	1027.456	.742	.400 ^a
	Residual	24921.494	18	1384.527		
	Total	25948.950	19			

a. Predictors: (Constant), CHGPA

b. Dependent Variable: CHGNA

Dependent t-test in individuals of African descent

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	BMI_B	27.9692	13	3.50366	.97174
	BMI_F	26.9462	13	3.00600	.83371
Pair 2	TFAT_B	40.3273	11	10.19148	3.07285
	TFAT_F	39.9455	11	10.57964	3.18988
Pair 3	LBW_B	44390.18	11	11018.55958	3322.221
	LBW_F	43879.73	11	10863.43736	3275.450
Pair 4	SBP_B	130.4800	10	9.95945	3.14945
	SBP_F	134.0800	10	13.40786	4.23994
Pair 5	DBP_B	85.3000	9	4.83580	1.61193
	DBP_F	84.0222	9	6.08169	2.02723
Pair 6	NA_B	114.0000	13	37.80873	10.48625
	NA_F	117.5385	13	39.81963	11.04398
Pair 7	K_B	57.3538	13	28.46450	7.89463
	K_F	69.1000	13	30.02788	8.32823
Pair 8	TV_B	2002.0769	13	774.31738	214.75700
	TV_F	1957.3846	13	744.22975	206.41219
Pair 9	PA_B	63.5714	14	34.54476	9.23248
	PA_F	61.2143	14	33.41851	8.93147
Pair 10	VO2REL_B	22.3154	13	3.57301	.99098
	VO2REL_F	25.8536	13	5.79773	1.60800
Pair 11	WGT_B	79.4923	13	12.17685	3.37725
	WGT_F	76.5538	13	10.87908	3.01731

Paired Samples Test

	Paired Differences					t	df	Sig. (2-tailed)
	Mean	Std. Deviation	Std. Error	95% Confidence Interval of the Difference				
				Lower	Upper			
Pair 1 BMI_B - BMI_F	1.0231	1.80377	.50028	-.0669	2.1131	2.045	12	.063
Pair 2 TFAT_B - TFAT_F	.3818	2.11840	.63872	-1.0413	1.8050	.598	10	.563
Pair 3 LBW_B - LBW_F	10.4545	1370.37304	3.18302	10.1746	31.0837	1.235	10	.245
Pair 4 SBP_B - SBP_F	-3.6000	9.93714	3.14240	-10.7086	3.5086	-1.146	9	.281
Pair 5 DBP_B - DBP_F	1.2778	5.36884	1.78961	-2.8491	5.4046	.714	8	.496
Pair 6 NA_B - NA_F	-3.5385	43.97464	2.19637	-30.1121	23.0351	-.290	12	.777
Pair 7 K_B - K_F	-11.7462	28.40242	7.87742	-28.9096	5.4173	-1.491	12	.162
Pair 8 TV_B - TV_F	44.6923	584.67945	2.16090	38.6260	98.0106	.276	12	.788
Pair 9 PA_B - PA_F	2.3571	29.94766	8.00385	-14.9341	19.6484	.295	13	.773
Pair 10 VO2REL_B - VO2REL_F	-3.5382	2.55077	.70746	-5.0796	-1.9968	-5.001	12	.000
Pair 11 WGT_B - WGT_F	2.9385	5.06993	1.40615	-.1253	6.0022	2.090	12	.059

Univariate Analysis in individuals of African descent

Covariate: baseline plasma aldosterone

Dependent variable: change in plasma aldosterone

Descriptive Statistics

Dependent Variable: CHGPA

Mean	Std. Deviation	N
-2.3571	29.94766	14

Tests of Between-Subjects Effects

Dependent Variable: CHGPA

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	2580.522 ^a	1	2580.522	3.411	.090	.221
Intercept	1673.723	1	1673.723	2.212	.163	.156
PA_B	2580.522	1	2580.522	3.411	.090	.221
Error	9078.693	12	756.558			
Total	11737.000	14				
Corrected Total	11659.214	13				

a. R Squared = .221 (Adjusted R Squared = .156)

Grand Mean

Dependent Variable: CHGPA

Mean	Std. Error	95% Confidence Interval	
		Lower Bound	Upper Bound
-2.357 ^a	7.351	-18.374	13.660

a. Covariates appearing in the model are evaluated at the following values: PA_B = 63.5714.

Regression in individuals of African descent

Predictor: baseline plasma aldosterone

Dependent variable: change in plasma aldosterone

Descriptive Statistics

	Mean	Std. Deviation	N
CHGPA	-2.3571	29.94766	14
PA_B	63.5714	34.54476	14

Correlations

		CHGPA	PA_B
Pearson Correlation	CHGPA	1.000	-.470
	PA_B	-.470	1.000
Sig. (1-tailed)	CHGPA	.	.045
	PA_B	.045	.
N	CHGPA	14	14
	PA_B	14	14

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.470 ^a	.221	.156	27.50559

a. Predictors: (Constant), PA_B

ANOVA^b

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	2580.522	1	2580.522	3.411	.090 ^a
	Residual	9078.693	12	756.558		
	Total	11659.214	13			

a. Predictors: (Constant), PA_B

b. Dependent Variable: CHGPA

Univariate Analysis in individuals of African descent
Covariate: baseline 24-hour Na⁺ excretion
Dependent variable: change in 24-hour Na⁺ excretion

Descriptive Statistics

Dependent Variable: CHGNA

Mean	Std. Deviation	N
3.5385	43.97464	13

Tests of Between-Subjects Effects

Dependent Variable: CHGNA

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	6631.897 ^a	1	6631.897	4.402	.060	.286
Intercept	6636.698	1	6636.698	4.405	.060	.286
NA_B	6631.897	1	6631.897	4.402	.060	.286
Error	16573.334	11	1506.667			
Total	23368.000	13				
Corrected Total	23205.231	12				

a. R Squared = .286 (Adjusted R Squared = .221)

Grand Mean

Dependent Variable: CHGNA

Mean	Std. Error	95% Confidence Interval	
		Lower Bound	Upper Bound
3.538 ^a	10.766	-20.156	27.233

a. Covariates appearing in the model are evaluated at the following values: NA_B = 114.0000.

Regression in individuals of African descent
Predictor: baseline 24-hour Na⁺ excretion
Dependent variable: change in 24-hour Na⁺ excretion

Descriptive Statistics

	Mean	Std. Deviation	N
CHGNA	3.5385	43.97464	13
NA_B	114.0000	37.80873	13

Correlations

		CHGNA	NA_B
Pearson Correlation	CHGNA	1.000	-.535
	NA_B	-.535	1.000
Sig. (1-tailed)	CHGNA	.	.030
	NA_B	.030	.
N	CHGNA	13	13
	NA_B	13	13

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.535 ^a	.286	.221	38.81581

a. Predictors: (Constant), NA_B

ANOVA^b

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	6631.897	1	6631.897	4.402	.060 ^a
	Residual	16573.334	11	1506.667		
	Total	23205.231	12			

a. Predictors: (Constant), NA_B

b. Dependent Variable: CHGNA

Univariate Analysis in individuals of African descent

Covariate: change in 24-hour Na⁺ excretion

Dependent variable: change in DBP

Descriptive Statistics

Dependent Variable: CHDBP

Mean	Std. Deviation	N
-1.2778	5.36884	9

Tests of Between-Subjects Effects

Dependent Variable: CHDBP

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	100.424 ^a	1	100.424	5.400	.053	.435
Intercept	24.116	1	24.116	1.297	.292	.156
CHGNA	100.424	1	100.424	5.400	.053	.435
Error	130.172	7	18.596			
Total	245.290	9				
Corrected Total	230.596	8				

a. R Squared = .435 (Adjusted R Squared = .355)

Grand Mean

Dependent Variable: CHDBP

Mean	Std. Error	95% Confidence Interval	
		Lower Bound	Upper Bound
-1.278 ^a	1.437	-4.677	2.121

a. Covariates appearing in the model are evaluated at the following values: CHGNA = 4.0000.

Independent T-test in the Caucasian TT vs. TC+CC Group
Group Statistics

	1=TT:2 TC+CC	N	Mean	Std. Deviation	Std. Error Mean
AGE	1.00	13	56.08	4.663	1.293
	2.00	18	60.11	6.398	1.508
BMI_B	1.00	13	28.9077	4.71301	1.30715
	2.00	16	28.3938	4.03410	1.00852
BMI_F	1.00	9	29.1111	2.35502	.78501
	2.00	14	28.0286	4.40951	1.17849
MDRD	1.00	12	74.6192	14.36136	4.14577
	2.00	18	74.8117	9.71508	2.28987
SCR	1.00	12	.9833	.18505	.05342
	2.00	17	1.0176	.16672	.04043
TFAT_B	1.00	12	37.1250	8.91374	2.57318
	2.00	18	33.5389	8.98581	2.11798
TFAT_F	1.00	7	35.4286	6.15866	2.32775
	2.00	8	30.3250	7.86670	2.78130
LBW_B	1.00	12	48920.750 0	11778.76270	3400.2359 1
	2.00	18	53786.277 8	11636.48809	2742.7465 5
LBW_F	1.00	8	54852.375	13138.65419	4645.2157 4
	2.00	8	56038.125 0	13493.80238	4770.7795 8
SBP_B	1.00	12	130.4417	11.12201	3.21065
	2.00	17	133.7176	10.39130	2.52026
SBP_F	1.00	8	130.4625	11.14014	3.93863
	2.00	12	130.2000	8.39740	2.42412
DBP_B	1.00	11	83.3727	8.83585	2.66411
	2.00	16	85.8375	5.95011	1.48753
DBP_F	1.00	8	86.1250	8.85676	3.13134
	2.00	12	82.6250	7.44765	2.14995
NA_B	1.00	12	123.1667	65.67251	18.95802
	2.00	17	107.9412	56.17881	13.62536
NA_F	1.00	8	157.3750	86.89885	30.72338
	2.00	13	122.6154	53.12805	14.73507
K_B	1.00	12	75.0333	20.31333	5.86395
	2.00	17	65.8059	26.04255	6.31625
K_F	1.00	8	73.6250	22.18447	7.84339
	2.00	13	68.2000	17.86342	4.95442
TV_B	1.00	12	2371.3333	789.91062	228.02755
	2.00	17	1984.4118	885.02380	214.64980
TV_F	1.00	8	2400.6250	422.45133	149.35910
	2.00	13	2368.6923	1133.80571	314.46113
PA_B	1.00	11	119.0000	63.91870	19.27221
	2.00	16	98.8125	67.11504	16.77876

PA_F	1.00	8	98.2500	22.37186	7.90965
	2.00	12	70.1667	31.62805	9.13023
VO2REL_B	1.00	13	24.4154	4.57381	1.26855
	2.00	15	25.6533	5.60202	1.44644
VO2REL_F	1.00	9	29.1222	4.81762	1.60587
	2.00	13	29.6392	5.31955	1.47538
WGT_B	1.00	13	86.2692	18.05526	5.00763
	2.00	16	87.5250	13.76302	3.44075
WGT_F	1.00	9	89.6667	12.86439	4.28813
	2.00	14	84.7500	14.25271	3.80920
CHGSBP	1.00	8	-1.5000	5.78644	2.04581
	2.00	12	-4.4083	6.26817	1.80946
CHDBP	1.00	8	3.3875	6.39563	2.26120
	2.00	12	-2.1083	4.68507	1.35246
CHGK	1.00	8	-8.1250	29.58343	10.45932
	2.00	13	4.4615	16.48077	4.57094
CHGNA	1.00	8	20.1250	45.57705	16.11392
	2.00	13	6.7692	32.60919	9.04416
CHGPA	1.00	8	-35.7500	57.78470	20.42998
	2.00	12	-38.8333	71.93158	20.76486

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
AGE	Equal variances assumed	1.632	.212	-1.930	29	.063	-4.03	2.091	-8.310	.241
	Equal variances not assumed			-2.031	28.988	.052	-4.03	1.987	-8.097	.029
BMI_B	Equal variances assumed	.747	.395	.316	27	.754	.5139	1.62387	-2.81796	3.84584
	Equal variances not assumed			.311	23.794	.758	.5139	1.65099	-2.89510	3.92298
BMI_F	Equal variances assumed	.998	.330	.449	20	.659	.7188	1.60225	-2.62344	4.06104
	Equal variances not assumed			.498	19.137	.624	.7188	1.44298	-2.29993	3.73753
MDRD	Equal variances assumed	1.289	.266	-.044	28	.965	-.1925	4.38321	-9.17110	8.78610
	Equal variances not assumed			-.041	17.671	.968	-.1925	4.73613	-10.15601	9.77101
SCR	Equal variances assumed	1.081	.308	-.522	27	.606	-.0343	.06576	-.16925	.10062
	Equal variances not assumed			-.512	22.205	.614	-.0343	.06700	-.17318	.10455
TFAT_B	Equal variances assumed	.000	.989	1.074	28	.292	3.5861	3.33829	-3.25206	10.42429
	Equal variances not assumed			1.076	23.866	.293	3.5861	3.33273	-3.29434	10.46657
TFAT_F	Equal variances assumed	.004	.950	.946	14	.360	3.5625	3.76488	-4.51237	11.63737
	Equal variances not assumed			.946	13.884	.360	3.5625	3.76488	-4.51872	11.64372
LBW_B	Equal variances assumed	.006	.940	-1.117	28	.274	-4865.5278	4357.5703	-13791.6	4060.550
	Equal variances not assumed			-1.114	23.527	.277	-4865.5278	4368.5539	-13891.4	4160.334
LBW_F	Equal variances assumed	.212	.653	-.178	14	.861	-1185.7500	6658.7061	-15467.3	13095.75
	Equal variances not assumed			-.178	13.990	.861	-1185.7500	6658.7061	-15468.2	13096.71
SBP_B	Equal variances assumed	.000	.983	-.812	27	.424	-3.2760	4.03242	-11.54982	4.99786
	Equal variances not assumed			-.803	22.785	.430	-3.2760	4.08166	-11.72395	5.17199
SBP_F	Equal variances assumed	.660	.427	.060	18	.953	.2625	4.36261	-8.90301	9.42801
	Equal variances not assumed			.057	12.194	.956	.2625	4.62485	-9.79640	10.32140
DBP_B	Equal variances assumed	1.859	.185	-.869	25	.393	-2.4648	2.83717	-8.30804	3.37850
	Equal variances not assumed			-.808	16.160	.431	-2.4648	3.05126	-8.92796	3.99841
DBP_F	Equal variances assumed	.226	.640	.956	18	.352	3.5000	3.66294	-4.19554	11.19554
	Equal variances not assumed			.921	13.278	.373	3.5000	3.79836	-4.68846	11.68846

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
NA_B	Equal variances assumed	.342	.563	.670	27	.508	15.2255	22.70801	-31.36749	61.81847
	Equal variances not assumed			.652	21.378	.521	15.2255	23.34646	-33.27396	63.72494
NA_F	Equal variances assumed	.345	.564	1.145	19	.266	34.7596	30.36010	-28.78481	98.30404
	Equal variances not assumed			1.020	10.274	.331	34.7596	34.07416	-40.88921	110.40844
K_B	Equal variances assumed	1.597	.217	1.025	27	.314	9.2275	9.00174	-9.24260	27.69750
	Equal variances not assumed			1.071	26.660	.294	9.2275	8.61864	-8.46710	26.92200
K_F	Equal variances assumed	.137	.715	.617	19	.545	5.4250	8.79247	-12.97786	23.82786
	Equal variances not assumed			.585	12.536	.569	5.4250	9.27713	-14.69265	25.54265
TV_B	Equal variances assumed	.191	.665	1.211	27	.236	386.9216	319.56272	-268.767	1042.610
	Equal variances not assumed			1.236	25.413	.228	386.9216	313.16306	-257.519	1031.362
TV_F	Equal variances assumed	6.221	.022	.076	19	.940	31.9327	420.97358	-849.175	913.04051
	Equal variances not assumed			.092	16.579	.928	31.9327	348.12920	-703.980	767.84588
PA_B	Equal variances assumed	.012	.913	.783	25	.441	20.1875	25.79379	-32.93580	73.31080
	Equal variances not assumed			.790	22.346	.438	20.1875	25.55279	-32.75822	73.13322
PA_F	Equal variances assumed	1.711	.207	2.167	18	.044	28.0833	12.95789	.85981	55.30686
	Equal variances not assumed			2.325	17.881	.032	28.0833	12.07988	2.69228	53.47439
VO2REL_B	Equal variances assumed	1.642	.211	-.634	26	.532	-1.2379	1.95265	-5.25167	2.77577
	Equal variances not assumed			-.643	25.925	.526	-1.2379	1.92390	-5.19314	2.71724
VO2REL_F	Equal variances assumed	.692	.416	-.268	19	.792	-.6203	2.31480	-5.46522	4.22466
	Equal variances not assumed			-.274	18.505	.787	-.6203	2.26688	-5.37352	4.13297
VO2AB_B	Equal variances assumed	.114	.739	-.519	26	.608	-.1325	.25539	-.65742	.39250
	Equal variances not assumed			-.516	24.897	.610	-.1325	.25657	-.66098	.39606
VO2AB_F	Equal variances assumed	.008	.930	.329	19	.746	.0963	.29305	-.51703	.70970
	Equal variances not assumed			.325	16.657	.749	.0963	.29623	-.52964	.72231

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
WGT_B	Equal variances assumed	.553	.464	-.213	27	.833	-1.2558	5.90528	-13.37241	10.86087
	Equal variances not assumed			-.207	22.070	.838	-1.2558	6.07578	-13.85385	11.34231
WGT_F	Equal variances assumed	.126	.726	.578	20	.570	3.3128	5.73358	-8.64722	15.27286
	Equal variances not assumed			.583	17.873	.567	3.3128	5.68466	-8.63630	15.26195
CHGSBP	Equal variances assumed	.291	.596	1.047	18	.309	2.9083	2.77757	-2.92713	8.74380
	Equal variances not assumed			1.065	16.003	.303	2.9083	2.73121	-2.88147	8.69814
CHDBP	Equal variances assumed	.165	.689	2.224	18	.039	5.4958	2.47155	.30331	10.68836
	Equal variances not assumed			2.086	11.932	.059	5.4958	2.63480	-.24851	11.24017
CHGK	Equal variances assumed	4.483	.048	-1.260	19	.223	-12.5865	9.98730	-33.49021	8.31713
	Equal variances not assumed			-1.103	9.722	.297	-12.5865	11.41451	-38.11846	12.94538
CHGNA	Equal variances assumed	1.018	.326	.784	19	.443	13.3558	17.03362	-22.29600	49.00754
	Equal variances not assumed			.723	11.443	.484	13.3558	18.47851	-27.12408	53.83562
CHGPA	Equal variances assumed	.340	.567	.101	18	.921	3.0833	30.48398	-60.96112	67.12779
	Equal variances not assumed			.106	17.231	.917	3.0833	29.13011	-58.31310	64.47977

Univariate Analysis in Caucasians TT vs. TC+CC Genotype Group
Covariate: baseline plasma aldosterone levels
Dependent variable: change in plasma aldosterone levels

Between-Subjects Factors

		N
1=TT:2 TC+CC	1.00	8
	2.00	12

Descriptive Statistics

Dependent Variable: CHGPA

1=TT:2 TC+CC	Mean	Std. Deviation	N
1.00	-35.7500	57.78470	8
2.00	-38.8333	71.93158	12
Total	-37.6000	65.02421	20

Levene's Test of Equality of Error Variances^a

Dependent Variable: CHGPA

F	df1	df2	Sig.
3.273	1	18	.087

Tests the null hypothesis that the error variance of the dependent variable is equal across groups.

a. Design: Intercept+PA_B+CY_CODE

Tests of Between-Subjects Effects

Dependent Variable: CHGPA

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	67574.981 ^a	2	33787.490	45.015	.000	.841
Intercept	21180.470	1	21180.470	28.219	.000	.624
PA_B	67529.347	1	67529.347	89.970	.000	.841
CY_CODE	2816.438	1	2816.438	3.752	.070	.181
Error	12759.819	17	750.578			
Total	108610.000	20				
Corrected Total	80334.800	19				

a. R Squared = .841 (Adjusted R Squared = .822)

2. 1=TT:2 TC+CC

Dependent Variable: CHGPA

1=TT:2 TC+CC	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
1.00	-22.829 ^a	9.782	-43.466	-2.191
2.00	-47.448 ^a	7.961	-64.243	-30.652

a. Covariates appearing in the model are evaluated at the following values: PA_B = 119.0000.

Univariate Analysis in Caucasians TT vs. TC+CC Genotype Group

Covariate: baseline SBP

Dependent variable: change in SBP

Between-Subjects Factors

		N
1=TT:2 TC+CC	1.00	8
	2.00	12

Descriptive Statistics

Dependent Variable: CHGSBP

1=TT:2 TC+CC	Mean	Std. Deviation	N
1.00	-1.5000	5.78644	8
2.00	-4.4083	6.26817	12
Total	-3.2450	6.10077	20

Levene's Test of Equality of Error Variances^a

Dependent Variable: CHGSBP

F	df1	df2	Sig.
.004	1	18	.949

Tests the null hypothesis that the error variance of the dependent variable is equal across groups.

a. Design: Intercept+SBP_B+CY_CODE

Tests of Between-Subjects Effects

Dependent Variable: CHGSBP

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	257.025 ^a	2	128.512	4.853	.022	.363
Intercept	184.176	1	184.176	6.956	.017	.290
SBP_B	216.424	1	216.424	8.173	.011	.325
CY_CODE	21.245	1	21.245	.802	.383	.045
Error	450.145	17	26.479			
Total	917.770	20				
Corrected Total	707.170	19				

a. R Squared = .363 (Adjusted R Squared = .289)

2. 1=TT:2 TC+CC

Dependent Variable: CHGSBP

1=TT:2 TC+CC	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
1.00	-1.974 ^a	1.827	-5.828	1.880
2.00	-4.092 ^a	1.490	-7.235	-.950

a. Covariates appearing in the model are evaluated at the following values: SBP_B = 133.5500.

Dependent T-test in Caucasian TC+CC Genotype Group

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	BMI_B	28.8154	13	4.30443	1.19383
	BMI_F	28.3923	13	4.36548	1.21077
Pair 2	TFAT_B	32.7625	8	8.64621	3.05690
	TFAT_F	30.3250	8	7.86670	2.78130
Pair 3	LBW_B	55317.13	8	14539.27613	5140.410
	LBW_F	56038.13	8	13493.80238	4770.780
Pair 4	SBP_B	134.6083	12	10.48735	3.02744
	SBP_F	130.2000	12	8.39740	2.42412
Pair 5	DBP_B	84.7333	12	5.60817	1.61894
	DBP_F	82.6250	12	7.44765	2.14995
Pair 6	NA_B	115.8462	13	61.50860	17.05942
	NA_F	122.6154	13	53.12805	14.73507
Pair 7	K_B	63.7385	13	25.78984	7.15281
	K_F	68.2000	13	17.86342	4.95442
Pair 8	TV_B	1965.9231	13	1000.99679	277.62656
	TV_F	2368.6923	13	1133.80571	314.46113
Pair 9	PA_B	109.0000	12	72.24453	20.85520
	PA_F	70.1667	12	31.62805	9.13023
Pair 10	VO2REL_B	25.2417	12	5.97760	1.72559
	VO2REL_F	29.7425	12	5.54246	1.59997
Pair 11	VO2AB_B	2.2261	12	.64230	.18542
	VO2AB_F	2.5347	12	.64394	.18589
Pair 12	WGT_B	87.6462	13	13.44189	3.72811
	WGT_F	86.3538	13	13.45564	3.73192

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	BMI_B - BMI_F	.4231	.48158	.13357	.1321	.7141	3.168	12	.008
Pair 2	TFAT_B - TFAT_F	2.4375	1.65178	.58399	1.0566	3.8184	4.174	7	.004
Pair 3	LBW_B - LBW_F	-721.0000	1367.38771	483.44456	-1864.16	422.1647	-1.491	7	.179
Pair 4	SBP_B - SBP_F	4.4083	6.26817	1.80946	.4257	8.3909	2.436	11	.033
Pair 5	DBP_B - DBP_F	2.1083	4.68507	1.35246	-.8684	5.0851	1.559	11	.147
Pair 6	NA_B - NA_F	-6.7692	32.60919	9.04416	-26.4748	12.9363	-.748	12	.469
Pair 7	K_B - K_F	-4.4615	16.48077	4.57094	-14.4208	5.4977	-.976	12	.348
Pair 8	TV_B - TV_F	-402.7692	579.71202	160.78319	-753.0857	-52.4528	-2.505	12	.028
Pair 9	PA_B - PA_F	38.8333	71.93158	20.76486	-6.8698	84.5365	1.870	11	.088
Pair 10	VO2REL_B - VO2REL_F	-4.5008	1.88344	.54370	-5.6975	-3.3042	-8.278	11	.000
Pair 11	VO2AB_B - VO2AB_F	-.3086	.18335	.05293	-.4251	-.1921	-5.830	11	.000
Pair 12	WGT_B - WGT_F	1.2923	1.41978	.39378	.4343	2.1503	3.282	12	.007

Regression in Caucasian TT Genotype Group
Predictor: change in plasma aldosterone levels
Dependent variable: change in SBP

Descriptive Statistics

	Mean	Std. Deviation	N
CHGSBP	-.7143	5.77074	7
CHGPA	-40.0000	61.04916	7

Correlations

		CHGSBP	CHGPA
Pearson Correlation	CHGSBP	1.000	.565
	CHGPA	.565	1.000
Sig. (1-tailed)	CHGSBP	.	.093
	CHGPA	.093	.
N	CHGSBP	7	7
	CHGPA	7	7

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.565 ^a	.319	.183	5.21541

a. Predictors: (Constant), CHGPA

ANOVA^b

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	63.806	1	63.806	2.346	.186 ^a
	Residual	136.003	5	27.201		
	Total	199.809	6			

a. Predictors: (Constant), CHGPA

b. Dependent Variable: CHGSBP

Regression in Caucasian TT Genotype Group
Predictor: change in plasma aldosterone levels
Dependent variable: change in SBP

Descriptive Statistics

	Mean	Std. Deviation	N
CHDBP	3.6571	6.85878	7
CHGPA	-40.0000	61.04916	7

Correlations

		CHDBP	CHGPA
Pearson Correlation	CHDBP	1.000	-.117
	CHGPA	-.117	1.000
Sig. (1-tailed)	CHDBP	.	.401
	CHGPA	.401	.
N	CHDBP	7	7
	CHGPA	7	7

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.117 ^a	.014	-.183	7.46151

a. Predictors: (Constant), CHGPA

ANOVA^b

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	3.886	1	3.886	.070	.802 ^a
	Residual	278.371	5	55.674		
	Total	282.257	6			

a. Predictors: (Constant), CHGPA

b. Dependent Variable: CHDBP

Regression in Caucasian TT Genotype Group
Predictor: change in plasma aldosterone levels
Dependent variable: change in 24-hour Na⁺ excretion

Descriptive Statistics

	Mean	Std. Deviation	N
CHGNA	14.7143	46.37066	7
CHGPA	-40.0000	61.04916	7

Correlations

		CHGNA	CHGPA
Pearson Correlation	CHGNA	1.000	-.522
	CHGPA	-.522	1.000
Sig. (1-tailed)	CHGNA	.	.115
	CHGPA	.115	.
N	CHGNA	7	7
	CHGPA	7	7

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.522 ^a	.272	.127	43.33827

a. Predictors: (Constant), CHGPA

ANOVA^b

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	3510.402	1	3510.402	1.869	.230 ^a
	Residual	9391.027	5	1878.205		
	Total	12901.429	6			

a. Predictors: (Constant), CHGPA

b. Dependent Variable: CHGNA

Regression in Caucasian TC+CC Genotype Group
Predictor: change in plasma aldosterone levels
Dependent variable: change in SBP

Descriptive Statistics

	Mean	Std. Deviation	N
CHGSBP	-4.3273	6.56751	11
CHGPA	-42.9091	73.97493	11

Correlations

		CHGSBP	CHGPA
Pearson Correlation	CHGSBP	1.000	-.334
	CHGPA	-.334	1.000
Sig. (1-tailed)	CHGSBP	.	.157
	CHGPA	.157	.
N	CHGSBP	11	11
	CHGPA	11	11

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.334 ^a	.112	.013	6.52405

a. Predictors: (Constant), CHGPA

ANOVA^b

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	48.253	1	48.253	1.134	.315 ^a
	Residual	383.069	9	42.563		
	Total	431.322	10			

a. Predictors: (Constant), CHGPA

b. Dependent Variable: CHGSBP

Regression in Caucasian TC+CC Genotype Group
Predictor: change in plasma aldosterone levels
Dependent variable: change in DBP

Descriptive Statistics

	Mean	Std. Deviation	N
CHDBP	-1.9818	4.89220	11
CHGPA	-42.9091	73.97493	11

Correlations

		CHDBP	CHGPA
Pearson Correlation	CHDBP	1.000	-.586
	CHGPA	-.586	1.000
Sig. (1-tailed)	CHDBP	.	.029
	CHGPA	.029	.
N	CHDBP	11	11
	CHGPA	11	11

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.586 ^a	.344	.271	4.17721

a. Predictors: (Constant), CHGPA

ANOVA^b

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	82.294	1	82.294	4.716	.058 ^a
	Residual	157.042	9	17.449		
	Total	239.336	10			

a. Predictors: (Constant), CHGPA

b. Dependent Variable: CHDBP

Regression in Caucasian TC+CC Genotype Group
Predictor: change in plasma aldosterone levels
Dependent variable: change in 24-hour Na⁺ excretion

Descriptive Statistics

	Mean	Std. Deviation	N
CHGNA	7.1667	34.02628	12
CHGPA	-38.8333	71.93158	12

Correlations

		CHGNA	CHGPA
Pearson Correlation	CHGNA	1.000	-.068
	CHGPA	-.068	1.000
Sig. (1-tailed)	CHGNA	.	.417
	CHGPA	.417	.
N	CHGNA	12	12
	CHGPA	12	12

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.068 ^a	.005	-.095	35.60378

a. Predictors: (Constant), CHGPA

ANOVA^b

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	59.377	1	59.377	.047	.833 ^a
	Residual	12676.290	10	1267.629		
	Total	12735.667	11			

a. Predictors: (Constant), CHGPA

b. Dependent Variable: CHGNA

Independent T-test Among individuals of African descent in TT vs. TC+CC

Group Statistics

	1=TT:2 TC+CC	N	Mean	Std. Deviation	Std. Error Mean
AGE	1.00	14	57.79	3.534	.944
	2.00	12	57.08	6.694	1.932
BMI_B	1.00	12	28.1250	3.09578	.89368
	2.00	12	29.9500	3.84767	1.11073
BMI_F	1.00	6	25.5833	1.99741	.81544
	2.00	7	28.1143	3.36126	1.27044
MDRD	1.00	13	73.8438	10.23172	2.83777
	2.00	11	81.8227	23.01040	6.93790
SCR	1.00	13	1.0692	.11821	.03279
	2.00	11	1.0455	.29449	.08879
TFAT_B	1.00	11	36.6455	6.54788	1.97426
	2.00	8	43.7375	9.64275	3.40923
TFAT_F	1.00	6	35.1833	9.06651	3.70139
	2.00	5	45.6600	10.11103	4.52179
LBW_B	1.00	11	45341.27	8227.06300	2480.553
	2.00	8	46391.50	11271.96674	3985.242
LBW_F	1.00	6	43249.83	10191.83539	4160.799
	2.00	6	48223.17	14433.68900	5892.529
SBP_B	1.00	12	128.8667	9.16667	2.64619
	2.00	8	129.7625	11.99666	4.24146
SBP_F	1.00	6	128.0000	11.77268	4.80618
	2.00	4	143.2000	11.11246	5.55623
DBP_B	1.00	11	85.2909	4.98065	1.50172
	2.00	8	86.6750	5.20707	1.84098
DBP_F	1.00	5	81.4200	5.22178	2.33525
	2.00	4	87.2750	6.07310	3.03655
NA_B	1.00	13	126.1538	64.25450	17.82099
	2.00	11	89.4545	41.77646	12.59608
NA_F	1.00	6	110.1667	50.21719	20.50108
	2.00	7	123.8571	31.12571	11.76441

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
AGE	Equal variances assumed	4.760	.039	.342	24	.736	.70	2.056	-3.540	4.945
	Equal variances not assumed			.327	16.106	.748	.70	2.151	-3.855	5.260
BMI_B	Equal variances assumed	.735	.400	-1.280	22	.214	-1.8250	1.42561	-4.78154	1.13154
	Equal variances not assumed			-1.280	21.036	.214	-1.8250	1.42561	-4.78941	1.13941
BMI_F	Equal variances assumed	4.553	.056	-1.611	11	.136	-2.5310	1.57124	-5.98922	.92732
	Equal variances not assumed			-1.677	9.938	.125	-2.5310	1.50962	-5.89744	.83554
MDRD	Equal variances assumed	5.590	.027	-1.129	22	.271	-7.9789	7.06938	-22.63988	6.68212
	Equal variances not assumed			-1.064	13.315	.306	-7.9789	7.49582	-24.13372	8.17596
SCR	Equal variances assumed	7.439	.012	.268	22	.792	.0238	.08886	-.16050	.20805
	Equal variances not assumed			.251	12.716	.806	.0238	.09465	-.18118	.22873
TFAT_B	Equal variances assumed	1.139	.301	-1.915	17	.072	-7.0920	3.70295	-14.90458	.72049
	Equal variances not assumed			-1.800	11.571	.098	-7.0920	3.93961	-15.71113	1.52704
TFAT_F	Equal variances assumed	.000	.987	-1.813	9	.103	-10.4767	5.77970	-23.55127	2.59793
	Equal variances not assumed			-1.793	8.208	.110	-10.4767	5.84353	-23.89263	2.93930
LBW_B	Equal variances assumed	.729	.405	-.235	17	.817	-1050.2273	4460.0634	-10460.1	8359.684
	Equal variances not assumed			-.224	12.193	.827	-1050.2273	4694.1769	-11260.0	9159.535
LBW_F	Equal variances assumed	2.080	.180	-.689	10	.506	-4973.3333	7213.4699	-21045.9	11099.28
	Equal variances not assumed			-.689	8.993	.508	-4973.3333	7213.4699	-21293.2	11346.54
SBP_B	Equal variances assumed	.463	.505	-.189	18	.852	-.8958	4.72844	-10.82992	9.03825
	Equal variances not assumed			-.179	12.322	.861	-.8958	4.99923	-11.75675	9.96508
SBP_F	Equal variances assumed	.186	.677	-2.042	8	.075	-15.2000	7.44228	-32.36192	1.96192
	Equal variances not assumed			-2.069	6.863	.078	-15.2000	7.34650	-32.64201	2.24201
DBP_B	Equal variances assumed	.539	.473	-.587	17	.565	-1.3841	2.35820	-6.35946	3.59128
	Equal variances not assumed			-.583	14.821	.569	-1.3841	2.37579	-6.45329	3.68510
DBP_F	Equal variances assumed	.245	.636	-1.558	7	.163	-5.8550	3.75827	-14.74189	3.03189
	Equal variances not assumed			-1.528	6.019	.177	-5.8550	3.83067	-15.22116	3.51116
NA_B	Equal variances assumed	.987	.331	1.623	22	.119	36.6993	22.60747	-10.18573	83.58433
	Equal variances not assumed			1.682	20.766	.108	36.6993	21.82313	-8.71558	82.11418
NA_F	Equal variances assumed	5.700	.036	-.601	11	.560	-13.6905	22.76750	-63.80142	36.42046
	Equal variances not assumed			-.579	8.103	.578	-13.6905	23.63675	-68.07662	40.69567

Group Statistics

	1=TT:2 TC+CC	N	Mean	Std. Deviation	Std. Error Mean
K_B	1.00	13	47.3538	11.39339	3.15996
	2.00	11	55.2727	31.38070	9.46164
K_F	1.00	6	52.4167	12.65487	5.16633
	2.00	7	83.4000	33.95566	12.83403
TV_B	1.00	13	1822.8462	607.52090	168.49598
	2.00	11	1902.9091	926.38744	279.31632
TV_F	1.00	6	1621.8333	545.02456	222.50534
	2.00	7	2245.0000	806.91284	304.98439
PA_B	1.00	14	63.6429	42.42932	11.33971
	2.00	12	59.0000	33.01515	9.53065
PA_F	1.00	6	37.0000	19.35975	7.90359
	2.00	8	79.3750	30.44404	10.76359
VO2REL_B	1.00	12	22.1083	2.62902	.75893
	2.00	12	22.3750	3.52294	1.01699
VO2REL_F	1.00	6	25.5833	4.25602	1.73751
	2.00	7	26.0853	7.21090	2.72546
VO2AB_B	1.00	12	1.7345	.31795	.09178
	2.00	12	1.8707	.42520	.12274
VO2AB_F	1.00	6	1.8498	.44893	.18328
	2.00	7	2.1807	.74231	.28057
WGT_B	1.00	12	78.1417	8.54469	2.46664
	2.00	12	83.6417	12.43612	3.59000
WGT_F	1.00	6	71.4833	6.78157	2.76856
	2.00	7	80.9000	12.27341	4.63891
CHGSBP	1.00	6	-1.2833	7.55630	3.08485
	2.00	4	10.9250	9.04668	4.52334
CHDBP	1.00	5	-3.3200	5.03905	2.25353
	2.00	4	1.2750	5.23155	2.61578
CHGK	1.00	6	4.9167	18.97329	7.74581
	2.00	7	17.6000	35.02551	13.23840
CHGNA	1.00	6	-9.1667	42.22519	17.23836
	2.00	7	14.4286	45.62842	17.24592
CHGPA	1.00	6	-16.5000	29.11185	11.88486
	2.00	8	8.2500	27.57198	9.74817

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
K_B	Equal variances assumed	6.391	.019	-.849	22	.405	-7.9189	9.32777	-27.26348	11.42572
	Equal variances not assumed			-.794	12.228	.442	-7.9189	9.97536	-29.60839	13.77063
K_F	Equal variances assumed	3.302	.097	-2.102	11	.059	-30.9833	14.73742	-63.42017	1.45350
	Equal variances not assumed			-2.240	7.855	.056	-30.9833	13.83486	-62.98967	1.02300
TV_B	Equal variances assumed	1.081	.310	-.254	22	.802	-80.0629	315.05053	-733.438	573.31186
	Equal variances not assumed			-.245	16.754	.809	-80.0629	326.20316	-769.064	608.93775
TV_F	Equal variances assumed	.346	.568	-1.600	11	.138	-623.1667	389.51300	-1480.48	234.14567
	Equal variances not assumed			-1.651	10.513	.128	-623.1667	377.52365	-1458.81	212.47824
PA_B	Equal variances assumed	1.260	.273	.307	24	.761	4.6429	15.10728	-26.53705	35.82276
	Equal variances not assumed			.313	23.811	.757	4.6429	14.81291	-25.94233	35.22804
PA_F	Equal variances assumed	1.614	.228	-2.972	12	.012	-42.3750	14.25623	-73.43665	-11.31335
	Equal variances not assumed			-3.173	11.786	.008	-42.3750	13.35371	-71.52881	-13.22119
VO2REL_B	Equal variances assumed	1.231	.279	-.210	22	.835	-.2667	1.26895	-2.89831	2.36498
	Equal variances not assumed			-.210	20.352	.836	-.2667	1.26895	-2.91073	2.37739
VO2REL_F	Equal variances assumed	6.208	.030	-.149	11	.884	-.5020	3.36559	-7.90956	6.90565
	Equal variances not assumed			-.155	9.905	.880	-.5020	3.23220	-7.71312	6.70922
VO2AB_B	Equal variances assumed	1.270	.272	-.888	22	.384	-.1362	.15327	-.45402	.18169
	Equal variances not assumed			-.888	20.372	.385	-.1362	.15327	-.45550	.18317
VO2AB_F	Equal variances assumed	3.842	.076	-.950	11	.363	-.3309	.34840	-1.09771	.43595
	Equal variances not assumed			-.987	10.023	.347	-.3309	.33512	-1.07735	.41559
WGT_B	Equal variances assumed	1.139	.297	-1.263	22	.220	-5.5000	4.35573	-14.53324	3.53324
	Equal variances not assumed			-1.263	19.493	.222	-5.5000	4.35573	-14.60107	3.60107
WGT_F	Equal variances assumed	1.309	.277	-1.667	11	.124	-9.4167	5.64824	-21.84835	3.01502
	Equal variances not assumed			-1.743	9.577	.113	-9.4167	5.40227	-21.52605	2.69272
CHGSBP	Equal variances assumed	.476	.510	-2.321	8	.049	-12.2083	5.25900	-24.33561	-.08106
	Equal variances not assumed			-2.230	5.700	.070	-12.2083	5.47512	-25.77838	1.36172
CHDBP	Equal variances assumed	.007	.935	-1.337	7	.223	-4.5950	3.43623	-12.72040	3.53040
	Equal variances not assumed			-1.331	6.444	.228	-4.5950	3.45264	-12.90428	3.71428
CHGK	Equal variances assumed	.401	.539	-.790	11	.446	-12.6833	16.05513	-48.02043	22.65377
	Equal variances not assumed			-.827	9.478	.429	-12.6833	15.33795	-47.11512	21.74845
CHGNA	Equal variances assumed	.568	.467	-.961	11	.357	-23.5952	24.54279	-77.61356	30.42309
	Equal variances not assumed			-.968	10.910	.354	-23.5952	24.38407	-77.31831	30.12783
CHGPA	Equal variances assumed	.144	.711	-1.624	12	.130	-24.7500	15.24260	-57.96077	8.46077
	Equal variances not assumed			-1.610	10.573	.137	-24.7500	15.37130	-58.74962	9.24962

Univariate Analysis in African descent TT vs. TC+CC
Covariate: baseline plasma aldosterone levels
Dependent variable: change in plasma aldosterone levels

Between-Subjects Factors

		N
1=TT:2 TC+CC	1.00	6
	2.00	8

Descriptive Statistics

Dependent Variable: CHGPA

1=TT:2 TC+CC	Mean	Std. Deviation	N
1.00	-16.5000	29.11185	6
2.00	8.2500	27.57198	8
Total	-2.3571	29.94766	14

Levene's Test of Equality of Error Variances^a

Dependent Variable: CHGPA

F	df1	df2	Sig.
1.839	1	12	.200

Tests the null hypothesis that the error variance of the dependent variable is equal across groups.

a. Design: Intercept+PA_B+CY_CODE

Tests of Between-Subjects Effects

Dependent Variable: CHGPA

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	6335.668 ^a	2	3167.834	6.546	.013	.543
Intercept	2566.653	1	2566.653	5.303	.042	.325
PA_B	4235.453	1	4235.453	8.752	.013	.443
CY_CODE	3755.146	1	3755.146	7.759	.018	.414
Error	5323.547	11	483.959			
Total	11737.000	14				
Corrected Total	11659.214	13				

a. R Squared = .543 (Adjusted R Squared = .460)

2. 1=TT:2 TC+CC

Dependent Variable: CHGPA

1=TT:2 TC+CC	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
1.00	-21.953 ^a	9.168	-42.132	-1.774
2.00	12.340 ^a	7.900	-5.048	29.727

a. Covariates appearing in the model are evaluated at the following values: PA_B = 63.5714.

Univariate Analysis in African descent TC+CC

Covariate: baseline plasma aldosterone levels

Dependent variable: change in plasma aldosterone levels

Descriptive Statistics

Dependent Variable: CHGPA

Mean	Std. Deviation	N
-16.5000	29.11185	6

Tests of Between-Subjects Effects

Dependent Variable: CHGPA

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	2992.085 ^a	1	2992.085	9.610	.036	.706
Intercept	660.950	1	660.950	2.123	.219	.347
PA_B	2992.085	1	2992.085	9.610	.036	.706
Error	1245.415	4	311.354			
Total	5871.000	6				
Corrected Total	4237.500	5				

a. R Squared = .706 (Adjusted R Squared = .633)

Grand Mean

Dependent Variable: CHGPA

Mean	Std. Error	95% Confidence Interval	
		Lower Bound	Upper Bound
-16.500 ^a	7.204	-36.500	3.500

a. Covariates appearing in the model are evaluated at the following values: PA_B = 53.5000.

Univariate Analysis in African descent TT
Covariate: change in BMI
Dependent variable: change in 24-hour Na⁺ excretion

Descriptive Statistics

Dependent Variable: CHGNA

Mean	Std. Deviation	N
14.4286	45.62842	7

Tests of Between-Subjects Effects

Dependent Variable: CHGNA

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	8298.154 ^a	1	8298.154	9.894	.026	.664
Intercept	7317.986	1	7317.986	8.725	.032	.636
CHGBMI	8298.154	1	8298.154	9.894	.026	.664
Error	4193.560	5	838.712			
Total	13949.000	7				
Corrected Total	12491.714	6				

a. R Squared = .664 (Adjusted R Squared = .597)

Grand Mean

Dependent Variable: CHGNA

Mean	Std. Error	95% Confidence Interval	
		Lower Bound	Upper Bound
14.429 ^a	10.946	-13.709	42.566

a. Covariates appearing in the model are evaluated at the following values: CHGBMI = -1.6000.

Dependent T-test Individuals of African descent TT Genotype Group

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	BMI_B	25.9333	6	2.15932	.88154
	BMI_F	25.5833	6	1.99741	.81544
Pair 2	TFAT_B	35.3167	6	7.34586	2.99893
	TFAT_F	35.1833	6	9.06651	3.70139
Pair 3	LBW_B	43485.00	6	9850.52496	4021.460
	LBW_F	43249.83	6	10191.83539	4160.799
Pair 4	SBP_B	129.2833	6	9.14799	3.73465
	SBP_F	128.0000	6	11.77268	4.80618
Pair 5	DBP_B	84.7400	5	5.08360	2.27346
	DBP_F	81.4200	5	5.22178	2.33525
Pair 6	NA_B	119.3333	6	44.69750	18.24768
	NA_F	110.1667	6	50.21719	20.50108
Pair 7	K_B	47.5000	6	15.01666	6.13052
	K_F	52.4167	6	12.65487	5.16633
Pair 8	TV_B	1668.8333	6	384.75988	157.07756
	TV_F	1621.8333	6	545.02456	222.50534
Pair 9	PA_B	53.5000	6	35.67492	14.56423
	PA_F	37.0000	6	19.35975	7.90359
Pair 10	VO2REL_B	22.1333	6	2.20151	.89876
	VO2REL_F	25.5833	6	4.25602	1.73751
Pair 11	VO2AB_B	1.6140	6	.27134	.11077
	VO2AB_F	1.8498	6	.44893	.18328
Pair 12	WGT_B	72.5000	6	7.68583	3.13773
	WGT_F	71.4833	6	6.78157	2.76856

Paired Samples Test

		Paired Differences				t	df	Sig. (2-tailed)	
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	BMI_B - BMI_F	.3500	.78166	.31911	-.4703	1.1703	1.097	5	.323
Pair 2	TFAT_B - TFAT_F	.1333	2.76164	1.12744	-2.7648	3.0315	.118	5	.910
Pair 3	LBW_B - LBW_F	235.1667	1065.39165	34.94432	-882.8933	353.2266	.541	5	.612
Pair 4	SBP_B - SBP_F	1.2833	7.55630	3.08485	-6.6465	9.2132	.416	5	.695
Pair 5	DBP_B - DBP_F	3.3200	5.03905	2.25353	-2.9368	9.5768	1.473	4	.215
Pair 6	NA_B - NA_F	9.1667	42.22519	17.23836	-35.1460	53.4793	.532	5	.618
Pair 7	K_B - K_F	-4.9167	18.97329	7.74581	-24.8279	14.9946	-.635	5	.553
Pair 8	TV_B - TV_F	47.0000	628.41579	56.54967	-612.4819	706.4819	.183	5	.862
Pair 9	PA_B - PA_F	16.5000	29.11185	11.88486	-14.0510	47.0510	1.388	5	.224
Pair 10	VO2REL_B - VO2REL_F	-3.4500	2.38390	.97322	-5.9518	-.9482	-3.545	5	.016
Pair 11	VO2AB_B - VO2AB_F	-.2358	.17882	.07300	-.4235	-.0482	-3.230	5	.023
Pair 12	WGT_B - WGT_F	1.0167	2.19765	.89719	-1.2896	3.3230	1.133	5	.309

Regression Among the Individuals of African descent: TT Genotype Group
Predictor: change in plasma aldosterone levels
Dependent variable: change in SBP

Descriptive Statistics

	Mean	Std. Deviation	N
CHGSBP	-1.2833	7.55630	6
CHGPA	-16.5000	29.11185	6

Correlations

		CHGSBP	CHGPA
Pearson Correlation	CHGSBP	1.000	-.383
	CHGPA	-.383	1.000
Sig. (1-tailed)	CHGSBP	.	.227
	CHGPA	.227	.
N	CHGSBP	6	6
	CHGPA	6	6

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.383 ^a	.147	-.067	7.80467

a. Predictors: (Constant), CHGPA

ANOVA^b

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	41.837	1	41.837	.687	.454 ^a
	Residual	243.652	4	60.913		
	Total	285.488	5			

a. Predictors: (Constant), CHGPA

b. Dependent Variable: CHGSBP

Regression Among the Individuals of African descent: TT Genotype Group
Predictor: change in plasma aldosterone levels
Dependent variable: change in DBP

Descriptive Statistics

	Mean	Std. Deviation	N
CHDBP	-3.3200	5.03905	5
CHGPA	-20.4000	30.74573	5

Correlations

		CHDBP	CHGPA
Pearson Correlation	CHDBP	1.000	-.678
	CHGPA	-.678	1.000
Sig. (1-tailed)	CHDBP	.	.104
	CHGPA	.104	.
N	CHDBP	5	5
	CHGPA	5	5

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.678 ^a	.460	.280	4.27553

a. Predictors: (Constant), CHGPA

ANOVA^b

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	46.727	1	46.727	2.556	.208 ^a
	Residual	54.841	3	18.280		
	Total	101.568	4			

a. Predictors: (Constant), CHGPA

b. Dependent Variable: CHDBP

Regression Among the Individuals of African descent: TT Genotype Group
Predictor: change in plasma aldosterone levels
Dependent variable: change in 24-hour Na⁺ excretion

Descriptive Statistics

	Mean	Std. Deviation	N
CHGNA	-9.1667	42.22519	6
CHGPA	-16.5000	29.11185	6

Correlations

		CHGNA	CHGPA
Pearson Correlation	CHGNA	1.000	-.501
	CHGPA	-.501	1.000
Sig. (1-tailed)	CHGNA	.	.156
	CHGPA	.156	.
N	CHGNA	6	6
	CHGPA	6	6

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.501 ^a	.251	.063	40.86498

a. Predictors: (Constant), CHGPA

ANOVA^b

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	2235.046	1	2235.046	1.338	.312 ^a
	Residual	6679.788	4	1669.947		
	Total	8914.833	5			

a. Predictors: (Constant), CHGPA

b. Dependent Variable: CHGNA

Regression Among the Individuals of African descent: TC+CC Genotype Group
Predictor: change in plasma aldosterone levels
Dependent variable: change in SBP

	Mean	Std. Deviation	N
CHGSBP	10.9250	9.04668	4
CHGPA	2.2500	24.39091	4

Descriptive Statistics

Correlations

		CHGSBP	CHGPA
Pearson Correlation	CHGSBP	1.000	-.353
	CHGPA	-.353	1.000
Sig. (1-tailed)	CHGSBP	.	.323
	CHGPA	.323	.
N	CHGSBP	4	4
	CHGPA	4	4

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.353 ^a	.125	-.313	10.36502

a. Predictors: (Constant), CHGPA

ANOVA^b

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	30.660	1	30.660	.285	.647 ^a
	Residual	214.867	2	107.434		
	Total	245.527	3			

a. Predictors: (Constant), CHGPA

b. Dependent Variable: CHGSBP

Regression Among the Individuals of African descent: TC+CC Genotype Group
Predictor: change in plasma aldosterone levels
Dependent variable: change in DBP

Descriptive Statistics

	Mean	Std. Deviation	N
CHDBP	1.2750	5.23155	4
CHGPA	2.2500	24.39091	4

Correlations

		CHDBP	CHGPA
Pearson Correlation	CHDBP	1.000	-.723
	CHGPA	-.723	1.000
Sig. (1-tailed)	CHDBP	.	.139
	CHGPA	.139	.
N	CHDBP	4	4
	CHGPA	4	4

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.723 ^a	.522	.284	4.42814

a. Predictors: (Constant), CHGPA

ANOVA^b

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	42.891	1	42.891	2.187	.277 ^a
	Residual	39.217	2	19.608		
	Total	82.107	3			

a. Predictors: (Constant), CHGPA

b. Dependent Variable: CHDBP

Regression Among the Individuals of African descent: TC+CC Genotype Group
Predictor: change in plasma aldosterone levels
Dependent variable: change in 24-hour Na⁺ excretion

Descriptive Statistics

	Mean	Std. Deviation	N
CHGNA	14.4286	45.62842	7
CHGPA	3.8571	26.58589	7

Correlations

		CHGNA	CHGPA
Pearson Correlation	CHGNA	1.000	.588
	CHGPA	.588	1.000
Sig. (1-tailed)	CHGNA	.	.082
	CHGPA	.082	.
N	CHGNA	7	7
	CHGPA	7	7

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.588 ^a	.346	.215	40.42110

a. Predictors: (Constant), CHGPA

ANOVA^b

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	4322.388	1	4322.388	2.645	.165 ^a
	Residual	8169.326	5	1633.865		
	Total	12491.714	6			

a. Predictors: (Constant), CHGPA

b. Dependent Variable: CHGNA

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