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PHYSICAL ACTIVITY AND ANGIOTENSIN I-CONVERTING ENZYME POLYMORPHISM EFFECT ON CARDIOVASCULAR DISEASE RISK FACTORS IN YOUNG ADULTS

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Jeremy Lynn Knous

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PHYSICAL ACTIVITY AND ANGIOTENSIN I-CONVERTING ENZYME POLYMORPHISM EFFECT ON CARDIOVASCULAR DISEASE RISK FACTORS IN YOUNG ADULTS

By

Jeremy Lynn Knous

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ABSTRACT

PHYSICAL ACTIVITY AND ANGIOTENSIN I-CONVERTING ENZYME POLYMORPHISM EFFECT ON CARDIOVASCULAR DISEASE RISK FACTORS IN YOUNG ADULTS

By

Jeremy Lynn Knous

Cardiovascular disease (CVD) is a multifactorial malady with several modifiable risk factors including high blood pressure, unhealthy cholesterol levels, smoking, obesity, and physical inactivity. While CVD primarily affects older adults, younger individuals are not immune from the disease and/or its risk factors. Young adulthood (20-30 years of age) may be a pivotal time in the progression of CVD and its risk factors due to lifestyle choices related to food and beverage consumption, smoking, aerobic fitness, and physical activity (PA). Of interest was whether the well-documented decline in PA from youth to adulthood may trigger a genetic predisposition to CVD, resulting in a negative effect on health. For example, it is possible that a person's PA behavior may influence the expression of genetic components such as the angiotensin I-converting enzyme (ACE) polymorphisms. The purpose of this study was to determine how PA and the ACE polymorphism affect CVD risk factors in young adults. METHODS: 191 college students enrolled in a healthy lifestyles class completed: 1) an online survey assessing demographics and PA participation, 2) a CVD risk factor assessment of blood pressure, blood cholesterol, body mass index, percent fat and aerobic fitness and 3) collection

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of mouth buccal cells for genetic testing of the ACE polymorphism. RESULTS: The majority of the sample was female (69%), Caucasian (84%), non-smoking (85%), and of normal weight based on BMI (72%). Ninety percent reported meeting PA guidelines. There was a gender effect for PA and percent fat, with males being more active and having less body fatness. Physical activity showed an inverse relationship with percent fat, but was not associated with other risk factors. Those with the *DD* genotype of the ACE polymorphism had the highest levels of PA and aerobic fitness. There was no association between the ACE polymorphism and other modifiable CVD risk factors. In addition, PA did not modify relationships between the ACE polymorphism and any CVD risk factors. CONCLUSION: We showed that PA may affect body composition but not other modifiable CVD risk factors in healthy young adults. While the ACE *DD* genotype in healthy young adults may associate with higher PA and aerobic fitness, relationships did not exist with other modifiable CVD risk factors. Potential reasons for our findings include the multifactorial nature of CVD risk factors, the nature of the PA and ACE I/D polymorphism relationship, the relative good health of the study participants, and/or the inadequate statistical power with final sample size. Further studies should focus on the understanding of gene by environmental interaction, the nature of the interaction, and timing or triggers of the interaction.

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CHAPTER 1

INTRODUCTION

Cardiovascular disease (CVD) is the number one killer in the United States, accounting for greater than 831,000 deaths or approximately 34% of all mortalities in 2005.[109,136] It is estimated that more than one in every three adults has at least one form of CVD, that may manifest as myocardial infarction, stroke, heart failure, etc.[109] CVD is a multifactorial disease with several modifiable risk factors including high blood pressure (BP), elevated total cholesterol (TC), elevated low density lipoprotein (LDL) cholesterol concentration, low high density lipoprotein (HDL) cholesterol concentration, smoking, obesity, and physical inactivity.[9] The prevalence of these risk factors is staggering in adults. It is estimated that one in three adults has high blood pressure, while an additional 28% of Americans are diagnosed as pre-hypertensive.[109] In 2006, the prevalence of individuals 20 years of age or greater having borderline (≥200 mg/dl) or high (≥240 mg/dl) total cholesterol was 47% and 16%, respectively.[109] Further, 16% of adults over 20 years of age had low HDL cholesterol (<40 mg/dl)[109] and 21% of Americans greater than 18 years of age are current smokers.[109] According to body mass index (BMI) criteria, 66% of US adults are considered overweight (BMI = 25.0-29.9 kg/m^2) with approximately 32% being classified as obese (BMI ≥ 30 kg/m^2).[109] In addition, only 32.5% percent of Americans meet American College of Sports Medicine (ACSM) recommended guidelines for physical activity (PA),[109]

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defined as ≥30 minutes of moderate intensity activity performed on five or more days per week or ≥20 minutes of vigorous PA performed on three or more days of the week.[86] These modifiable risk factors are often accompanied by other morbidities such as type II diabetes mellitus, stress, alcohol consumption or non-modifiable risk factors such as age, race and gender. Thus, it is not surprising that the incidence and mortality of CVD is high in American adults.

While CVD is considered a disease of older age, younger individuals are not immune from the disease and/or its risk factors. Although CVD risk factors in children and adolescents do not have the same effect on morbidity and mortality as in older adults,[62] the presence of these risk factors in early life leads to earlier and/or more severe development of CVD later in life. Children, even infants, can have high BP that could potentially contribute to early development of CVD.[111] The prevalence of high BP in US children has been reported to be 1-3%, but this statistic appears to be increasing.[84] The National Health and Nutrition Examination Survey (NHANES) III reported that approximately one fourth of children have borderline high (170-199 mg/dL) TC, with another 11% being in the elevated (>200 mg/dL) TC category.[11,204] This finding is alarming since high plasma lipid values tend to track into adulthood. To further illustrate the importance of controlling cholesterol levels in children, the American Academy of Pediatrics issued a position stand stating that pharmacologic intervention be considered for children with LDL cholesterol concentration ≥190 mg/dL.[57] Further, the prevalence of overweight and

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obesity in US children is rising and has become one of the most common pediatric disorders.[155] According to the 2003-2004 NHANES data, 17% of children and adolescents are overweight, as defined by having an age- and sex-specific BMI greater than or equal to the 95th percentile.[160] Further, since the early 1970's, the prevalence of adolescents classified as overweight has increased. This documented evidence is alarming given that children are demonstrating the same co-morbidities as their adult counterparts, only decades earlier.[60] Among high school adolescents, only 44% of males and 28% of females meet the recommended level of leisure time PA.[65,160]

CVD risk factors act independently or cluster synergistically to cause disease.[148] Clustering is the phenomenon of risk factors occurring together more frequently than expected by chance.[148] For example, overweight and obesity have been shown to cluster with other CVD risk factors such as high BP and unfavorable lipid profiles. These risk factors have also been found to cluster in children.[74] From the Bogalusa Heart study data, Berenson et al.[31] reported that as the number of CVD risk factors increased, so did the severity of atherosclerosis in youth. Specifically, 60% of overweight children 5-10 years of age had one additional CVD risk factor while more than 20% of them had two or more CVD risk factors.[74] Further, it has been shown that overweight children and adolescents are approximately 2-3 times more likely to have high TC, seven times more likely to have elevated triglycerides, and approximately three times more likely to have high BP.[74,175] This demonstrates the detrimental effects of pediatric obesity and risk factor clustering.

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Research shows that clustered CVD risk factors track strongly from adolescence to young adulthood,[26] signifying a need for increased research in this area. The Cardiovascular Risk in Young Finns study reported that 25% of the participants initially identified as at risk for CVD during adolescents (i.e., having three risk factors - high TC, low HDL cholesterol, and high diastolic BP) remained in the highest categories as young adults.[148] In support of these findings, the Danish youth study showed that participants with clustered risk factors at baseline had a higher probability (odd ratio 6.0) of clustering during the follow-up.[26] Thus, not only do risk factors cluster in youth and adolescence, they tend to track into adulthood. Individual risk factors during childhood can predispose an individual to an increased risk of developing CVD, but when clustered together, risk factors can accelerate the onset and/or severity of the disease. Those experiencing risk factors during childhood and adolescents are at the highest risk as an adult.[156] Therefore, reducing the prevalence of modifiable CVD risk factors in children, adolescents and young adults could lead to delay and reduce severity of CVD in the future.

As has been discussed previously, CVD risk factors often originate during childhood and their prevalence intensifies until the disease manifestation in adulthood. Young adulthood (20-30 years of age) is a pivotal time in the progression of CVD and its risk factors. However, there is a paucity of research on this "generational change" with respect to cardiovascular health. Studies on college students can provide data that is currently lacking to bridge the "generational change" gap from adolescence to adulthood.

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According to The US Department of Education's Digest of Education Statistics 2006, a record 17.5 million students were enrolled in colleges or universities and 28.4% of all young adults between ages 25-29 have completed a bachelor's degree or higher.[6] In addition, the 2003 US Census Bureau report showed that nearly 53% of the population has completed some college.[178] The young adult population represents an important key in how CVD risk factors track and/or intensify into adulthood, as young adults are exposed to significant changes associated with moving away from home and living with others going through similar experiences. This "generational change" consists of transforming from parental dependence to virtual independence and all the accompanying consequences.[170] Examples of this independence include paying bills, managing money, living independently and choosing lifestyle behaviors.[170] Choices related to sleep cycles, food and beverages consumption, smoking and PA behavior could all affect the health of young adults. Thus, environmental exposures for a college student may act independently or interact with other factors, both environmental and genetic, to influence health. While new lifestyle influences may not manifest themselves immediately as disease, it is possible that lifestyle decisions made during this "generational change", like smoking, alcohol use, unhealthy eating or lack of PA, could drastically affect the development of chronic ailments such as CVD.

A decline in PA from adolescence to adulthood has been documented.[146,181] According to Youth Risk Behavior Surveillance System (YRBSS) data, 28% and 44% of adolescent females and males, respectively, are meeting

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current PA recommendations.[7,65] A Finnish longitudinal study of 12 to 27 year olds showed a decrease (20-55% gender dependent) in PA with age.[187] Physical activity participation decreases from youth to adulthood.[46,165,187]

The decline in PA during the "generational change" may potentially have a negative effect on cardiovascular health. It is suspected that environmental factors such as diet, body composition, physical inactivity, smoking, alcohol consumption, etc., account for the majority of chronic health conditions; accounting for at least 60% of CVD.[36] Physical inactivity has been shown to increase the incidence of CVD both independently and in combination with other risk factors.[71]

Environmental factors are thought to influence genes. An environmental influence may alter gene expression, resulting in a phenotype that surpasses a threshold of biological significance, invoking the appearance of pathologic states.[36] Further, responses to environmental factors are often variable among individuals due to genetic differences. In many cases, a genetic profile results in disease development only after being triggered by the presence of necessary environmental factors.[36,159] Thus, an individual's genotype combined with environmental factors leads to individualized susceptibilities to health problems, such as CVD.

Genetic differences may be due to the functional role of polymorphisms, or variations in the DNA sequence. A CVD related polymorphism of interest is the angiotensin-I converting enzyme (ACE) insertion (*I*)/deletion (*D*) polymorphism. The ACE I/D polymorphism has been associated with the

development of CVD and associated with risk factors of high BP, high cholesterol and obesity. Specifically, the *DD* genotype is shown to be detrimental to cardiovascular health.[47,56,195]

Since genetic predisposition to a particular disease can be affected by the interaction of triggering environmental factors, it is possible that a person's PA behavior may influence the expression of the ACE I/D polymorphisms, potentially altering the phenotype. The interaction between environmental factors, such as PA, and genetic factors, like the ACE I/D polymorphism, could facilitate the early phenotypic expression of CVD or its risk factors.

To date, most ACE I/D polymorphism and CVD related studies have assessed older adults (> 50 years of age) suffering from myocardial infarctions, strokes or diagnosed CVD. Cambien et al.[42] reported that the *DD* genotype was more frequent in participants who suffered a myocardial infarction than those who had not. Furthermore, a meta-analysis by Sayed-Tabatabaei et al.[168] reported a significant positive association between the *D* allele and vessel wall thickness in the common carotid artery, a measure of atherosclerosis. Thus, studies indicate that older CVD patients with the ACE I/D polymorphism are associated with the expression of a diseased phenotype.

Although it is known that atherosclerosis begins in childhood with most individuals having some level of arterial plaque buildup by age fifteen,[180] research on the ACE I/D polymorphism and CVD risk factors in young adults is scarce. While the plausible association between ACE I/D polymorphism and

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CVD risk factors has been studied preliminarily [42,76,167,171] there are few definitive results.

It is important to evaluate CVD risk factors associated with genetic and environmental factors in young adults (college aged) during the "generational change," as this is a critical time for developing lasting health behaviors affecting CVD progression and/or severity. An understanding of the interaction between genetic (ACE I/D polymorphism) and environmental factors, such as PA, on the development of CVD risk factors in a young adult can assist in reducing the development and/or severity of diseased phenotypes and the future burden of CVD. Increased knowledge of CVD risk factors associated with the ACE I/D polymorphism and PA independently and their interaction (ACE-PA) will assist future researchers and clinicians to better understand the protective effect of PA, determine the change in risk factor profiles in response to PA, and facilitate effective intervention strategies.

CVD risk factors occur through a complex association between genetic factors, environmental factors, and their interactions. A major environmental effect on these risk factors is PA. With the decline of PA from youth to adulthood, the college years may represent a "generational change" that could significantly affect the development of CVD. Genetic determinants of CVD factors, such as the ACE I/D polymorphism, may be affected by physical inactivity. Therefore determining the contribution of PA, ACE I/D polymorphism, and their interaction during the "generational change" could provide information to curb the development and burden of CVD.

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Aims:

Specific Aim 1: To measure and describe physical activity, blood pressure, blood lipid (total cholesterol and HDL cholesterol) concentration, body mass index, and body fatness in young adults.

- The presentation of the distribution of these study participant characteristics will be descriptive and not hypothesis driven.

Specific Aim 2: To determine the association between physical activity and blood pressure, blood lipid concentration, body mass index and body fatness in young adults.

- Physical activity will be inversely associated with blood pressure, total cholesterol, body mass index, percent body fatness and directly associated with HDL cholesterol.

Specific Aim 3: To determine the association between ACE I/D polymorphism and blood pressure, blood lipid concentrations, body mass index, and body fatness in young adults.

- The *DD* genotype will be associated with increased blood pressure, total cholesterol, body mass index, percent body and inversely associated with HDL cholesterol.

Specific Aim 4: To explore whether physical activity modifies the effect of the ACE I/D polymorphism on blood pressure, blood lipid concentrations, body mass index and body fatness in a college students.

- Increased physical activity will suppress the relationships between the *DD* genotype and blood pressure, lipid concentration, body mass index, and body fatness.

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CHAPTER 2

LITERATURE REVIEW

L1 Brief Cardiovascular Disease Overview

It is well recognized that cardiovascular disease (CVD) is the number one killer in the United States.[136,160] Risk factors for CVD included high blood pressure (BP), high total cholesterol (TC), low high density lipoprotein cholesterol (HDL), obesity, smoking and physical inactivity. Cardiovascular disease and associated risk factors are present in younger individuals including children, adolescents, or young adults; without the same effect on morbidity and mortality as in older adults.[62] Risk factors and disease progression is multifactorial, with both environmental and genetic factors having significant roles. A primary environmental factor associated with CVD and risk factors is physical activity (PA) or aerobic fitness. The beneficial effects of PA or exercise on CVD risk factors may slow the progression and onset of CVD risk factors and disease. Physiologic responses to environmental factors, like PA, are often variable among individuals possibly due to genetic differences. In many cases, a genetic profile results in disease development only after being triggered by the presence of necessary environmental factors.[36,159] Thus, an individual's genotype combined with environmental factors leads to individualized susceptibilities to health problems, such as CVD.

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Genetic differences may be due to polymorphisms, or variations in the DNA sequence. A CVD related polymorphism of interest is the angiotensin-I converting enzyme (ACE) insertion (*I*)/deletion (*D*) polymorphism. The ACE I/D polymorphism has been associated with the development of CVD and linked with risk factors of high BP, high cholesterol and obesity. Understanding the relationship between the ACE I/D polymorphism, PA, and CVD is important for increased understanding of CVD progression.

L2 Angiotensin I-converting enzyme (ACE)

Angiotensin I-converting enzyme (ACE) is a major part of the renin-angiotensin system (RAS), a highly complicated hormonal / autocrine / paracrine system that controls the cardiovascular system, kidneys and adrenal glands. [100] This system regulates BP and fluid/electrolyte homeostasis. [66,100] ACE is responsible for the chief reaction in RAS, converting angiotensin I, an inactive decapeptide, into the active octapeptide angiotensin II. [43,56] Angiotensin II functions as a potent vasoconstrictor and has been linked to additional physiologic functions (to be discussed in subsequent sections). An additional function of ACE is to inactivate bradykinins and other peptides that are potent vasodilators.[58]

ACE is an acidic glycoprotein, composed of a metallopeptidase with an active zinc center,[66,69] that functions as a dipeptidyl carboxypeptidase to catalyze the hydrolysis of carboxy-terminal dipeptides from substrates.[66] ACE is located in a variety of tissues including, but not limited to, vascular, heart,

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brain, kidney, adipose as well as macrophages and smooth muscle cells of human atherosclerotic plaques.[58,87] ACE can be found in solution as part of blood plasma, lymph, amniotic and cerebrospinal fluids,[21,69] though it is thought that circulating ACE originates from endothelial cells.[56]

Results from ACE inhibitor studies have demonstrated that disrupting the RAS system can lead to reversals of cardiac fibrosis and vascular remodeling along with reductions in cardiac myocyte apoptosis.[153] Reductions in ACE levels have led to reduction in free radical generation and oxidative stress, both of which lead to atherosclerosis.[87]

L2.1 ACE role in the Renin-angiotensin system (RAS) and Kinin-Kallikrein system

RAS plays a role in the homeostasis of the cardiovascular system by functioning as a long-term regulator of BP and blood volume.[169] Renin is made and stored in the juxtoglomerular cells of the kidney and is released into the circulation under conditions of increases sodium, volume loss, and/or sympathetic activation.[56,169] Circulating renin converts angiotensinogen into, the inactive decapeptide, angiotensin I.[43,56,169] The key RAS reaction is the conversion of the angiotensin I into the active angiotensin II via ACE.[43,56,169] This conversion, that occurs in most tissues of the body,[2] is what generates the functional octapeptide, angiotensin II.

Angiotensin II functions to control BP directly by binding to receptors, predominately in the heart, smooth muscle, kidneys, and adrenal glands,

which leads to peripheral vasoconstriction and sodium re-absorption in the kidneys. Angiotensin II increases BP indirectly through the stimulating adrenal cortex and increases aldosterone production.[58,70,100,102] Additionally, angiotensin II augments the formation of endothelin, a peptide released by the lining of blood vessels, which has powerful vasoconstriction properties and has an established position in the development of hypertension as well as other cardiac conditions.[58]

Angiotensin II also plays a role in vascular smooth muscle cell modulation by directly inducing vascular endothelial growth factor.[58,70] This cell proliferation is mediated by the angiotensin II receptor type I[56] as a response to vascular inflammation and leads to the development of atherosclerosis. Angiotensin II interacts with pro-inflammatory cytokines to boost free radical production by activating oxidase.[58,100] Free radical generation can damage endothelial cells or stimulate vascular endothelial dysfunction. Nitric oxide, a potent vasodilator that also inhibits platelet aggregation, is decreased by the production of free radicals.[58] Thus, the beneficial effects of nitric oxide are suppressed by angiotensin II.[100]

ACE also functions as a type II kininase in the Kinin-Kallikrein system to breakdown kinins, which function as powerful vasodilators.[124] Kinins are a group of polypeptides that influence smooth muscle contraction, induce hypotension and increase blood flow. Kinins lead to the release of nitric oxide and exert anti-proliferation effects while opposing vasoconstrictor effects of angiotensin II.[173]

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The Kinin-Kallikrein system disrupts the coagulation/fibrinolysis process (making of/breaking down of blood clots) in a way that can lead to acute ischemic coronary events. Kinins, when bound to platelets, inhibit platelet aggregation thus blocking the effects of thrombin which functions to convert fibrinogen into fibrin.[121] Further, kinins inhibit the coagulation process by blocking the attachment of fibrinogens, precursors to fibrin, to platelets.[182] Kinins counter the coagulation processes by increasing fibrinolysis via bradykinin increasing the release of tissue plasminogen activator,[40] which when active, functions to create enzymes that breaks down clots into smaller pieces.

Within the Kinin-Kallikrein system, ACE functions at the surface of endothelial cells,[124] degrading 95% exogenous bradykinin after a single pass through the pulmonary system.[34] Bradykinin is a potent vasodilator that acts as an antagonist to proliferation, can increase coronary blood flow, and improves myocardial metabolism. ACE inhibitor studies suggest that some cardioprotective effects are elicited by the increase in bradykinin as well as other kinins.

L2.2 ACE ID polymorphism

Serum ACE levels are influenced by the ACE gene, located on the long arm of chromosome 17 at band 23 (17q23) and is divided into 26 exons that span 21 kilobases. This area contains a polymorphism characterized by either insertion (*I*) or deletion (*D*) of a 287 base pair fragment in intron 16.[197] Due to

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the I/D polymorphism being located in an intron, it is thought to be a neutral marker. However, the I/D polymorphism is in strong linkage disequilibrium (statistical association between alleles at separate but neighboring loci) with one, or possibly more, unknown variants.[147]

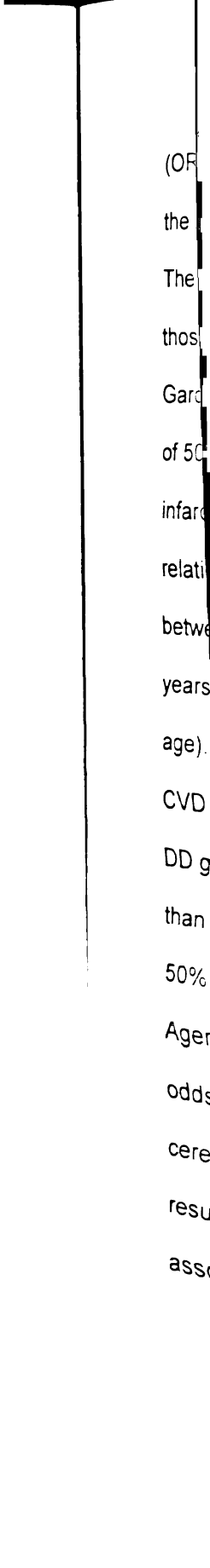
Conventional belief is that the *D* (deletion) allele of the ACE polymorphism is associated with CVD through increasing serum ACE levels, which would increase angiotensin II and decrease bradykinin activity thus enhancing the ACE double effect.[147] Rigat et al.[158] were the first to show that the ACE I/D polymorphism was associated with the amount of ACE enzyme available, with the *D* allele linked to higher enzyme levels. The presence of the *D* allele is associated with elevated vascular smooth muscle tone along with vasoconstriction.[147] Homozygotes for the *D* allele have been shown to have higher levels of plasminogen activator inhibitor-1(inhibitor of fibrinolysis), von Willebrand factor (marker of coagulation), and thromobomodulin (marker of coagulation),[98] thus associating the *D* allele with endothelial dysfunction. Further, the *D* allele is associated with increased CVD, including coronary artery disease and myocardial infarction.[56]

The ACE I/D polymorphism is not only associated with CVD but has been associated with PA and aerobic fitness. The *I* allele has been associated with improved endurance performance by enhancing the mechanical efficiency of trained muscle.[130,202] Further, the *I* allele has been associated with increased type 1 skeletal muscle fibers,[210] potentially explaining the association between the *I* allele and increased endurance performance. The

ACE I/D polymorphism has been associated with PA. A study on mild hypertensive individuals showed that a sedentary lifestyle was more common among those with the *DD* genotype and followed a linear trend of increased PA from *DD*→*ID*→*II*.[205] This result in no way implies that there is causal relationship between PA and the ACE polymorphism; it merely provides a potential explanation as to the association of the ACE polymorphism with CVD and PA.

L2.3 ACE ID Polymorphism Association with Hard CVD Endpoints

The ACE I/D polymorphism has been associated with hard CVD endpoints, like myocardial infarction (MI), stroke or mortality, as well as CVD risk factors (to be discussed later). Volzke et al.[200] assessed the hard endpoints of mortality, cardiovascular mortality, and recurrent coronary artery bypass graft in 249 patients who underwent an initial coronary artery bypass graft. The *DD* genotype was an independent predictor of total mortality after coronary artery bypass graft, accounting for approximately 10% of the variance. Further, the *DD* genotype accounted for approximately 18% of the total variance in mortality from cardiovascular events or recurrent coronary artery bypass graft.[200] Cambien et al[42] examined the association between the ACE ID polymorphism in a group of 610 patients with who suffered a myocardial infarction and compared them to 733 health controls. They found that the *DD* genotype was more frequent in those who suffered a myocardial infarction as compared to health controls, with the association being stronger



(OR: 3.2) in low risk cases.[42] Margaglione et al.[114] assessed the relationship of the ACE genotype with in 210 participants with and without a history of stroke. The *DD* genotype was more common in participant who suffered stroke than those who were stroke free.[114] In 2267 male Caucasian participants, Gardemann et al.[76] examined coronary artery disease, defined as a minimum of 50% stenosis of the coronary vessels, angina pectoris and acute myocardial infarction, defined using World Health organization criteria, to assess their relationship with the ACE ID polymorphism. They reported an association between the *D* allele and coronary artery disease in younger participant (< 61 years of age) and non-fatal myocardial infarction in older adults (>61 years of age).[76] Both of these associations were stronger in participants without other CVD risk factors.[76] Similar, Sekuri et al.[171] reported that participants with the DD genotype had higher risk (OR: 2.82) of developing premature (younger than 55 years of age for men) coronary heart disease (defined as greater than 50% stenosis of a major coronary artery).[171] Finally, in a meta-analysis Agerholm-Larsen et al.[17] compared the *DD* genotype to the *I* allele and found odds ratio for myocardial infarction, ischemic heart disease and ischemic cerebrovascular disease to be 1.21, 1.16, and 1.18, respectively. These results demonstrate that the DD genotype of the ACE ID polymorphism is associated with increased cardiovascular disease endpoints.

L2.4 ACE summary

The activation of the RAS, through the conversion of angiotensin I to angiotensin II via ACE, results in systemic vasopressor response and blocks the hypotensive effects of Kinin-Kallikrein system by ACE degrading bradykinin.[34,43,56,70,169] ACE activity also triggers free radical formation and release while decreasing nitric oxide activity.[58] Through these physiologic responses, ACE may have a role in CVD development.

The ACE I/D polymorphism is associated with the amount of ACE enzyme produced while also determining the metabolism of bradykinin.[134,147,158] The ACE I/D polymorphism is associated with many forms of CVD as well as PA and aerobic fitness.[56,130,205] Due to the formation of and degradation of vasoactive substances, future studies are need to tease out the effect, if any, of the ACE I/D polymorphism and its role with PA/fitness on CVD risk factors.

L3 ACE ID Polymorphism and Blood Pressure/Hypertension

The RAS regulates blood pressure (BP) through increases in blood volume and constriction of the vessels.[70] Homeostatic control of the RAS is mediated primarily through the production of angiotensin II. Angiotensin II increases BP via sodium re-absorption, by binding to specific receptors or by increasing aldosterone secretion, and through peripheral vasoconstriction.[70,100,102] The production of angiotensin II is controlled by ACE, which converts angiotensin I to angiotensin II. The concentration of

angiotensin II is dependent on the amount of angiotensin I and, more importantly, circulating ACE levels.[43] The greater the presence of ACE, the greater the production of angiotensin II. Plasma ACE levels have high inter-individual variability, with as much as a five-fold difference.[22,43,56] The ACE I/D polymorphism has been shown to be associated with differences in ACE levels,[191] accounting for 47% of the total variance.[158] Specifically, the *D* allele appears to be associated with higher ACE concentrations than the *I* allele.[158,191]

Genetic variability in ACE I/D polymorphism could partially explain the differences in resting BP and could contribute to the pathogenesis of hypertension. The *D* allele leads to more ACE enzyme which in turn produces more angiotensin II and an increase BP. In the Nancy study of 87 families (2 parents < 60 years of age and at least 1 child of > 10 years of age), similarities in plasma ACE levels were explained more by genetic factors than by the environment.[41] Genetics accounted for 29% of the ACE variance in parents and up to 75% in offspring.[41]

L3.1 Evidence for an association between ACE ID Polymorphism and BP/Hypertension

The ACE I/D polymorphism has been found to be associated with higher resting BP. The Rochester Family Heart Study performed linkage analyses among a cross-sectional sample of 1488 participants, to evaluate the contribution of the ACE gene region in systolic, diastolic and mean arterial

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pressures.[73] Participants were siblings with a mean age 15 years who were 3rd generation members of the Rochester Family Heart study. Investigators established that the ACE gene region contributed significantly to interindividual differences in resting BP, accounting for variances of 14% and 17% of diastolic BP and mean arterial pressure.[73] Among males, the ACE gene region accounted for 38%, 38% and 54% of the variance in systolic BP, diastolic BP and mean arterial pressure, respectively.[73] Results for females did not show significant differences in BP outcomes based on ACE genotype. Data collected on more than 3000 men and women participating in Framingham Heart Study found the odds of being hypertensive statistically greater in men having the *DD* genotype (OR=1.59, 95%CI=1.13–2.23) as compared to those having the *II* genotype.[138] Further, increases in diastolic BP were associated with the *DD* genotype.[138] Similarly, in a case-control study of 244 Croatian adults who were matched on age, sex, and place of residence, the *DD* genotype was more prevalent in hypertensive participants (OR=2.50, 95%CI=1.19–5.25) than in normotensive participants.[27] During a 6 year follow up study to the familial occurrence of hypertension, investigators assessed BP in a cohort 3500 Finnish children and reported that higher systolic BP in girls ≥ 9 years of age and boys ≥ 12 years of age was positively associated with family history of hypertension.[184] Further, boys with the *DD* genotype had higher systolic BP than those heterozygous or homozygous *II*.[184]

The ACE I/D polymorphism association with increased BP has also been shown in other ethnic samples, such as African Americans. In a case-

control study of 89 Black Americans from the Detroit Metropolitan area, the *D* allele frequency was more prevalent in hypertensive participants compared to the normotensive controls.[64] In a separate study of participants of Afro-Caribbean decent, the frequency of the *D* allele increased, with increases in BP from normal (52%) to hypertensive (70%) participants.[28] In a case-control study of Chinese participants > 40 years of age, the *D* allele was significantly associated with hypertension.[49] In the study sample, the hypertensive group was 1.46 (95%CI=1.08-1.99) times more likely to have a *D* allele then the normotensive group.[49] In subgroup analysis of elderly participants, defined as ≥65 years of age, the hypertensive group was 2.28 (95%CI=1.17-4.45) times more likely to have the *D* allele and the *DD* genotype than normotensives.[49] Further, when the elderly subgroup was stratified by gender, the odds of the hypertensive group having the *D* allele was 4.57 (95%CI=1.58-13.21) times that of the normotensive group.[49] A unique study by Uemura et al.[195] looked at the association between ACE I/D polymorphisms and BP patients post hospitalization. Patients showed a significantly higher systolic BP after being discharged from the hospital when they had the *D* allele.[195] In 197 healthy Israeli men between the age of 40 and 70 years, those who possessed the *D* allele had higher BP, both systolic and diastolic, than the *II* genotype group.[142] This result was unchanged even after controlling for age and BMI.[142]

The studies in this section illustrate a positive association between the ACE I/D polymorphism and BP or hypertension. This association is found in various ages and populations. Further, this association was found when using

several research strategies and various samples sizes. Thus, an association between BP and the ACE I/D Polymorphism is evident.

L3.2 Evidence against an association between ACE ID Polymorphism and BP/Hypertension

Not all studies have shown an association between the ACE genotype and BP or hypertension. In a cross-sectional study of 1975 non-Hispanic whites from Minnesota, the ACE I/D polymorphism was not associated with hypertension.[194] In separate Finnish adults studies the ACE genotypes were not associated with systolic or diastolic BP.[75,91]

In various case-control studies with participants including Germans, Hungarians, and Greeks ranging in age from 14 to 65, there was no association found between the ACE I/D polymorphism and BP or hypertension.[126,127,144,199] Within 178 Hungarian adolescents and young adults (14-20 years of age), the distribution of ACE genotypes did not differ between the hypertensive (N=120) and normotensive (n=58) groups.[144] Both groups had similar genotypic frequencies, with approximately 25% being *DD*, 45% being *ID* and 33% being *II*.[144] In 182 Greek participants, 98 of whom were hypertensive, the ACE I/D polymorphism had no association with presence of hypertension.[199] The genotype distribution (*DD*: 30.6% vs. 34.5%, *ID*: 45.9% vs. 47.6%, *II*: 23.5% vs. 17.8% for hypertensive vs. normotensive participants, respectively) and allele frequency (0.45 in hypertensive and 0.42 in normotensive participants) was similar between groups[199], establishing that

the ACE polymorphism was not involved in causing the hypertension in the case group. Mondorf et al.[126] found that in 246 Germans (53.6% male), 121 of whom were hypertensive, no difference was seen in genotype distribution between the hypertensive cases and healthy controls. The ACE concentration levels were graded within hypertensive patients based on genotype, with *DD* having the highest (46.7 ± 12.5 U/L), *ID* being intermediate (30.5 ± 9.3 U/L), and *II* having the lowest (20.3 ± 13.4 U/L) value.[126] In a separate study of 1358 Germans (48.4% male), 638 of whom were hypertensive, the genotypic frequency between the two groups, hypertensive and normotensive groups, was identical with 26% *DD*, 49% *ID*, and 23% *II*.[127]

Harrap et al.[85] utilized the four corners approach, which involves creating four groups representing different conditions to assess measured BP of both parental and adolescent participants. After grouping the participants based on high/low parental and high/low participants BP, no difference in genotype distribution was present among groups.[85] When regrouped by genotype, the *DD* group had the highest concentration of ACE enzyme while the *II* group had the lowest.[85]

In a study of 853 Hong Kong Chinese (41.6% male), the ACE I/D polymorphism had no relationship with BP.[188] In a Japanese cohort of approximately 1200 participants (36.1% male), the researchers looked at the association of the ACE I/D polymorphism and self-monitored home BP as well as ambulatory BP.[116] Neither the BP measures nor the prevalence of previous CVD differed among the three genotypes (*DD*, *ID*, *II*).[116] A cross-sectional

study involving BP screening of Japanese college students indicated that those who were found to be hypertensive did not differ in genotype distribution (17% *DD*, 33% *ID*, 50% *II*) or allele frequencies from that of the general Japanese population (10% *DD*, 43% *ID*, 47% *II*).[123]

Results of the various studies in this section, which were conducted using differing populations based on age and nationality, do not support the hypothesis that the ACE I/D polymorphism is associated with BP or hypertension. Inconsistencies in these negative findings compared to studies showing positive relationships may result from differing subject characteristics such as ethnicity, age, and sex.

L3.3 ACE ID Polymorphism and BP/Hypertension Summary

Based on the current literature, it is unclear whether the ACE I/D polymorphism is associated with BP or hypertension. In studies that declare an association, the nature of the relationship is the same with the *D* allele or the *DD* genotype having the highest BP value, the *ID* genotype being intermediate and the *I* allele or *II* genotype with the lowest BP values. There are numerous studies supporting[27,28,49,64,73,138,142,184,195] this association. However, there is also evidence among a variety of populations that refutes[75,85,91,116,123,126,127,144,188,194,199] an association that the ACE I/D polymorphism may have with BP or hypertension. The association between ACE polymorphism and BP remains ambiguous, possibly due to unidentified confounders and/or effect modifiers. Further work must be undertaken in this

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area to confirm or disprove the biologically plausible association between the ACE I/D polymorphism and BP or hypertension. By examining this association in young healthy, college-aged adults it may be possible to define the role of genetics with less chance of co-morbidities such as age, chronic disease, and other co-morbidities confounding the results.

L4 ACE ID Polymorphism and Lipids

Adverse levels of lipids and lipoproteins are recognized risk factors for CVD. Increases in TC, specifically through increases in low-density lipoproteins (LDL) and decreases in HDL concentrations, contribute to the development of CVD through the development of atherosclerosis. While hypercholesterolemia can lead to the development of atherosclerosis, this is not the only way for cholesterol to enhance the atherosclerotic process. Adverse molecular changes, such as oxidation of lipoproteins can also stimulate atherogensis. The ACE I/D polymorphism maybe associated with atherosclerotic process through increasing levels and/or changes made to the molecular structure of lipids.

At birth, TC, LDL and triglyceride levels are very low and increase sharply during the first year of life. By age two, cholesterol levels are equivalent to those of young adults,[31] with the bulk of this cholesterol consisting of LDL and HDL.[31] Total cholesterol and sub-fractions are similar in boys and girls until puberty, but a marked sex difference is observed during maturation. Boys tend to have reduced plasma HDL levels during puberty that

are not observed in girls.[62] While increases in lipid concentration are normal with growth, adverse levels, even at younger ages, are highly associated with atherosclerosis.

Elevations in lipids are associated with the development of atherosclerosis through mechanisms of initiation and progression.[35,145] By 15 years of age virtually all individuals have atherosclerosis,[180] which continues to develop silently for decades before becoming clinically significant.[149] Atherosclerosis is marked by cholesterol-lipid-calcium deposits in the intimal layer of the arterial wall.[4] Atherosclerotic lesions are established as flat fatty streaks and progress to raised lesions with intermediate raised fatty streaks.[117] The Pathobiological Determinates of Atherosclerosis in Youth (PDAY) examined the relationship of adult CVD risk factors to the development of atherosclerosis as autopsies were performed on approximately 3000 people between the age of 15 and 34 who died of external causes. Results from PDAY showed the amount of intimal surface with evidence of atherosclerosis was positively correlated with LDL concentration and negatively associated with HDL.[1] Raised fatty streaks were present in 20% of 15 - 19 year old subjects and increased to 40% by ages 30 - 34.[117] In the Bogalusa Heart Study, researchers examined children between the ages of 5 and 14 and followed them through their late 30s. The prevalence of fatty streaks in coronary arteries increased with age from about 8% at ages 2-15 to 69% at ages 21-39.[31] Thus, the percent of intimal surface involved with fatty streaks increases with age and is appreciably related to concentrations of lipoprotein

cholesterol.[117] Thus, fatty buildup in arteries or atherosclerosis begins in childhood, is associated with CVD risk factors in adolescents, and these CVD risk factors accelerate atherogensis in later decades.

Genetic factors affect the lipid levels and ultimately, atherogensis. Heritability estimates of TC and LDL cholesterol range from 0.43-0.97,[88] though this decreases as people age due to the increase in environmental variance.[35] Genetic influence appears to be stable after adolescence,[35] though genetics may still dictate molecular changes in cholesterol. It is estimated that genetics affects 1/4 - 1/2 of the total variance in cholesterols levels.[115] The ACE I/D polymorphism may participate in the development of atherosclerosis through its critical role in RAS. Evidence exists for an interaction between RAS and hypercholesterolemia in the atherogenic process.[107] Angiotensin II, the major vasoconstrictor component of RAS controlled by ACE, has been associated with arterial wall lipid deposition, lipid peroxidation, and modification/uptake of LDL.[72,96,97] Angiotensin II facilitates oxidized LDL uptake by endothelial cell through the increases in scavenger receptors, thus increasing the macrophage content of foam cells.[107,115] Angiotensin II interaction with LDL leads to a modified LDL form that is taken up at an enhanced rate, increasing cellular accumulation of cholesterol.[37,72,96] Further, angiotensin II displays pro-atherogenic properties as it has been found to stimulate smooth muscle cell proliferation, increased expression of inflammatory molecules and increased platelet activation.[72,97] Thus, the effect of the ACE I/D polymorphism on ACE and angiotensin II in turn effects

cholesterol content,[37] providing evidence for a possible association between the ACE I/D polymorphism and lipids.

L4.1 Evidence for an association between ACE ID Polymorphism and Lipids

Few studies have examined the association of the ACE I/D polymorphism with lipids. Of the studies that have assessed this association, only four have found positive associations between the ACE polymorphism and lipid levels. While all found a positive association there were few consistencies in the associations. In a cross-sectional study, Oren et al.[142] examined the correlation of the ACE polymorphism with CVD risk factors in 197 healthy Israeli men between the ages of 40 and 70. The investigators found a significant association between the ACE I/D polymorphism and LDL cholesterol, with the highest LDL levels in those who were *DD* homozygous. In a sample of 675 healthy Korean men, Choi et al.[50] found that TC and LDL levels where higher in men with the *DD* genotype. Both studies were conducted in apparently healthy, middle aged men, suggesting the ACE I/D polymorphism does play a role in the amount of cholesterol in the blood and that common genotypic variations could play a role in disease development. In a study of overweight or obese Canadian men between the ages of 25 and 55 years, Bosse et al.[37] reported that HDL levels were higher in those possessing the *DD* allele. This sample consisted of dyslipidemic young to middle age men. Thus, there may be some evidence that the ACE I/D

polymorphism was associated with lipid level, but it may be environment dependent. In a study comparing the association of ACE with cardiovascular risk factors, Vallejo et al.[196] compared 105 women from an urban community to 59 women from a rural community. Women residing in an urban community with the *II* genotype were more likely to have abnormal LDL levels compared to the urban community counterparts that had the *ID* genotype. The authors recommend caution when interpreting these results, as they found an association that is opposite (*II* allele being associated with abnormal lipids levels) too much of the literature. There explanation for this opposite finding is their samples ethnicity and small sample size.[196] While slightly different, these studies showed an associated with the ACE polymorphism and lipids.

In middle-aged, healthy men there was an association between the *DD* genotype and increase LDL levels. In slightly younger but dyslipidemic men, the *DD* allele was associated with higher HDL. Results may imply that the *DD* allele is protective due to high HDL level being protective, but the high HDL level could just be a result of their dyslipidemic state. The studies were all cross-sectional in design and limited to males. The only study finding an association in females was a case-control design looking at the ACE I/D polymorphism in Mexican women from urban and rural environments. The finding of the *II* genotype being associated with abnormal LDL levels is contrary to the finding in men.

L4.2 Evidence against an association between ACE ID Polymorphism and Lipids

Other studies have failed to find an association between the ACE polymorphism and lipid levels. Cross-sectional, case control and cohort studies involving participants from Asia, Europe, South and North American have not shown significant variation in lipid levels based on ACE genotype.

A cross sectional study of 136 Japanese participants, mostly male (n=106), found that males had higher levels of triglycerides but lower levels of HDL than females.[141] While participants having the *D* allele tended to have smaller LDL particle size, there was no difference between genotypes and little association was found between the ACE I/D polymorphism and lipids.[141] In a study of 274 American Samoan participants, Crews et al.[55] found that the ACE polymorphism was not associated with cholesterol levels. In a larger cohort sample, 1552 middle aged, healthy French participants were assessed for genetic and lipid profiles. Pallaud et al.[145] found no association between lipid levels and the ACE gene.

In a case control study of 147 Mexican coronary artery disease patients and 100 healthy controls between the ages of 20 and 84 years, Vargas-Alarcon et al.[198] reported an increased frequency of *D* allele in the patient group compared to controls. Further, the patient group had a slight, but non-significant increase in TC and LDL when the *D* allele was present.[198] Similar results were found in a case control study of post-menopausal Italian women with and without coronary artery disease. The *D* allele was found to be in

higher frequency in the coronary artery disease group, but there was no difference in TC, LDL, or HDL levels between cases and controls.[53] Thus, the ACE genotype had no association with lipid levels.

There are inconsistent findings for an association between the ACE I/D polymorphism and blood lipids. Differences in study participants, ethnicity and gender, may explain some of the variability in study results. Study participants' age (mostly middle-aged and older) may also explain some of the inconsistencies. With increased age comes an increase in confounders, like heart disease, that may mask any association. Further, increases in age may lead to changes in the contribution of genetic and/or environmental factors leading to increased variability in study finding.[35]

L4.3 ACE ID Polymorphism and Lipids summary

In summary, the current literature is inconsistent regarding a possible association between the lipid levels and the ACE I/D polymorphism. It is possible for the ACE I/D polymorphism to be associated with lipids levels through the affects of angiotensin II, but the true biological mechanism and it strength of association have yet to be ascertained.

The effect of ACE ID polymorphism on ACE and angiotensin II may affect cholesterol levels,[37] but evidence supporting this claim are minimal and inconsistent. Further research on the ACE I/D polymorphism is warranted to confirm or disprove the biological plausible association between the ACE I/D polymorphism and lipids. Researching this association in young healthy,

college-aged adults may assist in defining the role of genetics; as there is less chance of co-morbidities such as age, chronic disease, and other co-morbidities confounding the results.

L5 ACE ID Polymorphism and Overweight/Obesity

The prevalence of overweight and obesity is a growing epidemic, with estimates of more than 1 billion people globally considered overweight (BMI ≥ 25 kg/m^2), and at least 300 million considered obese (BMI ≥ 25 kg/m^2).[13] In the US the estimated prevalence of overweight is 64%, while the estimated prevalence of obesity is 32%.[109] The upward trend in overweight and obesity is startling since they are independent risk factors for increased morbidity and mortality, throughout the lifespan.[60]

Overweight and obesity occurs due to a complex interaction between environmental and genetic factors. Physical activity, an environmental exposure, generally shows an inverse relationship with overweight and obesity, with those with more PA or increased fitness being less likely to be overweight or obese.[163] While environmental exposures are pivotal to the development of overweight or obesity, there is significant interindividual variation, most likely due to genetics. It has been suggested that genetics accounts for approximately 5% of the variance in BMI and subcutaneous fat, while accounting for approximately 25% of the variance of percent body fat and fat mass measures.[38] ACE I/D is one of many polymorphisms associated with overweight or obesity. The genetic effect that the ACE I/D polymorphism

would have on being overweight or obese is thought to be related to the role that it plays in regulating RAS.

It is suggested that modulation of RAS lead to metabolic and biochemical changes in fat cells.[45] ACE, a functional enzyme of RAS, is associated with fat cell growth,[30] with stronger ACE expression being found in human visceral fat than subcutaneous fat.[68] Additionally, adipose tissue contains a functional RAS, with angiotensin II participating in adipocyte growth and differentiation.[68,89]

L5.1 Evidence for an association between ACE ID Polymorphism and Overweight/Obesity

Limited research is available on the association between the ACE I/D polymorphism and overweight or obesity, but there are a few studies that show a positive relationship. Moran et al.[131] studied 1016 Greek adolescents (11-18 years of age) to assess the influence of different variants of the ACE I/D polymorphism on measures of the subcutaneous fat mass. They reported in females, but not males, that the ACE *D* allele was strongly associated with increased fatness.[131] Further, the relationship was strongest in females who participated in little PA.[131] Strazzulo et al.[179] studied 959 Italian males (25-75 years) to determine the association between genetic polymorphisms and overweight /obesity and body fat distribution. The authors found that BMI was higher in participants with the *DD* genotype.[179] Additionally, those with the *DD* genotype had higher odds, 1.82 and 1.76 respectively, of increased abdominal

adiposity or being overweight compared to those with *I* allele.[179] Similarly, in a study of 457 Saudi men and women the *DD* genotype and the *D* allele occurred more frequently in overweight and obese participants.[67] Riera-Fortuny et al.[157] studied 185 participants, of Spanish decent, who had suffered at least one episode of coronary heart disease and compared them to 182 controls. Subjects with coronary heart disease and the *D* allele were heavier with larger measures of waist circumference and had a higher prevalence of obesity.

Finding from these few studies indicate that there may be some form of an association between the ACE ID polymorphism and overweight and obesity. These studies, by no means provide conclusive evidence for this association, but they suggest that further work should be done in determining the relationship the ACE I/D polymorphism and overweight and obesity.

L5.2 Evidence against an association between ACE ID Polymorphism and Overweight/Obesity

In contrast to the above studies, there are studies showing no relationship between the ACE I/D polymorphism and being overweight and obese. Bell et al.[30] studied 1054 severely obese adolescents and adults (BMI $\geq$ 40 kg/m^2 or > 97% for age and sex) and compared them to 918 non-obese controls to assess the effect of the ACE genotype. They reported no difference in allele frequency between cases and control groups. In a separate study of 24 obese females, Suchanek et al.[183] assessed the effect of regular aerobic

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PA on changes in BMI and body composition when stratified by genotype. There were no differences in BMI and no association to changes in body composition among any of the genotype groups,[183] suggesting that the ACE polymorphism has no effect on BMI or body composition. In a group of 449 Jamaican participants, Cooper et al.[52] examined the relationship between RAS and obesity. The ACE polymorphism was not associated with BMI, but was associated with levels of ACE which was found to be higher in obese individuals.[52]

These three studies found no association between the ACE I/D polymorphism and overweight and obesity. Inconsistencies between findings are unclear, but could be due to different study designs, sampling techniques and/or subject sampling.

L5.3 Associations between ACE ID Polymorphism and Overweight/Obesity Summary

In summary, overweight and obesity is a growing epidemic affecting billions of people worldwide. There is a small amount of research that suggests that the *D* allele may be associated with fatness or BMI,[67,131,157,179] but this body of research is small and limited. Further, there are a handful of studies that refute this association.[30,52,183]. Further research needs to be conducted to discern the role, if any, that the ACE I/D polymorphism has with fatness and/or BMI. Understanding the etiology of this disease is paramount to slowing the progression of overweight and obesity and associated co-

morbidities. Understanding the genetic and environmental variables that individually and through interaction affect overweight and obesity can help to slow this growing epidemic.

L6 ACE I/D polymorphism and Physical Activity/Aerobic Fitness

The development of chronic disease depends on many factors including the environment and genetics. It has been stated that physical inactivity is an initiating factor in the mechanism of disease.[36] Physical inactivity is an independent risk factor for a variety of health outcomes and the relative risk of CVD associated with physical inactivity ranges from 1.5 to 2.5.[152,160] In contrast, regular PA provides substantial protective effects against CVD and its risk factors.[36]

It is possible that our genome was programmed to expect PA, thus low levels of PA is an abnormal event that results in a differing phenotypic expression.[36] The response to PA and/or exercise is variable among individuals and it is suggested that the genetic make-up of the individual is a potential reason for this variability.[39,152] Genetic factors may account for up to 30% of individual difference in the level of habitual PA.[101]

There are numerous studies identifying an association between the ACE I/D polymorphism and PA and aerobic fitness.[190] Some of these studies have found an association between the ACE *I* allele and PA and fitness, while other have reported an association between the ACE *D* allele and PA and fitness.[24,25,51,54,78,130,135,189,205,211] Finally, there are studies that have found no

association with the ACE I/D polymorphism and PA or aerobic fitness.[59,75,150,151]

While there are inconsistencies in the strength and specifics of relationships found between ACE I/D polymorphism and PA and aerobic fitness, there are a number of biologically plausible mechanisms for their existence. Rigat et al.[158] were the first to show that the ACE I/D polymorphism was associated with ACE availability, with the *D* allele linked to higher enzyme activity. ACE is responsible for converting angiotensin I into the active angiotensin II,[43,56] and inactivating bradykinins and other vasodilator peptides.[58] The ACE *II* genotype is related to lower ACE levels which in turn means potentially higher kinin levels.[205] Kinins are a group of polypeptides that influence smooth muscle contraction and increase blood flow and capillary density, all of which lead to increased oxygen and substrate delivery to muscles.[205] These effects may improve the efficiency of muscle aerobic metabolism and the contractile function of both skeletal and cardiac muscle.[94] The *DD* genotype is associated with increases in angiotensin II, which is reported to inhibit glucose uptake of smooth muscle, inhibit and lipolysis in both skeletal and adipose tissue, and cause hypertrophy in cardiac myocytes.[25,29,81,164] The hypertrophic response to physical training may be attenuated by the presence of the ACE *D* allele, resulting in left ventricular hypertrophy, which in turn would increase VO_2max via increased stroke volume.[211] The ACE *D* allele has been associated with increases in left ventricular mass as a response to training.[129] Further, angiotensin II has been

shown to regulate oxygen consumption and affect muscle energy expenditure.[25] Thus, there a number of biologically plausible reasons the ACE I/D polymorphism could be related to PA or aerobic fitness.

L6.1 Evidence for an association between ACE ID Polymorphism and Physical Activity/Aerobic Fitness

In a Portuguese twin study, Maia et al.[112] reported that 63% and 32% of total variance in leisure time PA in males and females was explained by genetic factors.[112] Specifically, the ACE I/D polymorphism has been shown to be associated with PA. Winnicki et al.[205] studied a group of 355 young (mean age ~33 years), mildly hypertensive Italian adults. They found that the *D* allele was associated with lower levels of PA.[205] Further, those participating in sports had an increased frequency of the *II* allele as did those who were sedentary.[205] They also stated that the ACE I/D polymorphism was the strongest contributor to PA status.[205] The authors assessed PA through a questionnaire that dichotomized PA (sedentary and exerciser) based on relative intensity while also assessing hypertensive adults.[205]

Numerous studies have shown associations between the ACE *I* allele and aerobic fitness.[24,51,78,130,135,189] Alvarez et al.[24] studied a group of 60 professional Spanish, male athletes that participated in cycling, long distance running, and hand ball and compared them to 400 healthy controls. They found an increased frequency of the ACE *I* in athletes compared to healthy controls.[24] This result implies that there is an association between those with

higher levels of aerobic fitness, the "endurance athletes", and the ACE *I* allele. Collins et al.[51] studied 447 Caucasian male triathletes who completed the 2000 or 2001 South Africa Ironman and compared them to 199 Caucasian healthy controls. After subdividing the triathletes into homogenous groups (fastest 100 finishers, slowest 100 finishers, South African born finishers, 100 fastest South African born finishers and the slowest 100 South African born finishers) they reported that frequency of the ACE *I* allele was higher in the fastest South African born finishers compared to controls.[51] However, there was no difference in allele frequency or genotype between the fasted 100 finishers and the slowest 100 finishers or controls.[51] These results suggest that the ACE *I* allele is associated with performance in the highly aerobic event of triathlons, but the association between the ACE *I* allele and aerobic performance or fitness may be dependent on genetic origin. Regardless, the results of Collins et al.[51] show that there is an association between the ACE *I* allele and fitness. Gayagay et al. [78] performed a case-control study, in which they assessed the association of the ACE I/D polymorphism with cardiovascular adaptations to exercise in Olympic level rowers (n=64) and matched controls (n=118). They found that the rowers had an increased frequency of the ACE *I* allele and the *II* genotype compared to controls.[78] Myerson et al.[135] assessed 495 Olympic level athletes from varying sport disciplines. They found in runners, as the distance of the event increased so did the frequency of the *I* allele.[135] Further, there was no association reported in athletes who participated in sporting disciplines that were not aerobic in

nature. Tsianos et al.[193] studied 35 elite swimmers and found that the ACE *I* allele was more prevalent in those competing in long distance events (25 km races), while the ACE *D* allele was more prevalent in those competing in short distance events (1-10km distance races). Montgomery et al.[130] compared 25 male, British high-altitude mountaineers, considered to have high aerobic fitness, to 1,906 healthy British, male controls. They found the genotype distribution to be significantly different between the mountaineers and controls, with more mountaineers having the *II* genotype.[130] Additionally, none of the climbers who ascended greater than 8,000 meters were homozygous for the DD genotype.[130] Thompson et al.[189] also studied high-altitude mountaineers (n=141) to assess the association of the *I* allele with the success of ascending greater that than 8000 meters. The authors determined that the distribution of the ACE genotype was significantly different between mountaineers who successfully ascended 8000 meters and those who were unsuccessful.[189] These studies demonstrate an association between the *I* allele of the ACE I/D polymorphism and aerobic fitness. A caveat to these results is that none of these studies included actual measured or estimated aerobic fitness, but rather aerobic fitness was implied based on the type of athlete chosen, including cyclist, triathletes, Olympic level runners and rowers, as well as high altitude mountaineers. While these studies did find an association between the ACE *I* allele and aerobic fitness, they specifically looked at the genotype frequency of elite athletes and the findings could potentially be due to selection bias.

Other studies have found an association between aerobic fitness and the ACE I/D polymorphism, but instead of the *I* allele these studies report an association with the *D* allele.[25,54,205,211] Costa et al.[54] reported that the allele distribution was significantly different between Portuguese elite short distance swimmers, considered anaerobic, and elite middle distance swimmers, considered a mix between anaerobic and aerobic. They reported that the *D* allele was more prevalent in the long distance swimmers, who may have had higher aerobic fitness levels.[54] Amir et al.[25] compared the ACE allele frequency in 121 (male and female) Israeli elite endurance athletes, elite power athletes and healthy controls. The *D* allele was more frequent in endurance sport athletes than in power sports athletes or controls.[25] Again, the endurance athletes with an assumed higher aerobic fitness, had a significantly higher frequency of the *D* allele. Similar to the association between the ACE *I* allele and aerobic fitness, these associations between the ACE *D* allele and aerobic fitness are reported in selected groups of athletes who are assumed, but not measured, to have high levels of aerobic fitness.

Only one study was found that included a direct measure of aerobic fitness. In a sample of 63 young (23 years of age) Chinese men, Zhao et al.[211] assessed aerobic fitness using a treadmill running test to volitional exhaustion. In this study, the *DD* genotype was found to be associated with higher levels of measured aerobic capacity, [211] thus providing measured evidence that the ACE *D* allele may be associated with aerobic fitness.

The ACE I/D polymorphism has been shown to be associated with PA or aerobic fitness. The majority of these studies have been conducted in selected athletes to assess the association with aerobic fitness. While there are a number of studies showing an association, some show an association with the *I* allele [24,51,78,130,135,189] while others show an association with the *D* allele [25,54,205,211] and PA or aerobic fitness.

L6.2 Evidence against an association between ACE ID Polymorphism and Physical Activity/Aerobic Fitness

In contrast to findings reported in the previous section, there are studies that report no association between the ACE I/D polymorphism and PA or aerobic fitness. Fuentes et al.[75] assessed moderate intensity leisure time PA in 721 middle-aged Finnish adults and found no association with the ACE I/D polymorphism. In this study, Fuentes et al.[75] assessed leisure time PA through the use of a single self-reported question. A singular question may not provide the validity needed to investigate gene effects.[203]

Other investigators have also reported no association between aerobic fitness and the ACE I/D polymorphism. In a study of 60 untrained women (mean age 24 years), Day et al.[59] assessed the ACE I/D polymorphism and maximal oxygen uptake and found no association. Similarly, Rankinen et al.[150] found no association between measured VO_2max values and the ACE I/D polymorphism in 724 sedentary adults. In a comparison of 192 aerobically fit male athletes, VO_2max ≥ 75 ml/kg/min, and 182 sedentary males controls

there was no difference in genotype or frequency between groups.[151] Further, there was no difference in genotype frequency among the athlete quartiles.[151] In 147 US army recruits, Sonna et al.[174] performed pre and post basic training fitness tests, including a timed 2 mile run. The authors found no difference in pre basic training or post basic training estimated VO_2max values among the ACE I/D polymorphism. Taylor et al.[186] assessed frequency of the ACE polymorphism in a group of 120 aerobically trained, Australian national athletes and compared them to 685 healthy controls. Their analysis revealed no differences in genotype distribution between the aerobic athletes and the healthy controls.

L6.3 ACE ID polymorphism and Physical Activity/Aerobic Fitness Summary

Based on the current literature it is unclear how the ACE I/D polymorphism is associated with PA or aerobic fitness. In the studies that have shown an association, the nature of the relationship is still ambiguous with a number of studies reporting that the *I* allele is associate with PA or aerobic fitness[24,51,78,130,135,189] with others report that the *D* allele is associated with PA or aerobic fitness.[25,54,205,211] Further, there is evidence that refutes an association between the ACE I/D polymorphism and PA and aerobic fitness.[59,75,150,151] Further work must be completed in this area to confirm or disprove the biologically plausible association between the ACE I/D polymorphism and PA and aerobic fitness.

L7 ACE ID Polymorphism and Cardiovascular Disease Risk Factor Literature Review Summary

In summary, the ACE I/D polymorphism is highly researched, but few definitive results exists in terms of its relationship with CVD risk factors, like BP, lipids, and fatness. There are several biologically plausible mechanisms for how the ACE I/D polymorphism could be related to modifiable CVD risk factors, which is why further studies must be completed to discern this relationship. Further, understanding the relationship that the ACE I/D polymorphism has with PA or aerobic fitness is necessary as they both have been shown to be associated with CVD risk factors. Understanding an interaction between these genetic and environmental factors is important, since this could affect risk factors or disease progression and/or severity. By examining this association in young healthy adults it may be possible to define the role of genetics with less chance of co-morbidities such as age, chronic disease, or other co-morbidities confounding the results.

CHAPTER 3

METHODOLOGY

M1 Research Design

A cross-sectional study design was used to assess PA, aerobic fitness, ACE genotype, and other CVD risk factors of college students enrolled in a healthy lifestyles class at Michigan State University (MSU). All students enrolled in class during fall, 2007 and spring, 2008 semesters were eligible for the study. The research protocol had three major elements: 1) completion of an online survey with standardized questions, 2) completion of CVD risk factor assessment including measures of BP, cholesterol, BMI and percent fat, and 3) collection of mouth buccal cells for genetic testing. Written informed consent from all study participants was obtained prior to data collection. This study was approved by the Michigan State University Biomedical and Health Institutional Review Board.

M2 Subject recruitment

Study investigators attended the lecture portion of one class period for each of the healthy lifestyle class sections (total of 6 sections). A lecture on CVD, associated risk factors, and prevention strategies was presented to each class and followed by a detailed description of study objectives, requirements, and incentives. Students interested in the study completed and signed a consent form that included a randomly assigned survey ID number and

instructions for accessing the online data collection instrument (Appendix A). For those who consented, DNA was collected (explained below) for data analysis.

M3. Eligibility Criteria (inclusion/exclusion)

All students enrolled in the healthy lifestyles class during the fall 2007 and spring 2008 semesters were eligible for participation in this study. All participants had to be 18 years of age, but there were no other exclusion criteria applied to the undergraduate enrollees.

M4 Survey data collection

The majority of data collection utilized the Longitudinal Study Engine (LSE), an internet-based surveillance system developed at MSU, within the first two weeks of each semester. The LSE is a menu-driven web page, accessible by any major internet browser, with main features detailed below:

a) Each study participant was provided online access to the LSE (http://lse.commtechlab.msu.edu/login.php)
b) LSE software uses a unique survey ID number (provided to participants) to keep track of each individual survey protocol and also to keep track of each individual designated participant's responses at baseline and/or over time. (The LSE builds a unique ID number that tracks both the survey protocol and the individual participant.)

c) At the time of completion of the initial baseline assessment, the designated participant accesses the LSE website, keys in his/her LSE ID number, and is prompted to key in a personal user_id, and password. Prompting questions will be asked to help the individual recall the user_id and password if these should be lost or forgotten (e.g., 'What is the first initial of your mother's maiden name (surname before marriage)?')

d) Thereafter, the web browser displays the study's disclosure statement, which invites participation, and the participant can key a NEXT button to proceed to complete the survey, or can log out at any time. The LSE is designed to allow the participant to terminate participation and to skip over any survey item.

e) Survey elements used in this investigation included true/false, multiple choice, and short answer items.

f) The LSE captures keyed responses as well as time elements (e.g., time of log-in, time of log-out), with storage of these responses within a protocol-specific EXCEL spreadsheet stored on the LSE secure server.

M5 Demographics and Physical Activity Data

Participant demographic information such as age, gender, race and academic major were collected using the LSE. Physical activity during the past month was assessed using a questionnaire, based on questions taken from previously validated instruments,[16,19,92] and was administered through the LSE. If the participant indicated that s/he performed leisure-time PA, a series

of three questions were asked to assess type (mode), frequency (sessions per week), and duration (average minutes per session) of PA. The same series of questions were repeated for up to two more activities. Each activity was assigned a metabolic equivalent value (MET) using the Compendium of Physical Activities.[19] One MET is equivalent to one kilocalorie expended per week indexed by body weight (kcal/kg/week). Weekly energy expenditure (kcal/kg/week) was extrapolated using the MET values for each activity, the number of sessions per week, and the number of minutes per session. Sedentary behaviors were assessed by determining the number of hours per day spent watching television, playing video games, using the computer, or reading/studying (Appendix B).

M6 Cardiovascular Disease Risk Factors and Fitness

As part of enrollment in healthy lifestyles class, each student was required to complete a health assessment at the beginning of the semester. Employees of Olin Health Center at MSU conducted the health assessment. The health assessment included measures of height (cm) via stadiometer, weight (kg) via a triple beam balance, and a BMI (kg/m^2) calculation. Seated resting systolic and diastolic BP were assessed via an automated cuff placed on the right arm. Values that exceed 140 (systolic) or 90 (diastolic) millimeters of mercury were repeated for accuracy. Body fatness was assessed by four site skinfold (triceps, abdomen, ilium, and thigh). Skinfold measures were used because of their feasibility, reliability, and validity of estimating body

composition in the field.[132] Skinfold sites were marked and measured on the right side of the body in accordance to American College of Sports Medicine guidelines (ACSM).[8] All measures were taken in a triplicate series by the same person using a Lange caliper (Beta Technology Incorporated, Santa Cruz, CA). Average of the three measures taken at each site was calculated and then summed. The sum of four site skinfold was then used to estimate percent fat using a standard equation (Appendix C).[132] Nurses from Sparrow Hospital (Lansing, Michigan) collected non-fasting blood samples to assess total cholesterol and total high-density lipoproteins. Smoking was assessed by a specific question via the LSE. Aerobic capacity (fitness) was estimated via an instructor administered one and half mile endurance test completed during class. The one and half mile endurance test is a valid field tool (correlation of R=0.79-0.92[80,103,119,212]) in predicting VO_2max and a very expeditious technique to use with large groups.[8,103] The objective of the endurance test is to cover one and half miles in the shortest time possible, with time being placed in an equation (VO_{2max} (ml/kg/min) = 3.5 + 483/(time in minutes) to estimate aerobic capacity.[8] Following the completion of the health/aerobic fitness assessment, students recorded their results on a data sheet that was returned to the principal investigator (Appendix D).

M7 Genetic Sampling

Indicator FTA® cards (Whatman Plc.) were used to collect and store participants' deoxyribonucleic acid (DNA), obtained on the day of recruitment.

Indicating FTA® cards are saturated with a chemical that lyses cell membranes and denature proteins on contact while trapping, stabilizing and protecting nucleic acids in a cellulose fiber. Fresh buccal epithelial cells were removed, non-invasively, from the participant's mouth using a foam tipped applicator and placed onto the indicating circle (changes color when sample is applied) of the FTA® card. Once the sample was applied to the indicating FTA® card, the card was individually sealed in a plastic bag to be stored at room temperature until further use.[122]

M8 Genetic Testing

Immobilized DNA on each FTA® card was prepared for polymerase chain reaction (PCR) via modified manufacture's protocol (Whatman Plc.).[10] Four discs per card (one card per subject) were punched into a 0.2 ml PCR reaction tube using a 1.58 mm hole punch (approximately 1/16 inch). Participant samples were prepared in batches of 24. To minimize contamination the punch was cleaned between samples using a clean lint free wipe moistened with 70% ethanol and allowed to air dry for 30 seconds between samples. After punching the FTA® card, samples were washed per manufacture's protocol (Whatman Plc.)[10] with a five minute incubation being completed on a Multi-Microplate Genie Rotator (Scientific Industries) at approximately 1800-1900 RPM. After completing the final wash all liquid was aspirated off the disc. This step was performed in duplicate to ensure removal

of all buffers. At this point, 5 µl of double distilled water was add to each PCR tube and held at 80°C for approximately 30 minutes.

DNA was then amplified by PCR according to modified methods of Lindpainter et al.[108] as described in detail. After a 30 minute hold, a 50 µL PCR reaction master mix containing: 1 µM of each primer (hace3s, 5'GCCCTGCAGGTGTCTGCAGCATGT3'; hace3as, 5'GGATGGCTCTCCCCGCCTTGTCTC3'), 200 µM each of deoxynucleotide triphosphates, 1.3mM magnesium chloride, 50mM potassium chloride, 10 mM TRIS-hydrochloric acid (pH 8.4 at 23°C) and 0.35 units of *Thermus aquaticus* polymerase (Applied Biosystems). DNA was amplified for 35 cycles with denaturing at 94°C for 30 seconds, annealing at 56°C for 45 seconds, and extension at 72°C for 2 minutes using a GeneAmp PCR system 9700 (Applied Biosystems).

Ten µL of PCR product was combined with 0.5 µL of 5X glycerol based loading buffer and loaded into a 1.5% agarose gel submerged in TAE buffer. The products were then electrophoresed to separate DNA fragments of differing length (597 bp for *I* allele and 319 bp for *D* allele) for 0.7 hours at approximately 5V/cm. The gel was then stained with a 0.02% to 0.1% solution of ethidum bromide (0.1 ml diluted in 500-750 ml H_2O) for ten to fifteen minutes and visualized with UV transillumination.

Due to the preferential amplification of the *D* allele in heterozygous samples,[172] all samples found to be the *DD* genotype were subjected to a second independent PCR amplification using an insertion specific primer pair

(hace5a, 5'TGGGACCACAGCGCCCGCCACTAC3', hace5c, 5'TCGCCAGCCCTCCCATGCCCATAA3'). PCR conditions were the same with the exception of an annealing temperature of 67°C. Positive reaction produced amplification of a 335 base pair amplicon only in the presence of an *I* allele. All assays were performed in the Histocompatibilty and Immunogenetics Laboratory in the Department of Medicine at Michigan State University.

M9.1 Statistical Analysis - Data Management

Collected FTA® cards and completed health assessment forms were separated from identifying information and stored in locked filing cabinets. Data from the FTA® card and health assessment forms were entered into in Excel™ database and stored on a password protected computer. Responses from the LSE online questionnaire were abstracted directly into the data file. Data files were backed up regularly and stored in secure locations.

M9.2 Statistical Analysis - Data Linkage

Each participant was assigned a unique coupon number that was recorded on the study questionnaire, health assessment form, and FTA® card. Data were linked in a single anonymous database using this coupon number. No personal identification could be accessed from the database. The de-identified database was used for statistical analysis.

M9.3 Statistical Analysis - Data Cleaning

All data were evaluated for miscoded, erroneous, and missing values. Individual erroneous variables were replaced by imputing the sample mean. Frequency distributions were assessed indicating that no recoding and/or transformations were necessary.

M9.4 Statistical Analysis - Variable Definitions

Main exposure variables:

Angiotensin Converting Enzyme I/D Polymorphism: ACE I/D polymorphism data were obtained through the genetic testing. For analysis, ACE I/D polymorphism was categorized by genotype (*II, ID, DD*).

Physical activity: Participation in leisure time PA was assessed by asking each participant if they participated in leisure-time PA or exercise in the past month. If yes, participants where then asked to assess the type, frequency, and duration of the activity. Physical activity was expressed as a continuous variable calculated as kilocalories expended per week indexed by body weight (kcal/kg/week).

Primary outcome variables:

Blood pressure: BP was recorded as millimeters of mercury (mm Hg) and analyzed as a continuous variable.

Total cholesterol: TC was recorded in units of milligrams per deciliter (mg/dL) and analyzed as a continuous variable.

High Density Lipoprotein cholesterol: HDL cholesterol was recorded as mg/dL and analyzed as a continuous variable.

Body Mass Index: Weight (kg) and height (m) were collected and body mass index (kg/m^2) calculated. For analysis, BMI was assessed as a continuous variable as well as was a categorical variable definitions of normal weight (≤ 24.9 kg/m^2), overweight (25-29.9 kg/m^2), and obese (≥ 30 kg/m^2).

Body fatness: Percent body fatness was measured via skinfolds and analyzed as a continuous variable.

Additional variables:

Gender: Gender was be defined as a dichotomous variable (male/female).

Age: Because of the age range of recruited participants, age was defined as a categorical variable (18 years, 19 years, 20 years and ≥ 21 years).

Race: Due to the relatively low numbers of non-Caucasian minorities, race was dichotomized as Caucasian or Other

Total seat time: A variable composed of various sedentary behaviors including study time, television viewing time, playing of video games and computer use. This variable was analyzed as a continuous variable (hours)

Smoking: Current smoking was defined as a yes/no dichotomous variable

Estimated VO_2max: An estimate of maximal oxygen consumption (VO_2max) was determined based on the time it took to cover one and half miles (Appendix C for formula). The VO_2max (ml/kg/min) value was analyzed as a continuous variable.

M9.5 Statistical Analysis - Sample Size Calculation

Linear regression was used to evaluate the relationships between PA and ACE I/D polymorphism on the presence of CVD risk factors (blood pressure, total cholesterol, HDL cholesterol, BMI, and percent body fat). Power analysis to detect significant linear regression coefficients for the prediction of outcome variables was performed with the conservative assumption that regressions would include nine predictors. Based on the means and standard deviations of the study sample, and a fairly large effect size of 0.30 will enable us to detect a difference beyond measurement error for all outcome variables.

For this study there were 600 potential participants. Two hundred and seventy five participants completed all data collection (PA questionnaire, CVD data sheet, and a FTA® card). Based on a PCR success rate of 80%,[122] our study sample size would be 220. Assuming alpha equal to $P \leq 0.05$ and at sample size of 220, we will be adequately powered (Power > 0.99) to detect an effect size of 0.30. This sample size should be large enough to provide the power necessary to effectively assess study aims two and three.

In assessing study aim four, the interaction of PA and the ACE I/D polymorphism on CVD risk factors, the allele frequency and the association between the exposure and outcome variables must be considered. It is suggested that to detect interactive effects that you need at least four times as many participants than needed for the evaluation of main effects.[90] With a power of 0.8 and significance 0.05, it is estimated that a minimum of 1279 participants would be need to complete this portion of the study. Due to that fact that the maximal sample size could only be 600 participants, study aim #4 will be exploratory.

M9.6 Statistical Analysis

Aim One: To assess the PA and CVD risk factor profile of college students, basic descriptive analysis were used. Means, standard deviations, medians, intra-quartile ranges and histograms were calculated for each of the following: systolic and diastolic BP, TC, HDL, BMI, percent body fat, PA, and estimated VO_2max. Percent distributions were calculated for gender, age groups, race, PA quartiles and BMI categories. Due to the fact that previous investigators[3,104] have shown male students to be more likely than female students to report high levels of PA, we used univariate logistic regression analysis to develop odds ratios for differences in categorical variables between genders. Further, an analysis of variance was used to assess differences in continuous variables between genders and among the PA quartiles.

Aim Two: A student's test was conducted to assess mean differences of continuous variables between each dichotomized variable (gender, race, major, and smoking). To determine the associations between PA and BP, TC, HDL, BMI, and percent body fat, linear regression analysis was utilized. Univariate linear regression was used to assess the relationships between PA and a single dependent variable (BP, TC, HDL, BMI, or percent body fat). Five separate multiple linear regressions models were developed to assess both the main effects and interaction effects (based on post hoc statistical analysis) of PA on each dependent variable while controlling for covariates (Appendix E). The first step in the development of these multiple linear regression models was the generation of a baseline model which included the main effect of PA, plus two a-priori confounders - gender and race. All other covariates were then tested by adding them to this base model and then removing them using backwards elimination methods if their associated p-value was > 0.10. Non-significant variables were not removed if their removal changed the estimate for the remaining variables by more than 10%. Modeling was completed with interactions being assessed for significance first, followed by main effect terms. No main effect term was eliminated if it was included in a significant interaction term. The final regression model was tested for significance (p-value $\leq$ 0.05) and included all variables that had a p-value $\leq$ 0.10. The same modeling procedure was repeated for each of the five multiple linear regressions.

Aim Three: Univariate linear regression models were developed to assess the relationship between the ACE I/D polymorphism and a single dependent variable (BP, TC, HDL, BMI, percent body fat, PA or eVO_2max). Multiple linear regression models were developed to assess the impact of the ACE I/D polymorphism on a dependent variable (BP, TC, HDL, BMI, percent body fat, PA or eVO_2max) while controlling for covariates (Appendix E). The first step in the development of these multiple linear regression models was the generation of a baseline model which included the main effect of the ACE I/D polymorphism. All other covariates were then tested by adding them to this base model and then removing them using backwards elimination methods if their associated p-value was > 0.10. Non-significant variables were not removed if their removal changed the estimate for the remaining variables by more than 10%. Modeling was completed with interactions being assessed for significance first, followed by main effect terms. No main effect term was eliminated if it was included in a significant interaction term. The final regression model was tested for significance (p-value ≤ 0.05) and included all variables that had a p-value ≤ 0.10.The same modeling procedure was repeated for each of the five multiple linear regressions.

AIM Four: Multiple linear regression analysis was used to determine whether PA affects the association between ACE I/D polymorphism and BP, TC, HDL, BMI and percent body fat in college students. Multiple linear regression models assessing the effects of ACE I/D polymorphism and PA on individual

dependent variables (BP, TC, HDL, BMI, or percent body fat) was developed. To obtain the most parsimonious model, all covariates (Appendix E) were entered into the model and removed in a backwards stepwise fashion based on the level of the p-value. Finally, five multiple regression models were developed, containing both the main and interactive effects of the ACE I/D polymorphism and PA on each dependent variable while controlling for the covariates (Appendix E).

CHAPTER 4

RESULTS

R1 Descriptive Data (Study Aim #1)

Among the 600 students eligible to participate, a total of 191 (32%) completed both the online assessment and the CVD risk factor profile, while 149 (24.8%) completed all aspects of data collection (i.e., online assessment, CVD risk factor profile and genotyping). The sample was similar to an earlier sample of 388 participants from the same class studied during the 2005-2006 academic year.[139] The only significant difference between the two study samples was that systolic BP was higher in the 2005-2006 cohort (SBP=124±15 bpm) as compared to the 2007-2008 sample (SBP=118±14 bpm). For the sample, 90% reported meeting the American College of Sports Medicine (ACSM) minimal PA recommendation of 7.5 kcals/kg/wk (Table 1A).[133] Descriptive data for age, race, major, smoking status, BMI categories, PA quartiles, and genotype are shown in Table 1A. The majority of the sample was female (69%), Caucasian (84%), non-smokers (85%) and of normal weight (72%) based on BMI. Chi square analysis was used to assess gender differences for BMI categories, PA levels and genotype. A significant difference ($p<0.05$) was found between genders for BMI categories and PA levels. Males were 3.3 times and 1.8 times more likely than females to be overweight or obese, respectively. Further, males were 3.7 time and 6.4 times more likely than females to be in the 3rd (28.0-54.7 kcals/kg/week) and 4th

(>54.7 kcals/kg/week) PA quartile, respectively. The ACE polymorphism was found to be in Hardy-Weinberg equilibrium (p=0.08). There was no difference in genotype based on gender (p=0.097), although when Hardy-Weinberg equilibrium was analyzed by gender, the genotype in males deviated (p=0.032) from Hardy-Weinberg, while the females remained in equilibrium (p=0.36).

Cardiovascular disease risk factor measures for the sample and by gender are found in Table 1B. Systolic and diastolic BP, eVO_2max and PA were significantly ($p<0.05$) higher in men (SBP:130±13 mmHg; DBP:76±7 mmHg; eVO_2max 41.9±6.4 ml/kg/min; PA 48.2±28.9 kcals/kg/wk) than women (SBP:114±11 mmHg; DBP:72±9 mmHg; eVO_2max 34.5±7.2 ml/kg/min; PA 31.5 kcals/kg/wk). Body mass index had a small (1.7 kg/m^2) but significant ($p<0.05$) mean difference between the genders (males: 24.8±3.7; females: 23.1±3.7). Conversely, HDL and percent fat was significantly ($p<0.05$) lower in men (HDL:53±10 mg/dL; %fat:15.4±5.4) than women (HDL:64±14 mg/dL; %fat:26.7±5). No gender differences were found for other variables.

Further descriptive characteristics for the sample by PA quartile are shown in Table 1C. Systolic BP was significantly ($p<0.05$) lower in PA quartiles 1 (0–14.8 kcals/kg/wk) and 2 (14.9-28.0 kcals/kg/wk) when compared to the highest quartile (>54.7 kcals/kg/wk). Percent fat decreased as PA activity quartiles increased with percent fat in quartiles 1 and 2 being significantly ($p<0.05$) higher than both quartiles 3 and 4. Body mass index in quartile 1 was significantly ($p<0.05$) higher than all other quartiles. Estimated

maximal oxygen consumption increased linearly with increased PA quartiles, with those in quartiles 1 and 2 being significantly ($p<0.05$) lower than all other quartiles. Total seat time was not significantly different among quartiles.

R2.1 Association between Physical Activity and Systolic BP (Study aim #2)

Descriptive data for systolic BP (SBP) in terms of gender, race, age, major and smoking are shown in Table 2.1A. A Student's t-test was used to assess differences in mean SBP between each dichotomized variable (gender, race, major, smoking) while analysis of variance (ANOVA) was used to assess differences in SBP among age groups. The only significant finding was between SBP and gender, with males (SBP=130±13 mmHg) having a significantly ($p<0.05$) higher SBP than females (SBP=114±11 mmHg). Univariate linear regression showed that PA was significantly related to SBP ($\beta=0.09$ [0.20-0.159], $p<0.05$). Other variables including HDL, percent fat, BMI and eVO_2max were significantly related to SBP in univariate analysis (table 2.1B). Multiple regression was conducted to assess the outcome variable of SBP with a base model of PA, gender, and race. The remaining covariates (TC, HDL, percent fat, BMI, eVO_2max, total seat time, and smoking risk) were entered into the model and then removed if non-significant ($p>0.10$) via backwards elimination. A model with four predictors estimated 40% ($F_{4,152}=25.12$; $p<0.05$) of the total variance for SBP (Table 2.1C). Body mass index and gender were significantly related to SBP. Specifically, participants who

were male and /or had an increased BMI had higher SBP. Total PA was not a significant contributor to the model when controlling for covariates.

R2.2 Association between Physical Activity and Diastolic BP (Study aim #2)

Descriptive data for diastolic BP (DBP) are shown in Table 2.2A. Diastolic BP was significantly different ($p<0.05$) between genders (male:76±7 mmHg; females:72±9 mmHg). Univariate linear regression showed that PA was not significantly related to DBP (β=-0.008 [-0.046-0.031], p=0.702). Of the covariates, only BMI was significantly related to DBP (β=0.692 [0.394-0.990], p=0.00) during univariate analysis (Table 2.2B). Further, a five predictor multiple regression model ($F_{5, 151}$=6.104, $p < 0.05$) estimated 17% of DBP. Percent fat (β=0.485 [0.23-0.74], $p<0.05$) and gender (β=-9.65 [-13.39- -5.91], $p<0.05$) were significant predictors of DBP (Table 2.2C). PA remained a non-significant predictor of DBP when controlling for covariates.

R2.3 Association between Physical Activity and TC (Study aim #2)

Descriptive data for TC is shown in Table 2.3A. A significant difference ($p<0.05$) in mean TC between races (Caucasian:172±30 mg/dL; Other:151±39 mg/dL) was present. Physical activity was not related to TC (β =0.03 [-0.157-0.164], p=0.968). No other variables were related to TC during univariate analysis (Table 2.3B). Multivariate analysis revealed that all variables were not significant predictors of TC (Table 2.3C).

R2.4 Association between Physical Activity and HDL Levels (Study aim #2)

Males (53±39 mg/dL) had ($p<0.05$) lower mean HDL levels than females (64±39 mg/dL) (Table 2.4A). High density lipoprotein levels were different among age groups. Specifically, participants ≥21 year of age had a lower mean HDL concentration than all other age groups. Physical activity was not significantly related to HDL (β=-0.017 [-0.09-0.056], p=0.644) (refer to Table 2.4B). Univariate analysis found that both SBP and BMI were significantly related to HDL. On further analysis, BMI (β=-1.206 [-1.82- -0.59], $p<0.05$), smoking risk (β =7.64 [0.82-14.46], $p<0.05$) and gender (β =9.16 [4.59-13.73], $p<0.05$) were significant predictors of HDL cholesterol while SBP and PA where non-significant predictors Table 2.4C).

R2.5 Association between Physical Activity and Body Composition (Study aim #2)

Descriptive data for the sample related to body composition are displayed in Table 2.5A. Body composition (%fat) was significantly different ($p<0.05$) between genders (male:15±5; females:27±5). Univariate liner regression analysis indicated that PA was significantly related to percent fat (β=-0.098 [-0.133 - -0.063], $p<0.05$). Further, univariate linear regression indicated that SBP, HDL, and eVO_2max also had a significant ($p<0.05$) relationship with percent fat (Table 2.5B). In controlling for covariates, a multiple regression model with six predictors (DBP, BMI, eVO_2max, PA, race

and gender) estimated 82% ($F_{6,150}$=112.07; p<0.05) of the total variance in percent fat values. Due to issues of multicollinearity between PA and eVO_2max, data were re-analyzed with the removal of eVO_2max and a more parsimonious model with five predictors estimated 81% ($F_{5,154}$=132.27; p<0.05) of the total variance in percent fat values (Table 2.5C). Physical activity, BMI and gender were all significant contributors to the model. While controlling for other variables, PA had an inverse association with percent fat values, indicating that as PA participation increased percent fat value decreased.

R2.6 Association between Physical Activity and BMI (Study aim #2)

As shown in Table 2.6A, males (BMI =24.8) had a significantly higher (p<0.05) BMI values than females (BMI =23.3). There were no differences in BMI among the other categorical variables. Univariate linear regression indicated that PA activity was not significantly related with BMI (β=-0.01 [-0.028 - 0.08], p=0.259). Systolic BP, DBP and percent fat had significant positive relationship with BMI, while HDL and eVO_2max had inverse relationship with BMI (Table 2.6B). Using multivariate analysis to control for covariates, SBP (β =0.05 [0.02 - 0.08], p=0.003) and percent fat (β =0.48 [0.40 - 0.57], p≤0.05) remained positive determinants of BMI while HDL (β =-0.03 [-0.06 - -0.01], p=0.015) remained a significant inverse determinate of BMI. Thus, being male, having increased SBP, and/or increased percent fat value increased the BMI values while having a higher HDL level lowered BMI, while

controlling for other factors (Table 2.6C). PA remained a non-significant predictor of BMI.

R2.7 Summary of the association between Physical Activity and Cardiovascular Disease Risk Factors (Study aim #2)

The purpose of study aim #2 was to determine the association between PA levels and BP, blood lipid concentration, BMI and body fatness in young adults. It was hypothesized that PA would be inversely related with SBP, DBP, TC, BMI, and percent fat while be directly related with HDL. However, results showed that only percent fat values were significantly and inversely associated with PA.

R3. Association between ACE ID Polymorphism and Cardiovascular Disease Risk Factors (Study aim #3)

Of the 600 eligible study participants usable ACE I/D genotype data were obtained in 149 (24.8%). The genotype frequency for the sample was 24= *II,* 84=*ID*, and 41=*DD* (Figure 3.1). This was in Hardy-Weinberg equilibrium. Of those classified as *II*, 12.5% were male, 90.9% were non-smokers, and 75% were white. In the *ID* classification, 33.3% were male, 87.8% were non-smokers, and 85.7% were white. Finally, the *DD* genotype was 36.6% male, 94% non-smokers, and 85.4% white. There was no difference among genotype groups for gender, race, smoking, or BMI category (Table 3.1).

Cardiovascular disease risk factors and other continuous measures by genotype are found in Table 3.2. Physical activity, percent fat, and eVO_2max showed significant differences among the genotype groups. Specifically, those classified as *DD* had significantly greater amounts of PA (50.4±33 kcal/kg/wk) and higher eVO_2max (39±7 ml/kg/min) values than those classified as *ID* (PA:33.1±26 kcal/kg/wk; eVO_2max:36±8 ml/kg/min), who had significantly higher values than the *II* group (PA:25.5±17 kcal/kg/wk; eVO_2max:34±5 ml/kg/min). Conversely, participants possessing the *II* genotype had significantly higher ($p=0.04$) percent fat (26.5±5%fat) values than those with the *DD* genotype (21.7±7%fat), but there was no difference between homozygous groups and the heterozygous group. When similar analysis was conducted on the sample stratified by gender there were no significant differences among genotype groups, except for PA in females (Table 3.3). In females, there was a graded response with the *DD* genotype having the highest PA levels (50.9±37), the *ID* group being intermediate (26.6±22) and *II* group having the lowest PA levels (24.0±18). All were significantly different from each other ($p \leq 0.05$). The ACE I/D polymorphism was not significantly related to any other cardiovascular risk factors for the entire sample (Table 3.2).

R3.1 Association between ACE ID Polymorphism and Systolic BP (Study aim #3)

Univariate analysis showed no differences in SBP between genotype groups ($F_{2,144}$ =1.252; p=0.289). Further analysis indicated that race was not a significant predictor of SBP, but when gender was added to the genotype model it predicted 31% of the variance in SBP ($F_{3,143}$ =21.051; p=0.000). There was a difference between the genders (β=-17.205 [-21.610-12.801], p=0.000) with females having lower SBP than males, but there were no significant differences in SBP among the genotypes. The final multivariate model included five predictors that accounted for 41.5% ($F_{5, 115}$ =16.41; p<0.00) of the variance in SBP (Table 3.1A). When controlling for variables that remained in the model (Gender: β=-14.660 [-18.972- -10.347], p=0.000; BMI: β=1.271 [0.687 - 1.856], p=0.000; TC: β=0.059 [-0.007- 0.126], p = 0.079) there was no difference among the genotypes. Additional analysis revealed no significant interaction between the ACE genotype and gender.

When the data were stratified by gender, there was no difference in SBP among the genotypes in males or females. When controlling for covariates, SBP remained similar between genotype groups. Thus, there was no difference in SBP values between the *II, ID,* or *DD* genotype in males or females.

R3.2 Association between ACE I/D polymorphism and Diastolic BP (Study aim #3)

The ACE I/D polymorphism was not associated with DBP, as there was no difference in DBP values among the genotype groups. Further regression analysis with genotype and gender in the model predicted 8% ($F_{3,143}$=4.395; p=0.005) of the total variance for DBP. Similar to SBP, gender was a significant (β=-4.77 [-7.5- -2.02], p=0.000) predictor of DBP, with females having lower values than males. Additional analysis controlling for other covariates revealed a model that accounted for 19.5% ($F_{4,116}$=7.02; p=0.000) of the total variance in DBP with four predictors (Table 3.2A). Percent fat (β=0.56 [0.26-0.86], p<0.05) and gender (β=-10.47 [-14.76- -6.18], p<0.05) were significant predictors of DBP, but there was no difference in DBP among genotype groups. Thus, females had a lower DBP while increases in percent body fat increased DBP regardless of genotype.

Similar to SBP, genotype was a non-significant predictor of DBP when stratified by gender. In males, there was no difference in DBP among genotypes. Interestingly, while controlling for covariates in females the *ID* heterozygote (β=-4.88 [-8.58- -1.18], p<0.05) group was significantly different from the *DD* genotype group (referent group). Specifically, those classified as *ID* were more likely to have a lower DBP as compared to the homozygote groups. Further, percent fat (β=0.54[0.15- 0.93], p<0.05]) was a significant predictors of DBP. As percent fat increased so did DBP, which is similar to the finding for the overall sample.

R3.3 Association between ACE ID Polymorphism and TC (Study aim #3)

Similar to the finding for BP, there was no association ($F_{2,1\ 32}$ =0.077; p=0.926) between genotype and TC. When gender or race was added to the model neither were significant predictors of TC and there was no difference in TC among the ACE genotype. Further analysis controlling for covariates found similar non-significant results (Table 3.3A). Additional analysis on gender stratified data revealed a non-significant difference between genotype and TC.

R3.4 Association between ACE ID Polymorphism and HDL Levels (Study aim #3)

No differences in HDL were found between ACE I/D polymorphism groups in univariate analysis. Race was a non-significant predictor when added to the genotype model, but gender was a significant predictor of HDL (β=10.45 [5.67-15.22], p=0.000). Women had higher levels of HDL compared to men, independent of genotype. Further, analysis controlling for covariates revealed a model where both BMI (β-1.086 [-1.78 - -0.39], p=0.002) and gender (β=8.3 [3.19- 13.41], p=0.002) were significantly associated with HDL, but there were no differences among genotype groups (Table 3.4A). Thus, there was no difference in HDL among genotypes, but the female tended to have higher levels of HDL while those with increased BMI had decreasing levels of HDL.

When stratified by gender, the only significant results were found in females. Specifically, BMI (β=-1.33 [-2.20- -0.45], $p < 0.05$) was a significant

predictor of HDL, but there was no difference in HDL among the genotypes. This result is similar to the overall sample in that as BMI increased, HDL cholesterol decreased.

R3.5 Association between ACE ID Polymorphism and Body Composition (Study aim #3)

Univariate analysis revealed that percent fat values were significantly different among the genotype groups. Specifically, the *II* group had the highest percent fat (26.5%) with those classified as *ID* being intermediate (23.3%) and those classified as *DD* having the lowest percent fat (21.7%). However, only those classified as *II* had a significantly different value from the referent group (*II* %fat=26.5, *DD* %fat=21.7; $F_{2, 148}$=3.25; p=0.042). Analysis conducted with genotype and gender in the model accounted for 47% of the variance found in percent fat. While gender was a significant (β=10.614 [8.69- 12.54], p=0.000) predictor of percent fat, it also eliminated any significant difference that occurred in percent fat among genotypes. Specifically, females had higher percent fat values (26.2±5) than males (15.4±5.4) and this was independent of genotype. A multivariate regression model with five predictors accounted for 65% ($F_{5,115}$=42.025; p=0.000) of the variance in percent fat values (Table 3.5A). Specifically, females had higher percent fat values. In addition, percent fat was directly related to DBP and inversely related to eVO_2max. There was no interaction effect found between gender and genotype.

When stratified by gender, there was no significant difference in percent fat among the ACE I/D genotypes. When controlling for covariates in males there was no difference in percent fat among the genotypes. While non-significant, there was a trend for the *II* genotype to have the highest percent fat (18.3%fat) values with those classified as *ID* being intermediate (16.5%fat) and the *DD* genotype having the lowest percent fat (14.3%fat). While controlling for covariates in females, a similar but non-significant trend was seen with the *II* genotype group having the highest percent fat (27.6%fat) and the *DD* genotype having the lowest percent fat (25.9%fat). This is similar to the finding for the entire sample.

R3.6 Association between ACE I/D polymorphism and BMI (Study aim #3)

There was no difference in BMI among the genotypes. This did not change when the analysis controlled for race or gender. Gender was a significant predictor of BMI (β=-1.560 [-2.813- -0.307], p=0.015), with females having lower levels than males. In a multiple regression model controlling for other covariates, a significant difference (β=-1.781[-3.11- -0.451], p=0.009) was found between the *II* group and the *DD* (referent) group. Specifically, those classified as *II* had lower BMI than those of the *DD* group. This model contained six predictors and accounted for 60% ($F_{6,114}$=27.246; p=0.000) of the variance in BMI. Systolic BP (β=0.50 [0.013- 0.086], p=0.008), HDL (β=-0.040 [-0.71- -0.008], p= 0.014), percent fat (β=0.481 [0.387- 0.576], p=0.000), and gender (β=-5.311[-6.926- -3.695], p = 0.000)) were found to be significant

predictors of BMI (Table 3.6A). Specifically, females and/or having higher HDL lowered your BMI, while increases in percent fat and SBP increased BMI.

In gender stratified analysis, there was no difference in BMI among the genotype groups. When controlling for other covariates, BMI values remained similar among the genotypes for both males and females.

R3.7 Association between ACE ID Polymorphism and PA/eVO$_2$max (Study aim #3)

Univariate analysis revealed that PA and eVO$_2$max were significantly different among the genotype groups. Specifically, the *II* group had the lowest PA and eVO$_2$max (PA:25.53; kcals/kg/week; eVO$_2$max: 33.6 ml/kg/min) with those classified as *ID* being intermediate (PA:33.05 kcals/kg/week; eVO$_2$max: 36.08 ml/kg/min) and those classified as *DD* having the highest values (PA: 50.36 kcals/kg/week; eVO$_2$max:39.13 ml/kg/min). Analysis conducted with genotype and gender in the modes accounted for 14% and 21.7% of the variance found in PA and eVO$_2$max, respectively. Gender was a significant predictor of both PA (β=-12.33 [-21.72- -2.944) and eVO$_2$max (β=-6.89 [-9.41- -4.38). Specifically, females had lower PA and eVO$_2$max than males. For PA, a multivariate regression model with four predictors accounted for 22% ($F_{4,119}$ = 8.38; p=0.00) of the variance (Table 3.7A). A significant difference remained between the genotype while controlling for females having lower PA and PA being inversely related to DBP. For eVO$_2$max, a multivariate model with five predictors accounted for 38.8% of the total variance (Table 3.7B). While

controlling for percent fat and race, the ACE *II* genotype remained significantly different from the referent group (*DD* genotype), but the ID genotype was not longer significantly different. There was no interaction effect found between gender and genotype for either model.

When stratified by gender, there was no significant difference in PA or eVO_2max among the ACE I/D genotypes for males, when controlling for covariates. In females, there was a significant difference in PA among the genders, with II having the lowest (PA:24.0 kcals/kg/week) and DD (PA:50.9 kcals/kg/week) have the highest. Further, when controlling for covariates (Race) a significant difference in PA among the genotypes remained. This is similar to the finding for the entire sample.

R3.8 Summary of associations between the ACE ID Polymorphism and Cardiovascular Disease Risk Factors (Study aim #3)

The specific aim for this section was to determine the association between ACE I/D polymorphism and BP, blood lipid concentrations, BMI, and percent body fat in young adults. It was hypothesized that the *DD* genotype would be associated with increased BP, TC, BMI, percent body fat and inversely associated with HDL cholesterol. The data from this sample did not support the hypothesis. There was no association between SBP, DBP, TC, HDL, or BMI and the ACE genotype. There was a significant difference among genotype groups and percent fat, but this was not maintained when controlling

for covariates. There was a trend for percent fat to be higher in the *II* versus *ID* versus *DD* genotype groups.

R4. Determination of interaction effect between ACE ID Polymorphism and Physical Activity on Cardiovascular Disease Risk Factors (Study aim #4)

Cardiovascular disease is a multifactorial disease that can be affected by genotype and the environment factors. The focus of this section is to assess the interaction between the ACE I/D polymorphism and PA levels on CVD risk factors. Of the 600 eligible participants, usable PA assessment, CVD risk factor profile and ACE genotype data was obtained in 149 (24.8%) participants.

R4.1 Association between ACE ID Polymorphism and Physical Activity on Systolic BP (Study aim #4)

Linear regression with both genotype and PA in the model predicted only 4.3% ($F_{3,143}$=2.155; p=0.096) of the variance in SBP. There was no difference in SBP among genotype groups when controlling for PA. Further, when controlling for genotype, there was no difference in SBP by PA level. When gender was added to the base model of PA and genotype, the model predicted 31% ($F_{4,142}$=18.885; p=0.000) of the total variance in SBP. Genotype and PA remained non-significant predictors, while gender was significant (p=0.00). Specifically, females (113±11 mmHg) had lower SBP than males

(130±13 mmHg) independent of genotype and PA (β=-16.852 [-21.36- -12.35], p = 0.000). When assessed for covariates, BMI was a significant (β=1.262 [0.67- 1.85], p=0.000) predictor of SBP. There was no significant interaction between genotype and PA, but gender (β=-16.023[-20.53- -11.52], p = 0.000) and BMI (β= 1.442 [0.85- 2.03], p = 0.000) remained significant predictors of SBP (Table 4.1A). Specifically, when controlling for the interaction between PA and the ACE I/D genotype, females had a lower SBP while BMI was associated with higher SBP independent of all other variables.

R4.2 Association between ACE ID Polymorphism and Physical Activity on Diastolic BP (Study aim #4)

There was no difference among ACE I/D genotype groups when controlling for PA. Similar to SBP, gender was a significant predictor of DBP (β=-5.246 [-8.04- -2.45], p=0.000) independent of genotype and PA, with females (72±9 mmHg) having lower DBP than males (76±7 mmHg) (Table 4.2A). There was no significant interaction between the ACE I/D genotype and PA levels. Thus, no interaction effect was found between ACE I/D polymorphism and PA levels on DBP.

R4.3 Association between ACE ID Polymorphism and Physical Activity on TC (Study aim #4)

Similar to BP, there were no differences in TC among genotype groups when controlling for PA levels. Further, there was no difference among

genotype groups when controlling for both gender and PA. However, race was a significant predictor of TC (β=-15.485 [-30.39– -0.58], p=0.042) independent of PA and genotype. Specifically, Caucasians (172±29 mg/dL) had higher TC levels than other races (156±30 mg/dL) regardless of genotype group and PA level (Table 4.3A). Further, analysis revealed no interaction effect between genotype and PA for TC.

R4.4 Association between ACE ID Polymorphism and Physical Activity on HDL Levels (Study aim #4)

No differences among genotype groups were revealed for HDL levels when controlling for PA, though there was a trend for the *II* group to have a lower HDL level (57.3±12.1 mg/dL) as compared to those with the *D* allele (*ID*: 60.7±12.6 mg/dL; *DD*: 60.6±15.9 mg/dL). Independent of genotype and PA, gender was a significant predictor of HDL cholesterol, with males (52.8±10.3 mg/dL) having lower HDL levels in female (63.7±13.7 mg/dL) (Table 4.4A). For HDL cholesterol, there was no interaction effect found between the ACE I/D genotype and PA level.

R4.5 Association between ACE ID Polymorphism and Physical Activity on Body Composition (Study aim #4)

There were no differences in percent fat among genotype groups when controlling for PA level, though there was a trend for those with the *II* genotype to have greater percent fat (27±5 %fat) than those having the *D* allele (*ID*:

23±8 %fat; *DD*: 22±7 %fat). Independent of genotype, PA was a significant predictor (β=-0.079 [-0.12- -0.036], p=0.000) of percent fat values, with higher PA being associated with lower percent fat. Gender was found to be a significant predictor (β=10.093 [8.16- 12.03, p=0.000) of percent fat independent of both genotype and PA level. Specifically, males (15±5 %fat) had lower levels of percent fat than females (27±5 %fat). When controlling for covariates there was no differences among genotype, nor was PA a significant predictor of percent body fat. Gender, diastolic BP and eVO^2max were all significant predictors of percent body fat when controlling for PA and genotype (Table 4.5A). There was no interaction observed between PA levels and genotype on percent fat values.

R4.6 Association between ACE ID Polymorphism and Physical Activity on BMI (Study aim #4)

Body Mass Index was not shown to be affected by genotype or PA level. Gender was a significant predictor (β=-1.817[-3.09- 0.55], p=0.005) of BMI independent of genotype and PA levels, with females (23±4 kg/m^2) having lower BMI values than males (25±4 kg/m^2) (Table 4.6A). There was no interaction effect on BMI found between PA and genotype.

R4.7 Summary of association between ACE ID Polymorphism and Physical Activity on Cardiovascular Disease Risk Factors (Study aim #4)

The purpose of this specific aim was to determine whether PA modified the effect of the ACE I/D polymorphism on BP, lipid concentration, BMI and body fatness in college student. It was hypothesized that PA would suppress any relationship between the *DD* genotype and the CVD risk factors. Data from this sample did not support this hypothesis. There was no interaction observed between PA and genotype for any CVD risk factor.

CHAPTER 5

DISCUSSION

D1 Descriptive Data (Study Aim #1)

The reported PA for the sample was high, with 90% of the participants reporting meeting the minimal requirements (7.5 kcals/kg/week) of the ACSM. Our reported amount of physical activity is much higher than other reported values for young adults. According to Behavioral risk Factor Surveillance System Survey data, 64.5% of participants 18-24 years of age residing in Michigan reported meeting recommended PA guidelines.[12] Further, the U.S. National Center for Health statistics reported in 2007 that 37% and 30% of participants 18-24 years of age reported regular leisure-time activity and some leisure-time activity, respectively.[15] Additional, the National College Health Assessment reports in 2009 that 43.6% of participants meet recommended PA guidelines. This suggests that are sample of college adults was more active than other national and state reports. A potential reason for the increase in reported PA is that our sample was a convenience sample from a Kinesiology class. These participants may be participating in more PA activity due to the fact that they are in a class that promotes being PA.

The results of this study illustrate a gender effect for young adult PA participation. The impact of gender and PA participation in college students has been studied previously. Previous investigators have shown that male students were more likely to report higher levels of PA (43.7%) than female

students (33.0%).[3,106] However, others have found no differences in PA participation between males and females.[176] Inconsistencies in PA participation results could be due to data collection differences. Physical activity has been reported as the number of days per week of vigorous PA, the number of days of structured exercise, or as a categorical variable based on national PA recommendations. Leslie et al.[106] used a detailed assessment of PA which included frequency, intensity, and duration to determine weekly PA energy expenditure (kcals/week) in college aged students. They reported a higher proportion of females than males in the sedentary (< 100kcals/week) and low activity (100-799 kcals/week) categories, whereas males were more likely to be moderately active (>800 kcals/week of non-vigorous PA) or vigorous activity (>1600 kcals/week of PA including vigorous activity). Our results appear to support this finding of Leslie et al.[106]

Percent fat values in men (15±5%) were significantly lower than in women (27±5%) for our sample. This result is similar to a study conducted in the upper Midwest region of the US showed female college students had higher levels of percent fat (28.5±8), as measured by air displacement plethysmography, as compared to males (17.7±8).[140] Men typically have higher lean mass and more central fat distribution, whereas women typically have higher adiposity with more peripheral fat distribution.[79] However, data on participant fat distribution were not available in our sample for comparison.

D2 Association between Physical Activity and Cardiovascular Disease Risk Factors (Study aim #2)

Epidemiologic studies have demonstrated protective effects of PA, exercise and physical fitness on the prevention of CVD and reduction of risk factors. The relationship between physical fitness and CVD risk factors has been reported to be stronger than the relationship of PA and CVD. In the Quebec Family Study, Katzmarzyk et al.[95] investigated the relationship between in PA and CVD risk factors in 610 males and females between the ages of 9-18. They reported that 5-20% percent of the variation in CVD risk factors was explained by PA.[95] Further, it is suggested that the relationship between PA and CVD risk factors is not gender dependent.[95] This is supported by McGuire et al.[118] who concluded, by assessing 5882 adults using NHANES data, which PA was associated with a decrease in CVD risk factors but this association was not modified by gender.

D2.1 Association between Physical Activity and BP (Study aim #2)

In our study, the adjusted regression model indicated that PA was not associated with SBP ($p=0.71$) or DBP ($p=0.98$). However, SBP was predicted by BMI and gender, while DBP was only predicted by BMI. Our results are similar to those of Gaya et al.[77] who found in 167 adolescents, higher BMI was positively associated with both SBP and DBP. Gaya et al.[77] also found BMI to be the sole predictor of DBP. The fact that gender was a significant predictor

of SBP is not surprising considering that high BP is more common in males than females until the age of 45.[11]

We hypothesized that PA would be inversely related to BP, but this was not supported by our results. The sample consisted of relatively healthy, young adults who were enrolled in a kinesiology healthy lifestyles class. Selection bias is a potential concern since students enrolled in an activity based class may be more health conscious overall, and participate in other healthy behaviors (e.g., healthy diet) that might help lower BP. Also, the mean BP for the sample (119±14/73±9 mmHg) is equal to, if not better than, the recommended 120/80 mmHg. Therefore, it is possible that PA was not able to influence BP reductions any further in those whose values were already optimal. Numerous studies have found that PA reduces the risk of hypertension.[20,32,48,105,143,154] Most of these studies included participants who where older[20,154] or were prospective studies designed to examine participants who developed hypertension.[32,48,143] For example, Leary et al.[105] assessed BP and PA in 5505 11-12 year-old children. The authors used accelerometers to assess PA, a more objective measure of PA than self-report, and found that higher levels of PA were associated with lower BP. While it is widely accepted that regular PA is effective in reducing SBP and DBP, this effect is most beneficial for those diagnosed with mild hypertension and not those who are normotensive.[63] Since our sample had relatively low BP values, it is possible that any PA effect was minimized.

Another consideration is that for BP to be associated with PA there must be an intrinsic biologic threshold for the amount of PA needed to effect a change in BP.[36] Blair et al.[32] found in the Aerobic Center Longitudinal Study that those in the low fitness category were more likely to develop hypertension than those in the high fitness category. In the Coronary Artery Risk Development in Young Adults study, Carnethon et al.[44] reported that individuals with high cardiorespiratory fitness, as measured by treadmill test, had a lower relative risk of hypertension than those of low fitness. These studies differ from our study in that they examined fitness levels, where as our primary goal was to assess the PA level and association with BP. It could be possible that the level of PA activity was not enough to meet the intrinsic threshold needed for an association in this young adult sample. Further, since our study was cross-sectional and consisted of apparently healthy, young adults it is possible that the environmental variability necessary to find an association between PA and BP is still masked by a relatively high genetic component.

It is possible that while PA and BP are not associated currently in our sample, that an association will occur later in life. Blair et al.[32] in their prospective study of over 6000 normotensive men and women declared that the risk of developing hypertension increases substantially with increased higher baseline BP. This idea may be important for our study because while the mean BP was normal (119±14/73±9 mmHg), individual variability existed. Further, when BP was assessed by gender, males had a mean BP value

(130±13/77±7) that is in the pre-hypertensive range, while females had a mean BP value of (114±11/72±9) which is considered normal. In additional analysis, there were no trends for an association between PA and SBP by gender. Finally, it is possible that BP values measures for this study may not have represented true resting values. The testing was done as a part of health and wellness screening, thus due to moving from one station to another, the resting BP could have been elevated.

D2.2 Association between Physical Activity and Blood Lipid Concentration (Study aim #2)

Our results showed that PA was not associated with TC or HDL levels. On average, TC (169±32 mg/dL) and HDL (60±14 mg/dL) results for this sample were considered normal, and potentially favorable, based on the recommendation set by the National Cholesterol Education program for cholesterol management.[5] The HDL mean for the sample (60±14 mg/dL) and for females (63±14 mg/dL) would be considered protective, while the values for men (53±10 mg/dL) were normal. The TC and HDL levels found in this study are similar to those reported by National Health and Nutrition Examination Survey (NHANES) 2003-2004 among adolescents 12 to 19 years of age (TC:162 mg/dL; HDL: 55 mg/dL).[160] The similarity between the NHANES national TC and HDL statistics and our results are understandable considering that the majority of our sample was 20 years of age or younger.

The fact that PA was not associated with TC nor HDL is surprising considering that PA is frequently associated with increases in HDL levels. In the Atherosclerosis Risk in Communities study, Monda et al.[125] assessed the longitudinal effect of PA on lipids and reported that increases in PA level were associated with increases in HDL. In a review of literature, Tambalis et al.[185] concluded that the most frequently observed result of exercise training was increased HDL, with decreases in TC being less frequent.

It is possible that our results represent an age and PA level dependence. Ainslie et al.[18] found in a group of men, that the older group (mean age 56±4 years) had a greater reduction in TC and greater increase in HDL as compared to the younger group (mean age 24±4 years) after ten days of prolonged walking. Our study participants were young adults with favorable levels of TC and HDL, thus associations with PA could be hidden by other factors, such as diet and age.

Our results indicated that there was a racial difference in TC, with Caucasians having higher TC (172±30 mg/dL) than other races (157±30 mg/dL). Our results differ from McWilliams et al.[120] who reported, from NHANES data, that TC levels did not differ by race. It is possible that our results are due to small sample size resulting in a spurious distribution, since 26% of our study participants were not Caucasian.

We found gender to be a significant predictor of HDL values. Females had significantly higher levels of HDL (64±39 mg/dL) than did males (53±39 mg/dL). This finding is supported by the fact that boys tend to have a reduction

in plasma HDL levels during puberty that is not observed in girls.[62] Our data is similar to the NHANES 2003-2004 data for adolescent aged 12-19 years, where females had higher HDL levels (56 mg/dL) than males (50 mg/dL).[160] Further, NHANES data in adults supports the general trend that females have higher HDL levels than males.[160]

In summary, our results indicate that in a sample of young, apparently healthy adults PA was not associated with TC or HDL cholesterol. This result is most likely due to the optimal levels of HDL and TC found in most of our sample.

D2.3 Association between Physical Activity and BMI and Body Composition (Study aim #2)

Our results show that as PA quartile increased, percent fat values decreased, regardless of gender (refer to Table 1B). This finding supports our hypothesis that PA would be inversely associated with percent fat values. Our results are similar those of Zeno et al.[209] who found in a study of 142 African American and Caucasian adults that fitness was inversely associated with percent fat in both males and females. Zanovec et al.[208] found in 278 young adult students that prediction equations of percent fat are enhanced when PA is accounted for, implying that PA has an association with percent fat. Gregory et al.[82] reported that lower levels of PA in Guatemalan young adults were associated with higher percent fat values. Our result that PA is associated with

percent fat is consistent with the belief that PA plays a significant role determining body composition.[63]

While our data showed a significant association between PA and percent fat, we did not find a similar relationship between PA and BMI for either gender. This finding is in contrast to the results of Zeno et al.[209] who reported a significant association between PA and BMI. Our results are supported by the findings of Janssen et al.[93] which showed that for a given BMI, physically active individuals have lower levels of total fat mass than those who were less active. Further, in two separate randomized control trials, Ross et al.[161,162] observed that exercise without weight loss was associated with reductions in total and abdominal fat, even in individuals who did not lose weight. In our sample, both men and women had normal BMI values (men=24.8; women=23.3).[14] Since mean BMI was in the normal range, a significant PA effect would likely be minimal.

In summary, our results supported our hypothesis that PA would be inversely associated with percent fat but did not support an association between PA and BMI.

D3 Associations between the ACE ID Polymorphism and Cardiovascular Disease Risk Factors (Study aim #3)

The overall frequencies of genotypes *II*, *ID*, and *DD* were 16.1, 56.4 and 27.5, respectively for the entire sample. The observed genotype frequencies were in agreement with those predicted by Hardy-Weinberg

equilibrium. Our study is similar to studies done in Scottish young adults (mean age approximately 20 years) and Finish young adults (mean age approximately 34 years).[85,91] When stratified by gender, the overall frequencies for the *II*, *ID* and *DD* genotype were 20.3, 54.3 and 25.2 in women and 6.5, 60.8 and 32.6 in men, respectively. For women, the genotype frequencies were similar to results from a study done with US adolescents and young adults that reported a genotype frequency of 19.4 for *II*, 49.1 for *ID*, and 31.5 for *DD*.[194] In our study the gene frequency in males deviated from Hardy-Weinberg equilibrium for this sample, but this is most likely due to the low number of males (n=3) classified as having the *II* genotype.

In our sample we found a difference in the amount of PA based on genotype. Participants classified as *DD* reported higher levels of PA, with *ID* being intermediate and *II* having the lowest levels of PA. This finding does not support the results of Fuentes et al.[75] who found no association between the ACE I/D polymorphism and moderate intensity leisure time PA in middle aged (mean age 44 year) Finish adults. This difference may be due to differences in measured PA. Fuentes et al.[75] asked a single self-reported question on moderate leisure time PA. This single question may not have the precision to determine PA at the level needed to result in a relationship with gene effects.[203] We assessed PA through self-report, but were much more specific in terms of frequency, intensity and duration. An additional difference could be the age of the sample. Our sample consisted of young healthy adults, whereas the Fuentes et al.[75] studied middle aged Finnish adults. Our study results are

opposite of those found in a study conducted on young, Italian adults. Specifically, Winnicki et al.[205] found that the *D* allele was associated with lower levels of PA.[205] In contrast to our sample, Winnicki et al.[205] assessed the association between PA and the ACE I/D polymorphism in a group of hypertensive young (mean age ~33 years) adults. Further, they assessed PA through a questionnaire that dichotomized PA (sedentary and exerciser) based on relative intensity.[205]

We also found a graded response among genotypes for estimated VO_2max, with the *DD* genotype (39 ml/kg/min) having the highest estimated values and the *II* genotype having the lowest (33 ml/kg/min). Our finding of the ACE *DD* genotype being associated with higher VO_2max levels is in contrast to previous studies performed on elite and extremely fit athletes.[24,51,78,130,135,189] In studies of elite athletes, including Spanish runners and cyclists[24], 100 fastest South African born Ironman finishers,[51] and Australian rowers,[78] the *I* allele occurred at a higher frequency compared to healthy controls. In a study of potential British Olympic athletes there was a significant linear trend between the distance of the event and the frequency of the *I* allele; those who participated in longer distance running events had higher frequencies of *I* allele than athletes who participated in shorter distance events.[135] In a study of British high mountaineers, there was a significant difference in genotype distribution, with mountaineers having an increased representation of the *I* allele as compared to normal controls.[130,189]

The potential mechanism for the association between the ACE *I* allele and elite endurance performance may be due to increased substrate delivery,[130] modified absorptive function of the intestinal track,[128] increased anabolic response for fat-free mass,[24] and/or an increase in percentage of slow-twitch fibers.[210] These studies focused on the genotype frequency of elite athletes or high level mountaineers and the finding could potentially be due to selection bias. The difference in sample characteristics could explain why our results differed from these previous studies.

Our results are also in contrast to three previous studies reporting no association between the VO_2max and the ACE I/D polymorphism. In a study of 60 untrained women[59] (age= 24 years), and in 724 sedentary adults[150] there was no association between measured VO_2max values and the ACE I/D polymorphism. In another study, 192 aerobically fit male athletes (VO_2max ≥ 72 ml/kg/min) showed no difference in genotype distribution from 182 sedentary controls.[151]

In support of our findings, Costa et al.[54] reported that the allele distribution was significantly different between Portuguese elite short distance swimmers, considered anaerobic athletes, and elite middle distance swimmers, considered a mix between anaerobic and aerobic athletes. The authors reported that the *D* allele was more prevalent in the long distance swimmers, which is likely the group with the higher VO_2max values.[54] Amir et al.[25] compared the ACE allele frequency in Israeli elite endurance athletes, elite power athletes and healthy controls. The *D* allele was more frequent in

endurance athletes than in power athletes or controls.[25] Similar to our findings, endurance athletes with an assumed higher VO_2max had a significantly higher frequency for the *D* allele. In a sample of young (23 years of age) Chinese men, the *DD* genotype was found to be associated with higher levels of measured aerobic capacity.[211]

Rigat et al.[158] were the first to show that the ACE I/D polymorphism was associated with serum ACE levels, with the *D* allele linked to higher enzyme levels. ACE is responsible for converting angiotensin I into the active angiotensin II,[43,56] which reportedly causes hypertrophy in cardiac myocytes.[164] The hypertrophic response to physical training may be attenuated by the presence of the ACE *D* allele, resulting in left ventricular hypertrophy which in turn would increase VO_2max via increased stroke volume.[211] The ACE *D* allele has been associated with increased in left ventricular mass as a response to training.[129] Thus, our finding that the ACE *DD* genotype was associated with estimated VO_2max levels is biologically plausible.

In summary, we found the *DD* genotype to be associated with highest eVO_2max values and the *II* genotype having the lowest. Our findings are in agreement with other studies that have reported that the D allele is associated with higher VO_2max values,[25,54,211] but in opposition to those reporting that the *I* allele is associated with higher VO_2max values[24,51,78,130,135,189] or has no association. [59,150,151]

D3.1 Association between ACE ID Polymorphism and BP (Study aim #2)

In our sample there was no association between the ACE I/D polymorphism and BP. This result is not supported by studies that have shown an association between ACE I/D polymorphism and hypertension.[27,49,64,83,184] Case-control studies, including younger Croatian adults (mean age ~35 years)[27], older Chinese men (mean age ~57 years),[49] older Mongolian men and women (mean age ~52)[83] and African American men and women (age not reported)[64], have reported that the *D* allele of the ACE I/D polymorphism was associated with hypertension. Explanations of these findings include the regulation of BP through increased angiotensin II, a potent vasoconstrictor, as well as the degradation of bradykinin, a potent vasodilator.[27,49] The above studies[27,49,64,83] assessed participants who were greater than 35 years of age with hypertension. We assessed increased BP as a risk factor in young adults instead of hypertension in middle age to older adults, potentially explaining differences between the findings.

Our finding of no association between the ACE I/D polymorphism and BP is supported by other studies.[75,85,91,116,126,127,144,194,199]. In 885 Caucasian young (5-29 years of age) participants as well as in 721 young (mean age 34 years) Finnish adults, the ACE I/D polymorphism was not associated with SBP or DBP.[75,91,194] In a variety of case-control studies, including German, Hungarian and Greek participants ranging from youth to late adulthood, there was no association between the ACE I/D polymorphism and BP or hypertension.[126,127,144,199] Two meta-analyses, conducted to assess the

influence of the ACE I/D polymorphism on BP, reported no relationship between the ACE genotype and BP.[17,177] These studies support our finding of no association between ACE I/D polymorphism and BP in a sample of young adults.

While the ACE polymorphism has been shown to increase plasma ACE activity,[85,158,191] it is plausible that increased ACE levels do not result in increased production of angiotensin II. Such a result would provide some mechanistic evidence for the lack of association between the ACE polymorphism and BP.[17] Animal studies support the idea that hypertension genes may be activated only under certain conditions or during specific periods throughout the life span. This could explain why the ACE genotype has been found in association with hypertension in older adults, but not with BP in younger individuals. Further, it is possible that the ACE gene does not play a role in the initiation of increased BP but may contribute to the maintenance of high BP under situations of hypertension or older age.[49] Finally, multiple biochemical and physiological controls regulate BP levels through interactions with various genetic and environmental factors.[194] Thus, it is likely that variation in the ACE gene may have only small effects on BP.[83,194,199]

D3.2 Association between ACE I/D genotype and Blood Lipid Concentrations (Study aim #2)

We found that the ACE I/D polymorphism was not associated with TC or HDL cholesterol. Our findings differ from those who have found an association between impaired lipid levels and the *DD* genotype. Results from studies of Japanese and Israeli adolescents with type II diabetes showed those with the *D* allele had increased levels of TC and HDL.[23,99] In Polish adults (age~43 years) with coronary artery disease, the *DD* genotype was associated with elevated TC and low density lipoprotein cholesterol.[137] In two additional non-US samples (Korean and Israeli men) TC and low density lipoprotein cholesterol were highest in the those with the *DD* genotype compared the those with the *II* genotype.[50,142] In treatment intervention studies, researchers have found a gene by treatment effect, with those having the *DD* genotype having greater reduction in lipid levels in response to pharmaceutical treatment.[37,115] Current data do not provide a clear plausible mechanism for the association between ACE I/D polymorphism and lipid levels,[115] though it has been demonstrated that angiotensin II modifies LDL causing an enhanced rate of cellular uptake.[37,96] Thus, increases in ACE activity associated with the *DD* genotype could potentially increase angiotensin II production which could lead to increased modified LDL uptake. Our study results may differ from those that that showed an association because we did not assess older individuals, nor those with disease, and our design did not include an intervention. It is possible that the ACE I/D

polymorphism is a variability gene which contributes to the variability of lipid levels only under variation in environmental factors, such as age, pharmacological treatment or disease.[37]

Our results are supported by studies which have reported no association between ACE genotype and lipid profiles. For example, case-control studies of older (≥55 years of age) Italian and Mexican adults found no association of lipid levels among the genotypes.[53,198] In addition, studies of American Samoans and Japanese adults showed the ACE I/D polymorphism was not associated with lipid levels.[55,141] A lack of association between the ACE genotype and lipids levels in our study is likely due to our study participants having low TC (169 mg/dL) and high HDL (60 mg/dL) levels. Specifically, TC levels were well below the cut point, ≥200 mg/dL, set by the National Cholesterol Education Program.[5] Further, the mean HDL level for the sample would be considered protective since they are equal to the recommended level (60 mg/dL) set by the National Cholesterol Education Program.[5] Thus, TC and HDL levels in our sample may have limited our ability to find an association since they are already optimal. It is plausible that the ACE gene does not play a role in the initiation of high TC or decreased HDL, but may contribute to the maintenance of adverse lipid levels.[49] Further, the interaction of multiple genetic and physiologic factors may account for variable levels of blood lipids.[55]

D3.3 Association between ACE ID Polymorphism and BMI and Body Composition (Study aim #2)

There was no association between ACE I/D polymorphism and CVD risk factors in our sample, except for percent fat. Univariate analysis revealed that those with the *I* allele had higher percent fat values compared to the referent group of *DD* homozygotes. A plausible explanation for this is that in our sample of young adults those with the *DD* genotype were more physically active (50.4±33 kcals/kg/week) than those with the *I* allele (31.4±24 kcals/kg/week). Thus, the association between the ACE I/D polymorphism and percent fat values maybe driven by increased PA being inversely related to percent fat. The result of *I* allele being associated with increases in percent fat differs from others who have found the *D* allele to be associated with increases in percent fat. In Greek adolescent and Italian adult males, the *D* allele was associated with increased fatness [131] and increased incidence of being classified as overweight,[179] respectively. In Spanish coronary heart disease patients, those with the *D* allele, had an increased prevalence of obesity and abdominal fat.[157] Inconsistencies between findings are unclear, but could be due to different study designs, sampling techniques and/or subject sampling. Further, many of the reported findings have associated the ACE genotype with BMI, which is not a measure of body composition. We found the ACE genotype to be associated with percent fat, not with BMI.

Support for our finding is seen in the results of Wacker et al.[201] who studied middle-aged men and women classified by BMI as obese or normal

weight. The authors found that the *I* allele was more frequent in the obese males than in normal weight males.[201] A similar finding was found in females but it was not statistically significant.[201]

In summary, univariate analysis revealed an association between the *I* allele and percent fat level. However, this association disappeared after controlling for gender. The ACE gene may contribute to the regulation of body composition, but only through the complex interaction of multiple genes and environmental exposures. [89]

D4 Determination of interaction effect between ACE ID Polymorphism and Physical Activity on Cardiovascular Disease Risk Factors (Study aim #4)

We hypothesized that PA would suppress any relationship between the *DD* genotype and CVD risk factors, including increased BP, lipids and percent fat. However, we found no evidence of an interaction effect between PA levels, the ACE I/D polymorphism and CVD risk factors in our sample.

For biologic interaction to exist, the gene and environment must interdependently influence an expressed phenotype. There is abundant evidence that PA influences CVD risk factors, although individual responses to exercise differ. Thus, environmental exposure (PA) must interact to some degree with genetics (ACE I/D polymorphism) to produce different phenotypic responses. In our sample we were unable to substantiate this hypothesis.

There are a number of reasons why an interaction may not have occurred in our sample.

1) Cardiovascular disease and its risk factors are multifactorial in nature and as such, are unlikely to be controlled significantly by a single gene such as the ACE polymorphism. There are several complicated and life-long gene by environment interactions that may have to occur for PA to reduce the levels of CVD risk factors or decrease the manifestation of CVD.[39] Human diseases and associated risk factors likely involve multiple genes with modest effects and gene-environmental interactions.[207] Bray et al.[39] stated that findings of gene-environment interaction studies may be dependent on the context in which variables are analyzed, including assessing multiple polymorphisms in each participant. Due to the nature of our study we were unable to analyze more than one polymorphism, thus limiting our ability to assess potential findings in multifactorial risk factors.

2) The possibility exists that PA is associated with the ACE I/D polymorphism and that the relationship does impact CVD risk factors, but it could not be detected in this study. We assessed the relationship between PA and the ACE polymorphism under the hypothesis that if the *DD* genotype was present, high PA levels would be inversely associated with risk factor levels. The relationship could exist in the context that at a currently undetermined biologic threshold for PA, the interaction with ACE I/D polymorphism ceases to have biological significance. Booth et al.[36] stated that an intrinsic biologic requirement for PA is likely and not meeting this threshold (aka, physical

inactivity), leads to a disruption of normal homeostasis. In our sample of young adults, it could be possible that the high PA levels lead to no gene by environment interaction. Mudd et al.[133] reported that the minimum amount of energy expenditure needed to meet the PA recommendations of the ACSM or the Institutes of Medicine (IOM) was 7.5 kcals/kg/wk and 21 kcals/kg/wk, respectively. For our sample the median energy expenditure was 28 kcals/kg/wk; which is above both ACSM and IOM recommended levels. When stratified by gender, both males (43.0 kcals/kg/wk) and females (23.1kcals/kg/wk) reported median PA levels above the minimum required to meet both recommendations. Thus, it is possible that our participants' high PA levels were above the undetermined intrinsic PA threshold needed for an interaction to exist between PA and the ACE I/D polymorphism.

It is also possible that the PA and ACE I/D polymorphism interaction may be age dependent. Our participants were all under 25 years of age, which may impede our ability to detect a gene-environment interaction effect for CVD risk factors. Due to the sample's age we may have missed the opportune window of development that is needed for examining gene-environmental interactions.[207] After indentifying an age-varying association between ROBO1 and obesity, Lasky-Su et al.[104] reported that inconsistencies in replication of association findings may be due to age-genotype interactions. They stated that age-dependent effects are easily missed during cross-sectional studies because data collection may not occur when the genetic effect is strongest.[104] While genetics are constant, environmental exposures are not. Thus, age may

be a necessary trigger that must be met to amplify the modest genetic effect of the ACE I/D polymorphism or to allow for PA and the ACE polymorphism to have a significant biologic effect.[192]

3) A Type II error occurred, as the power needed to detect gene-environment interaction requires larger samples sizes than more basic environmental studies.[33,61] In a review of statistical associations between genes and disease, Manly[113] stated that most gene association studies will require hundreds or thousands of participants.[113] Dempfle et al.[61] stated that to detect a gene-environment interaction a 7x increase in sample size may be required over the sample size needed to assess genetic effects. Our final sample size for the gene-environment interaction was 149 participants. Post hoc power analysis suggested the power of our study to be 0.29, which significantly reduced our ability to show an interactive effect between PA and the ACE genotype. Thus, our Aim 4 was exploratory.

4) A final explanation for why an interaction between PA levels and the ACE I/D polymorphism did not occur is that the precision of measure for PA exposure or CVD risk factors may not been sufficient. A common complication of gene by environmental studies is low precision of environmental exposures, compared to genotype classification.[110] Boks et al.[33] stated that to prevent a decreases in power, measures of traits and environmental exposures must be completed as precisely as possible. In our study, PA was assessed via self-reported questionnaire to capture duration, frequency and intensity of activity. From these variables we were able to quantify PA into kcals/kg/week. This is a

valid measure of PA but is limited by report bias. Participants are likely to overestimate their vigorous PA level and underestimate their moderate PA level thus increasing measurement error.[166] Thus, a more precise measure of PA may be necessary when assessing PA as an environmental interaction with genetics. In research there is often a tension between the precision of measures and the size of the study. As the sample size increases selection of precise assessment measure decreases due to location, cost, and time. Errors in assessing the CVD risk factors such as participant BP potentially not being measured in a completely rested state, not assessing low density lipoprotein levels, or using skinfold caliper to assess body composition all potentially lead to increases in measurement error which subsequently could lead to decreases in statistical power. Any compromise in precision of exposure or outcome measures increases measurement error, and leads to a decreased likelihood of finding a true effect.[206,207] Thus, it is possible that no interaction was found between the ACE I/D polymorphism and PA on CVD risk factors due to the measures used to assess the various variables, despite the fact that all measures were obtained with as much precision and attention to detail as possible.

In summary, we found no interaction effect between PA and the ACE I/D polymorphism on CVD risk factors. Potential reasons for this finding include the multifactorial nature of CVD risk factors, the nature of the PA and ACE I/D polymorphism relationship, the inadequate power given the final sample size and the potential decrease in precision of variable measures.

While we did not find an interactive effect for the PA and the ACE I/D polymorphism, studies that investigate the environment and generic factors along with their interaction are necessary to determine the processes that lead to complex diseases and their associated risk factors.[33,39]

D5 Conclusions

Overall, study results showed a gender effect for PA and percent fat, with males having higher values of PA and lower values of percent fat than females. Physical activity showed an inverse relationship with percent fat, but was not associated with other CVD risk factors. The ACE I/D polymorphism was found to have an association with PA and estimated VO_2max values. Those with the *DD* genotype had the highest values of PA and estimated VO_2max values. There was no association between the ACE I/D polymorphism and other modifiable CVD risk factors. There was no interaction effect of PA with the ACE I/D polymorphism on CVD risk factors.

Young adulthood is a critical time in the development of disease. While young adults are typically considered healthy, lifestyle choices (e.g., exercise, diet, smoking) affect their future risk of disease development. Future studies should include larger samples, a wider range of health behavior profiles, and tracking should continue through older adulthood. The future of health research is dependent on the understanding of gene by environmental interaction. The nature of the interaction as well as timing or triggers of the interaction are critical to understanding disease progression and risk factors.

The study of gene by environmental interaction in young adults is central to the future understanding of disease progression.

FIGURES AND TABLES

Table 1A: Descriptive categorical data for study participants based on gender

	Total		Males		Females		Chi-square p-value
	n	%	n	%	n	%	
Race							
Caucasian	162	84.4	49	83.1	113	85.0	N/A
Other	30	15.6	10	16.9	20	15.0	
Age							
18	40	20.8	8	13.6	32	24.1	N/A
19	49	25.5	13	22.0	36	27.1	
20	62	32.3	25	42.0	37	27.8	
≥21	41	21.4	13	22.0	28	21.1	
Major							
Kinesiology	84	43.8	23	39.0	61	45.9	N/A
Other	106	55.2	35	59.3	71	53.4	
Smoking							
Non-Smoker	164	85.4	47	79.7	117	88.0	N/A
Smoker	18	9.4	8	13.6	10	7.5	
Physical Activity Cut point							
≤ 7.5 kcals/kg/week	18	9.4	3	5.1	15	11.4	N/A
> 7.5 kcals/kg/week	173	90.1	56	94.1	117	88.6	
BMI							
Obese	11	5.7	4	6.8	7	5.3	0.005
Overweight	39	20.3	20	33.9	19	14.3	
Normal Weight	139	72.4	34	57.6	105	78.9	
Physical Activity Quartile							
0-14.8 kcals/kg/week	48	25.1	7	11.9	41	30.8	0.000
14.8-28.0 kcals/kg/week	48	25.1	9	15.3	39	29.3	
28.0-54.7 kcals/kg/week	49	25.7	19	32.2	30	22.6	
> 54.7 kcals/kg/week	46	24.1	24	40.7	22	16.5	
Genotype							
II	24	12.5	3	6.5	21	20.4	0.097
ID	84	43.8	28	60.8	56	54.4	
DD	41	21.4	15	32.6	26	25.2	

Table 1B: Descriptive continuous data for study participants based on gender

	Total					Male					Female					Genders Comparison	
	n	Mean	SD	Median	IQR	n	Mean	SD	Median	IQR	n	Mean	SD	Median	IQR	F statistic	p-value
Height (in)	191	66.4	3.7	66.0	4.0	59	69.4	2.9	69.0	5.0	132	64.9	3.2	64.0	3.0	83.9	0.000
Weight (lbs)	191	147.9	28.2	145.0	30.0	59	170.2	29.1	162.0	37.0	132	138.1	21.5	136.0	26.7	72.8	0.000
SBP (mmHg)	190	119.0	13.9	119.0	19.0	58	130.0	12.7	130.0	16.0	132	114.0	11.1	113.0	16.7	82.0	0.000
DBP (mmHg)	190	73.0	7.6	73.0	10.0	58	76.0	7.2	77.0	10.0	132	72.0	7.5	72.0	10.0	12.4	0.001
TC (mg/dL)	175	169.0	30.1	166.0	35.3	54	167.0	30.5	165.0	45.8	120	171.0	29.8	167.0	35.2	0.6	0.431
HDL (mg/dL)	173	60.0	13.7	59.0	18.5	53	53.0	10.3	52.0	13.0	120	64.0	13.7	63.0	18.0	26.4	0.000
BMI (kg/m2)	191	23.7	3.5	23.2	3.8	59	24.8	3.7	24.1	4.4	131	23.3	3.3	22.8	3.4	8.4	0.004
%Fat	191	23.2	7.4	24.7	9.0	59	15.4	5.4	14.2	8.0	133	26.7	5.1	26.3	5.5	192.3	0.000
eVo2max (ml/kg/min)	188	36.8	7.7	36.0	7.0	57	41.9	6.4	42.0	9.5	131	34.5	7.2	36.0	9.0	44.9	0.000
PA (kcals/kg/week)	191	36.6	28.2	28.0	39.6	59	48.2	28.9	43.0	41.5	132	31.5	26.4	23.1	30.5	15.4	0.000
Total seat time (hours)	191	6.2	2.4	6.0	3.0	59	6.4	2.4	6.0	3.5	132	6.2	2.4	6.0	2.5	0.2	0.638

Table 1C: Descriptive characteristics of participant by physical activity quartile

	Quartile 1 0 - 14.8 kcal/kg/wk, n = 48	Quartile 2 14.9 - 28.0 kcal/kg/wk, n=48	Quartile 3 28.1 - 54.7 kcal/kg/wk, n=49	Quartile 4 > 54.7 kcal/kg/wk, n= 46
Female, %	85.4	81.3	61.2	47.8
White, %	72.9	85.4	91.8	87
Height (in)	66.3 ± 3.7	65.9 ± 4.6	66.9 ± 3.4	67.7 ± 3.5
Weight (lbs)	150.6 ± 32.8	138.8 ± 25.1	146.8 ± 23.8	155.4 ± 28.8
SBP (mmHg)	117 ± 14*	113 ± 13*	122 ± 11	122 ± 16
DBP (mmHg)	75 ± 7	72 ± 9	73 ± 7	73 ± 7
Total Cholesterol (mg/dL)	171 ± 32	169 ± 30	168 ± 28	171 ± 31
HDL (mg/dL)	62 ± 14	59 ± 12	59 ± 14	61 ± 14
BMI (kg/m^2)	25.1 ± 4.5‡	23.0 ± 2.6	23.1 ± 3.1	23.7 ± 3.1
%Fat	26.9 ± 4.6*¤	25.1 ± 7.6*¤	21.4 ± 6.8	19.2 ± 7.6
estimated Vo2max (ml/kg/min)	31.9 ± 6.1‡	35.2 ± 8.5‡	39.4 ± 6.9	40.4 ± 6.2
Total seat time (hours)	6.2 ± 2.5	6.2 ± 2.24	6.6 ± 2.2	5.9 ± 2.5

* Significant difference from highest quartile

¤ Significantly different from quartile 3

‡ Significantly different from all other quartiles

Table 2.1A: Descriptive categorical data for Systolic Blood Pressure (mmHg)

		N	Mean	SD	t-statistic	P-value
Gender						
	Male	58	130	13	9.056	0.000
	Female	132	114	11		
Race						
	Caucasian	161	119	14	0.215	0.830
	Other	29	118	12		
Major						
	Kinesiology	84	120	14	0.977	0.330
	Other	104	118	14		
Smoking						
	Non-Smoker	162	118	13	-0.649	0.517
	Smoker	18	120	14		

		N	Mean	SD	f statistic	P-value
Age						
	18	40	117	12	0.594	0.62
	19	48	119	14		
	20	62	120	16		
	≥21	40	118	13		

Table 2.1B: Descriptive continuous data for Systolic Blood Pressure (mmHg)

	β	Std. Err.	P-value	R	R square	R square Δ	P-value
Physical Activity (kcal/kg/wk)	0.09	0.035	0.012	0.182	0.033	0.028	0.012
TC (mg/dL)	0.021	0.036	0.562	0.044	0.002	-0.004	0.562
HDL (mg/dL)	-0.291	0.077	0.000	0.280	0.078	0.073	0.000
%Fat	-0.607	0.141	0.000	0.299	0.09	0.085	0.000
BMI (kg/m^2)	1.52	0.269	0.000	0.382	0.146	0.141	0.000
eVO2max (ml/kg/min)	0.419	0.139	0.003	0.217	0.047	0.042	0.003
Total Seat Time (hours)	0.447	0.428	0.297	0.076	0.006	0.000	0.297

Table 2.1C: Summary of multiple regression model for Systolic Blood Pressure (mmHg)

		95% CI for β			
	β	Lower	Upper	Std Error	p-value
Constant	113.92	98.25	129.58	7.93	0.000
BMI (kg/m^2)	1.22	0.71	1.73	0.26	0.000
Physical Activity (kcal/kg/wk)	-0.011	-0.07	0.05	0.03	0.711
Gender	-14.4	-18.23	-10.57	1.94	0.000
Race	-1.433	-6.25	3.38	2.44	0.557

Table 2.2A: Descriptive categorical data for Diastolic Blood Pressure (mmHg)

		N	Mean	SD	t-statistic	P-value
Gender						
	Male	58	76	7	3.52	0.001
	Female	132	72	7		
Race						
	Caucasian	161	73	8	-0.306	0.76
	Other	29	74	8		
Major						
	Kinesiology	84	74	8	0.269	0.788
	Other	104	73	7		
Smoking						
	Non-Smoker	162	73	8	-0.141	0.888
	Smoker	18	74	5		

		N	Mean	SD	f statistic	P-value
Age						
	18	40	72	8	1.198	0.312
	19	48	73	8		
	20	62	75	7		
	≥21	40	73	7		

Table 2.1B: Descriptive continuous data for Diastolic Blood Pressure (mmHg)

	β	Std. Err.	P-value	R	R square	R square Δ	P-value
Physical Activity (kcal/kg/wk)	0.001	0.023	0.976	0.002	0.000	-0.005	0.976
TC (mg/dL)	0.01	0.023	0.622	0.034	0.001	-0.005	0.622
HDL (mg/dL)	-0.08	0.052	0.125	0.117	0.014	0.008	0.125
%Fat	0.007	0.096	0.006	0.006	0.000	-0.005	0.939
BMI (kg/m^2)	0.746	0.181	0.289	0.289	0.083	0.078	0.000
eVO2max (ml/kg/min)	0.014	0.077	0.857	0.013	0.000	0.005	0.857
Total Seat Time (hours)	0.285	0.277	0.305	0.075	0.006	0.000	0.305

Table 2.2C: Summary of multiple regression model for Diastolic Blood Pressure (mmHg)

		95% CI for β			
	β	Lower	Upper	Std Error	p-value
Constant	63.79	55.45	72.13	4.22	0.000
Physical Activity (kcal/kg/wk)	-0.018	-0.060	0.024	0.021	0.401
Gender	-9.65	-13.39	-5.91	1.89	0.000
Race	1.31	-1.99	4.62	1.67	0.435
Percent Fat	0.49	0.23	0.74	0.13	0.000
TC (mg/dL)	0.033	-0.005	0.072	0.02	0.09

Table 2.3A: Descriptive categorical data for Total Cholesterol (mg/dL)

		N	Mean	SD	t-statistic	P-value
Gender						
	Male	54	167	31	-0.79	0.431
	Female	120	171	30		
Race						
	Caucasian	150	172	30	2.365	0.019
	Other	24	157	30		
Major						
	Kinesiology	73	170	30	-0.058	0.954
	Other	99	170	30		
Smoking						
	Non-Smoker	148	170	29	-0.214	0.831
	Smoker	16	171	30		
		N	Mean	SD	f statistic	P-value
Age						
	18	34	165	26	0.681	0.62
	19	45	167	33		
	20	57	172	32		
	≥21	38	173	27		

Table 2.3B: Descriptive continuous data for Total Cholesterol (mg/dL)

	β	Std. Error	P-value	R	R square	R square Δ	P-value
Physical Activity (kcal/kg/wk)	0.02	0.086	0.816	0.018	0.000	-0.005	0.816
Systolic BP (mmHg)	0.094	0.161	0.562	0.044	0.002	-0.004	0.562
Diastolic BP (mmHg)	0.109	0.249	0.662	0.034	0.001	-0.005	0.662
%Fat	-0.355	0.322	0.271	0.084	0.007	0.001	0.271
BMI (kg/m^2)	0.641	0.652	0.327	0.075	0.006	0.000	0.327
eVO2max (ml/kg/min)	-0.219	0.315	0.487	0.053	0.003	-0.003	0.487
Total Seat Time (hours)	0.794	1.044	0.448	0.058	0.003	-0.002	0.448

Table 2.3C: Summary of multiple regression model for Total Cholesterol (mg/dL)

		95% CI for β			
	β	Lower	Upper	Std Error	p-value
Constant	156.55	138.86	174.25	8.96	0.000
PA (kcals/kg/wk)	0.012	-0.155	4.02	0.085	0.883
Gender	3.10	-7.37	13.57	5.29	0.559
Race	-13.11	-26.82	0.60	6.94	0.06

Table 2.4A: Descriptive categorical data for High Density Lipoprotein levels (mg/dL)

		N	Mean	SD	t-statistic	P-value
Gender						
	Male	53	53	10	-5.14	0.000
	Female	120	64	14		
Race						
	Caucasian	149	61	14	1.329	0.186
	Other	24	57	9		
Major						
	Kinesiology	72	59	13	-0.983	0.327
	Other	99	61	14		
Smoking						
	Non-Smoker	147	60	14	-0.718	0.474
	Smoker	16	63	14		

		N	Mean	SD	f statistic	P-value
Age						
	18	34	58.9	13	2.770	0.043
	19	44	60	13		
	20	57	58	15		
	≥21	38	66	12		

Table 2.4B: Descriptive continuous data for High Density Lipoprotein Levels (mg/dL)

	β	Std. Error	P-value	R	R square	R square Δ	P-value
Physical Activity (kcal/kg/wk)	-0.017	0.037	0.644	0.036	0.001	-0.005	0.644
Systolic BP (mmHg)	-0.269	0.071	0.000	0.28	0.078	0.073	0.000
Diastolic BP (mmHg)	-0.173	0.112	0.125	0.177	0.014	0.008	0.125
%Fat	0.308	0.151	0.043	0.154	0.024	0.018	0.043
BMI (kg/m^2)	-1.3	0.297	0.000	0.315	0.099	0.094	0.000
eVO2max (ml/kg/min)	-0.032	0.147	0.830	0.017	0.000	-0.006	0.830
Total Seat time (hours)	0.028	0.453	0.951	0.005	0.000	-0.006	0.951

Table 2.4C: Summary of multiple regression model for High Density Lipoprotein levels (mg/dL)

		95% CI for β			
	β	Lower	Upper	Std Error	p-value
Constant	80.94	64.85	97.12	8.16	0.000
PA (kcals/kg/wk)	0.029	-0.043	0.101	0.04	0.43
Gender	9.16	4.59	13.73	2.31	0.000
Race	-2.78	-8.53	2.96	2.91	0.34
BMI (kg/m^2)	-1.21	-1.82	-059	0.31	0.000
Smoking risk	7.64	0.82	14.46	3.45	0.03

Table 2.5A: Descriptive categorical data for Percent Fat (%)

	N	Mean	SD	t-statistic	P-value
Gender					
Male	59	15	5	-13.87	0.000
Female	133	27	5		
Race					
Caucasian	162	23	7	-0.636	0.526
Other	30	24	10		
Major					
Kinesiology	84	23	7	-0.187	0.852
Other	106	23	8		
Smoking					
Non-Smoker	164	23	8	0.149	0.882
Smoker	18	23	6		

	N	Mean	SD	f statistic	P-value
Age					
18	40	23	7	1.608	0.189
19	49	25	8		
20	62	22	8		
≥21	41	23	6		

Table 2.5B: Descriptive continuous data for Percent Fat (%)

	β	Std. Error	P-value	R	R square	R square Δ	P-value
Physical Activity (kcal/kg/wk)	-0.098	0.018	0.000	0.374	0.14	0.135	0.000
Systolic BP (mmHg)	-0.148	0.034	0.000	0.299	0.09	0.085	0.000
Diastolic BP (mmHg)	0.009	0.066	0.894	0.01	0.000	-0.005	0.894
TC (mg/dL)	0.011	0.018	0.537	0.047	0.002	-0.004	0.537
HDL (mg/dL)	0.077	0.038	0.043	0.154	0.024	0.018	0.043
BMI (kg/m^2)	0.634	0.136	0.000	0.322	0.104	0.099	0.000
eVO2max (ml/kg/min)	-0.368	0.065	0.000	0.384	0.148	0.143	0.000
Total Seat Time (hours)	0.15	0.227	0.510	0.048	0.002	-0.003	0.510

Table 2.5C: Summary of multiple regression for Percent Fat (%)

		95% CI for β			
	β	Lower	Upper	Std Error	Sig
Constant	-11.37	-16.99	-5.76	2.84	0.000
BMI (kg/m^2)	0.94	0.78	1.09	0.08	0.000
Diastolic BP (mmHg)	0.07	-0.001	0.13	0.03	0.054
Physical Activity (kcal/kg/wk)	-0.03	-0.046	-0.01	0.01	0.001
Gender	12.38	11.27	13.51	0.57	0.000
Race	-1.10	-2.49	0.30	0.71	0.122

Table 2.6A: Descriptive categorical data for Body Mass Index (kg/m^2)

		N	Mean	SD	t-statistic	P-value
Gender						
	Male	59	24.8	3.7	2.91	0.004
	Female	131	23.3	3.3		
Race						
	Caucasian	161	23.6	3.2	-1.65	0.102
	Other	29	24.7	4.7		
Major						
	Kinesiology	84	23.7	3.6	-0.105	0.917
	Other	104	23.7	3.4		
Smoking						
	Non-Smoker	162	23.5	3.5	-1.531	0.127
	Smoker	18	24.8	3.1		

		N	Mean	SD	f statistic	P-value
Age						
	18	39	22.7	2.5	2.64	0.051
	19	48	24.3	3.3		
	20	62	24.4	3.9		
	≥21	41	23.2	3.4		

Table 2.6B: Descriptive continuous data for Body Mass Index (kg/m^2)

	β	Std. Error	P-value	R	R square	R square Δ	P-value
Physical Activity (kcal/kg/wk)	-0.01	0.009	0.259	0.083	0.007	0.002	0.259
Systolic BP (mmHg)	0.096	0.017	0.000	0.382	0.146	0.141	0.000
Diastolic BP (mmHg)	0.146	0.032	0.000	0.318	0.101	0.096	0.000
TC (mg/dL)	-0.008	0.009	0.345	0.072	0.005	-0.001	0.345
HDL (mg/dL)	-0.077	0.018	0.000	0.315	0.099	0.094	0.000
%Fat	0.163	0.035	0.000	0.322	0.104	0.099	0.000
eVO2max (ml/kg/min)	-0.105	0.035	0.003	0.216	0.047	0.042	0.003
Total Seat Time (hours)	0.146	0.107	0.177	0.099	0.01	0.004	0.177

Table 2.6C: Summary of multiple regression model for Body Mass Index (kg/m^2)

		95% CI for β			
	β	Lower	Upper	Std Error	p-value
Constant	12.10	7.474	16.73	2.34	0.000
PA (kcals/kg/wk)	0.012	-0.001	0.025	0.007	0.066
Gender	-5.60	-7.02	-4.18	0.72	0.000
Race	0.79	-0.21	1.79	0.51	0.12
SBP (mmHg)	0.05	0.02	0.08	0.02	0.003
HDL (mg/dL)	-0.03	-0.06	-0.007	0.01	0.015

Figure 3.1: Frequency of the ACE ID Polymorphism in a sample of 149 young adults

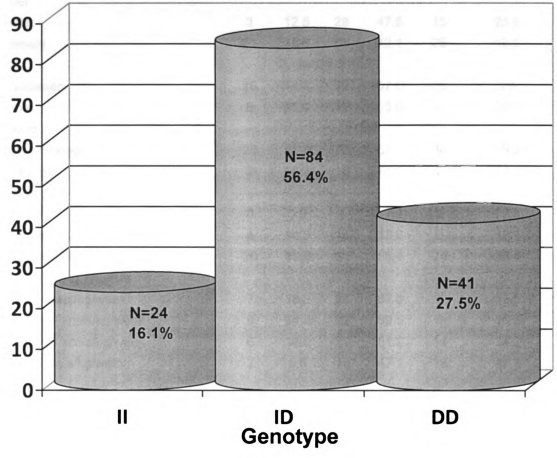

Table 3.0A: Descriptive categorical data based on the ACE ID Polymorphism

	II		ID		DD		χ^2	P-value
	N	%	N	%	N	%		
Gender								
Male	3	12.5	28	47.5	15	25.6	4.661	0.097
Female	21	15.8	56	42.1	26	19.5		
Race								
Caucasian	18	14.4	72	57.6	35	28	1.677	0.432
Other	6	25.0	12	50.0	6	25.0		
Smoking								
Non-smoking	20	15.8	72	57.1	34	26.9	1.249	0.535
Smoking	2	14.2	10	71.4	2	14.2		
BMI Category								
Obese	0	0.0	7	77.7	2	22.3	2.92	0.571
Overweight	4	14.3	15	53.6	9	32.1		
Normal Weight	20	18.2	61	55.4	29	26.4		
Physical Activity Quartile								
0-14.8 kcals/kg/week	7	18.9	25	67.6	5	13.5	15.323	0.018
14.8-28.0 kcals/kg/week	9	24.3	22	59.5	6	16.2		
28.0-54.7 kcals/kg/week	6	14.6	21	51.2	14	34.2		
> 54.7 kcals/kg/week	2	5.8	16	47.1	16	47.1		

Table 3.0B: Descriptive continuous data based on the ACE ID Polymorphism

	II	ID	DD	F statistic	P-value
N	22	72	39		
Physical Activity (kcal/kg/wk)	25.5 ± 17	33.1 ± 25.6	50.4 ± 33	8.14	0.00
Systolic BP (mmHg)	116 ± 13	119 ± 16	122 ± 12	1.25	0.29
Diastolic BP (mmHg)	74 ± 8	72 ± 8	74 ± 7	0.65	0.52
TC (mg/dL)	169 ± 30	171 ± 30	170 ± 31	0.08	0.93
HDL (mg/dL)	57 ± 12	61 ± 13	61 ± 16	0.58	0.57
%Fat	26 ± 5	23 ± 8	22 ± 7	3.25	0.04
BMI (kg/m^2)	23.1 ± 2.6	23.7 ± 3.8	24.0 ± 3.5	0.45	0.64
eVO2max (ml/kg/min)	33.7 ± 5.1	36.1 ± 8.2	39.1 ± 7.4	4.06	0.02
Total Seat Time (hours)	6.4 ± 2.8	6.1 ± 2.2	6.3 ± 2.3	0.29	0.74

Table 3.0C: Descriptive continuous data by gender and the ACE ID Polymorphism

	Males					Females				
	II	ID	DD	F statistic	P-value	II	ID	DD	F statistic	P-value
N	3	28	15			21	56	26		
PA (kcals/kg/wk)	36.2±4	46.0±28	49.3±26	0.3	0.74	24.0±18	26.6±22	50.9±37	9.31	0.00
Systolic BP (mmHg)	130±30	132±14	129±13	0.26	0.77	114±11	112±12	117±10	1.89	0.16
Diastolic BP (mmHg)	79±10	77±9	74±5	0.96	0.39	73±8	70±7	73±8	2.34	0.1
TC (mg/dL)	160±30	170±34	166±24	0.21	0.81	170±30	172±28	172±35	0.02	0.98
HDL (mg/dL)	45±8	55±10	53±10	1.26	0.29	59±12	64±13	65±17	1.02	0.37
%Fat	18±5	17±6	14±4	1.09	0.35	27.6±4	26.7±6	25.9±4	0.533	0.59
BMI (kg/m2)	23±5	25±4	24±3	0.54	0.58	23±2	23±3	24±4	0.43	0.65
eVO_2max (ml/kg/min)	42±2	40±6	44±6	2.08	0.14	32±4	34±8	36±7	1.53	0.22
Total Seat Time (hours)	8.3±2.7	6.5±2.7	6.1±2.7	0.88	0.42	6.2±2.8	5.8±1.9	6.4±2.1	0.67	0.51

Table 3.1A: Final multiple regression model for ACE ID Polymorphism and Systolic Blood Pressure (mmHg)

		95% CI for β			
	β	Lower	Upper	Std Error	p-value
Constant	89.94	70.90	108.97	9.61	0.000
ACE II	-1.08	-7.27	5.11	3.13	0.73
ACE ID	-3.47	-7.89	0.95	2.23	0.12
Gender	-14.66	-18.97	-10.34	2.18	0.000
TC (mg/dL)	0.06	-0.007	0.13	0.03	0.079
BMI (kg/m^2)	1.27	0.69	1.86	0.29	0.079

Table 3.2A: Final multiple regression model for ACE ID Polymorphism and Diastolic Blood Pressure (mmHg)

		95% CI for β			
	β	Lower	Upper	Std Error	p-value
Constant	68.56	63.10	74.02	2.75	0.000
ACE II	0.85	-3.51	5.21	2.2	0.70
ACE ID	-2.80	-5.88	0.27	1.55	0.074
Gender	-10.47	-14.77	-6.17	2.17	0.000
Percent Fat	0.56	0.26	0.86	0.15	0.000

Table 3.3A: Final multiple regression model for ACE ID Polymorphism and Total Cholesterol (mg/dL)

		95% CI for β			
	β	Lower	Upper	Std Error	p-value
Constant	169.37	159.18	179.55	5.14	0.000
ACE II	-1.34	-18.45	15.77	8.64	0.877
ACE ID	3.63	-8.59	15.85	6.17	0.588
Race	-8.85	-24.21	6.50	7.75	0.256

Table 3.4A: Final multiple regression model for ACE ID Polymorphism and High Density Lipoproteins Levels (mg/dL)

		95% CI for β			
	β	Lower	Upper	Std Error	p-value
Constant	81.16	63.15	99.167	9.09	0.000
ACE II	-6.86	-14.21	0.47	3.71	0.066
ACE ID	-0.072	-5.32	5.17	2.65	0.978
Gender	8.30	3.19	13.41	2.58	0.002
BMI (kg/m^2)	-1.087	-1.78	-0.39	0.35	0.002

Table 3.5A: Final multiple regression model for ACE ID Polymorphism and Percent Fat (%)

		95% CI for β			
	β	Lower	Upper	Std Error	p-value
Constant	12.32	2.07	22.57	5.18	0.019
ACE II	1.89	-0.52	4.29	1.22	0.123
ACE ID	0.95	-0.81	2.70	0.89	0.287
Gender	9.68	7.74	11.63	0.98	0.000
DBP (mmHg)	0.16	0.062	0.26	0.049	0.002
eVO_2max (ml/kg/min)	-0.23	-0.35	-0.09	0.065	0.001

Table 3.6A: Final multiple regression model for ACE ID Polymorphism and Body Mass Index (kg/m^2)

		95% CI for β			
	β	Lower	Upper	Std Error	p-value
Constant	13.26	8.07	18.44	2.62	0.000
ACE II	-1.78	-3.11	-0.452	0.67	0.009
ACE ID	-0.45	-1.38	0.49	0.47	0.347
Gender	-5.31	-6.93	-3.69	0.82	0.000
SBP (mmHg)	0.05	0.013	0.086	0.019	0.008
HDL (mg/dL)	-0.04	-0.07	-0.008	0.016	0.014
Percent Fat	0.48	0.39	0.58	0.048	0.000

Table 3.7A: Final multiple regression model for the ACE ID Polymorphism and Physical Activity (kcals/kg/week)

		95% CI for β			
	β	Lower	Upper	Std Error	p-value
Constant	114.173	67.21	161.14	23.72	0.000
ACE II	-26.82	-41.27	-12.37	7.29	0.000
ACE ID	-23.355	-34.06	-12.37	5.41	0.000
Gender	-15.37	-34.06	-12.65	5.26	0.004
DBP (mmHg)	-0.69	-1.29	-0.097	0.30	0.023

Table 3.7B: Final multiple regression model for the ACE ID Polymorphism and Estimated VO_2max (ml/kg/min)

		95% CI for β			
	β	Lower	Upper	Std Error	p-value
Constant	50.82	46.71	54.931	2.07	0.000
ACE II	-1.36	-4.63	1.91	1.65	0.411
ACE ID	-2.37	-4.66	-0.073	-0.172	0.043
Gender	-2.58	-5.80	0.63	1.62	0.114
Percent Fat	0.46	-0.68	-0.23	0.113	0.000
Race	-3.27	-6.15	-0.39	1.45	0.026

Table 4.1A: Final multiple regression model for ACE ID Polymorphism and Physical Activity on Systolic Blood Pressure (mmHg)

		95% CI for β			
	β	Lower	Upper	Std Error	p-value
Constant	91.07	70.95	111.18	10.16	0.000
ACE II	-1.45	-7.98	5.09	3.29	0.662
ACE ID	-3.76	-8.50	0.967	2.39	0.118
PA (kcals/kg/wk)	-0.013	-0.088	0.061	0.038	0.723
Gender	-14.83	-19.26	-10.39	2.24	0.000
TC (mg/dL)	0.06	-0.008	0.126	0.034	0.083
BMI (kg/m^2)	1.26	0.672	1.851	0.298	0.000

Table 4.2A: Final multiple regression model for ACE ID Polymorphism and Physical Activity on Diastolic Blood Pressure (mmHg)

		95% CI for β			
	β	Lower	Upper	Std Error	p-value
Constant	78.81	74.80	82.82	2.03	0.000
ACE II	0.778	-3.28	4.83	2.05	0.705
ACE ID	-1.78	-4.77	1.21	1.51	0.241
PA (kcals/kg/wk)	-0.039	-0.087	0.008	0.024	0.104
Gender	-5.25	-8.04	-2.45	1.413	0.000

Table 4.3A: Final multiple regression model for ACE ID Polymorphism and Physical Activity on Total Cholesterol (mg/dL)

		95% CI for β			
	β	Lower	Upper	Std Error	p-value
Constant	174.61	160.63	188.59	7.07	0.000
ACE II	-0.785	-17.49	15.92	8.45	0.926
ACE ID	0.527	-11.79	12.84	6.23	0.933
PA (kcals/kg/wk)	-0.062	-0.257	0.134	0.099	0.534
Race	-15.49	-30.39	-0.58	7.53	0.042

Table 4.4A: Final multiple regression model for ACE ID Polymorphism and Physical Activity on High Density Lipoproteins levels (mg/dL)

		95% CI for β			
	β	Lower	Upper	Std Error	p-value
Constant	53.32	46.15	60.49	3.62	0.000
ACE II	-5.36	-12.49	1.77	3.61	0.140
ACE ID	0.147	-5.13	5.43	2.67	0.833
PA (kcals/kg/wk)	0.009	-0.077	0.095	0.043	0.833
Gender	10.57	5.65	15.49	2.49	0.000

Table 4.5A: Final multiple regression model for ACE ID Polymorphism and Physical Activity on Percent Fat (%)

		95% CI for β			
	β	Lower	Upper	Std Error	p-value
Constant	12.54	2.13	22.95	5.25	0.019
ACE II	1.79	-0.712	4.297	1.26	0.159
ACE ID	0.86	-0.994	2.72	0.938	0.359
PA (kcals/kg/wk)	-0.004	-0.035	0.026	0.015	0.78
Gender	9.66	7.697	11.62	0.990	0.000
DBP (mmHg)	0.157	0.058	0.257	0.050	0.002
eVO_2max (ml/kg/min)	-0.22	0.058	0.257	0.068	0.001

Table 4.6A: Final multiple regression model for ACE ID Polymorphism and Physical Activity on Body Mass Index (kg/m^2)

		95% CI for β			
	β	Lower	Upper	Std Error	p-value
Constant	26.21	24.38	28.04	0.927	0.000
ACE II	-0.95	-2.81	0.901	0.938	0.311
ACE ID	-0.593	-1.96	0.77	0.69	0.39
PA (kcals/kg/wk)	-0.021	-0.043	0.001	0.011	0.057
Gender	-1.82	-3.09	-0.55	0.642	0.005

Appendix A: Consent Form

A cross-sectional assessment of physical activity behaviors, cardiovascular disease risk factors, and their association with genetic polymorphisms in college aged adults

Consent Form

Summary of the research protocol: You have been asked to participate in a study assessing physical activity behaviors, cardiovascular disease (CVD) risk factors, and their association with genetics in young adults. CVD has several known modifiable risk factors, including high cholesterol, high blood pressure, smoking, obesity, and a lack of physical activity. These CVD risk factors develop through a complex relationship between the environment and genetics. While CVD symptoms may not occur until later in life the risk factors begin during childhood. Your participation in this study will enable us to understand of how interactions between genetics, specifically the Angiotensin Converting Enzyme Insertion/Deletion polymorphism, and the environmental, specifically physical activity/physical fitness, affect the development of CVD risk factors in a young adult population. The ACE I/D polymorphism is shown to be associated with the development of CVD risk factors such as obesity, high cholesterol, and especially hypertension.

You are asked to complete an on-line survey and to donate, through non-invasive measures, some mouth epithelia cells to determine your Angiotensin Converting Enzyme Insertion/Deletion genotype.

Estimate of subject's time: Please take your time and fill out the survey completely. It should take approximately 10 minutes. The collection of the fresh mouth epithelia cells will take no longer than 5 minutes.

Experimental Procedures:

On-line Survey: The on-line utilize the Longitudinal Surveillance Engine (LSE), an internet-based surveillance system constructed at MSU. You will be provided online access to the LSE through a web page accessible via any major internet browser. A unique survey ID number will be given to you to keep track responses. You will be asked questions pertaining to basic demographics and your physical activity history.

Buccal cell collection: Buccal, relating to the check or mouth, epithelial cells are being collected to analyze your DNA to see which of the Angiotensin

Converting Enzyme polymorphisms you inherited. Special indicator cards will be used to collect and store your buccal epithelial cells. Fresh buccal epithelial cells are removed, non-invasively, from your mouth using a foam-tipped applicator, which is then placed onto the specially treated paper (changes color when sample is applied) of the indicator card. Once the sample is applied to the indicator card, it can be stored.

Risks/Discomforts: There are no known risks associated with participation in this study. Outside of receiving an ice-cream cone from The Dairy Store for your participation, you will not benefit from participation in this study. However, your participation in this study may contribute to the understanding of how physical activity associates with specific genotypes to affect cardiovascular risk factors.

Voluntary participation: Your participation in this study is completely voluntary, dependent upon your consent and will no way effect your grade in KIN 121. You may choose not to participate at all, you may refuse to answer certain questions or you may withdraw from this study at any time. Furthermore, any results of the testing that you complete will be available to you.

Confidentiality and anonymity: Your privacy will be protected to the maximum extent allowable by law. Presentation or publication of results will in no way identify you personally. The records and results obtained will only be available to the investigators of this study, Michigan State University biomedical institutional review board and to yourself if you wish and will not be shared with others.

Contact person: If you have any questions and/or concerns about your participation in this study, you may contact:

Dr. James Pivarnik, PhD.
3 IM Sports Circle
Michigan State University
East Lansing, MI 48824
517-353-3520
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If you have any questions pertaining to participant's rights as human subjects of research, please contact:

Dr. Peter Vasilenko, PhD.,
Director of Human Research Protection Programs
202 Olds Hall
Michigan State University
East Lansing, MI 48824

517-355-2180
irb@msu.edu

If you agree to join this study, please sign your name below. By completing and returning this form, you indicate your voluntary agreement to participate.

☐ I agree to allow my buccal epithelial cells to be stored and used for determination of the Angiotensin Converting Enzyme Insertion/Deletion polymorphism. Initials_____

☐ I agree to allow my buccal epithelial cells to be stored and used for future research studies. Initials_____

_____

Signature of participant Date

_____

Subjects Ticket Number

Appendix B: Questionnaire

A cross-sectional assessment of physical activity behaviors, cardiovascular disease risk factors, and their association with genetic polymorphisms in college aged adults

Questionnaire

1. What is your age?
    a. 17
    b. 18
    c. 19
    d. 20
    e. 21
    f. 22
    g. 23
    h. 24
    i. 25 or greater

2. What is your gender?
    a. Male
    b. Female

3. How do you describe yourself?
    a. American Indian or Alaskan Native
    b. Asian
    c. Black or African American
    d. Hispanic or Latino
    e. Native Hawaiian or Pacific Islander
    f. White
    g. Other

4. What best describes your current living status
    a. On-campus
    b. Off-campus apartment/house with friends
    c. Off-campus with parents/guardians
    d. Off-campus alone
    e. Other

5. Are you a Kinesiology Major?
    a. Yes
    b. No

6. During the past month, did you participate in any physical activities or exercises such as running, calisthenics, golf, gardening, walking, etc.?
    a. Yes
    b. No

    If yes, please continue to question #7

    If no, please continue to question #18

7. Using the list provided, click on the physical activity or exercise you currently participate in most often?

8. How many times per week do you usually participate in this activity
    a. Less than 1
    b. 1
    c. 2
    d. 3
    e. 4
    f. 5
    g. 6
    h. 7

9. Using 15 minute increments, how long do you usually participate in this activity?
    a. 15
    b. 30
    c. 45
    d. 60
    e. 75
    f. 90
    g. greater than 90

10. Do you regularly participate in a second physical activity or exercise other than what was previously mentioned?
    a. Yes
    b. No

    If yes, please continue to question #11

    If no, please continue to question #18

11. Using the list provided, click on the physical activity or exercise you currently participate in the second most often?

12. How many times per week do you usually participate in this activity

a. Less than 1
b. 1
c. 2
d. 3
e. 4
f. 5
g. 6
h. 7

13. Using 15 minute increments, how long do you usually participate in this activity?
    a. 15
    b. 30
    c. 45
    d. 60
    e. 75
    f. 90
    g. greater than 90

14. Do you regularly participate in a third physical activity or exercise other than what previously mentioned?
    a. Yes
    b. No

    If yes, please continue to question #15

    If no, please continue to question #18

15. Using the list provided, click on the physical activity or exercise you currently participate in the third most often?

16. How many times per week do you usually participate in this activity
    a. Less than 1
    b. 1
    c. 2
    d. 3
    e. 4
    f. 5
    g. 6
    h. 7

17. Using 15 minute increments (15, 30, 45, 60, 75, etc.), how long do you usually participate in this activity?
    a. 15
    b. 30
    c. 45
    d. 60
    e. 75
    f. 90
    g. greater than 90

18. On average, how many hours do you watch TV per day?
    a. Less than 1
    b. 1
    c. 2
    d. 3
    e. 4
    f. 5 or greater

19. On average, how many hours of video games do you play per day?
    a. Less than 1
    b. 1
    c. 2
    d. 3
    e. 4
    f. 5 or greater

20. On average, how many hours do you spend surfing the internet, writing e-mails, or completing class work on a computer per day (do not include playing video games)?
    a. Less than 1
    b. 1
    c. 2
    d. 3
    e. 4
    f. 5 or greater

21. Did you participate in any sponsored and/or club sports or activities (i.e., basketball, cheerleading, marching band, etc.) during high school?
    a. Yes
    b. No

22. Using the list provided, click on all of the sponsored or club sports/activities that you participated in.
    a. Baseball
    b. Basketball
    c. Cheerleading
    d. Cross Country
    e. Dance
    f. Field Hockey
    g. Football
    h. Golf
    i. Ice Hockey
    j. Marching Band
    k. Pom Pons
    l. Rugby
    m. Soccer
    n. Softball
    o. Swimming
    p. Track
    q. Volleyball
    r. Other

23. How many times per week did you usually participate in these sports/activities?
    a. Less than 1
    b. 1
    c. 2
    d. 3
    e. 4
    f. 5
    g. 6
    h. 7
    i. 8
    j. 9
    k. 10

24. When you took part in these activities, how many minutes did you usually participate?
    a. 30
    b. 60
    c. 90
    d. 120
    e. 150

25. During high school, did you participate in any non-school sponsored leisure-time physical activities or exercises such as running, sports, gardening, etc.?
    a. Yes
    b. No

26. Using the list provided, click on the leisure-time physical activities or exercises you participated in most often during high school (choose no more than 3 activities).

27. On average, how many times per week did you participate in these activities combined?
    a. 1
    b. 2
    c. 3
    d. 4
    e. 5
    f. 6
    g. 7
    h. 8
    i. 9
    j. 10 or greater

28. When you took part in these activities combined, how many minutes did you usually participate?
    a. 15
    b. 30
    c. 45
    d. 60
    e. 75
    f. 90
    g. greater than 90

29. What is your height in inches?_____

30. What is your weight in pounds? _____

31. What section of KIN 121 are you currently enrolled?

a. 001
b. 002
c. 003
d. 004
e. 005
f. 006

Appendix C: Equations

Generalized Skinfold Equations[132]

4-site skinfold equation (abdomen, suprailiac, tricep, and thigh):

Male: %Fat = 0.29288 (sum of four skinfolds) – 0.0005 (sum of four skinfolds)^2 + 0.15845 (age) – 5.76377

Female: % Fat = 0.29669 (sum of four skinfolds) – 0.00043 (sum of four skinfolds)^2 + 0.02963 (age) + 1.4072

Field test equation to estimate aerobic capacity

1.5 Mile Endurance test[14]

VO_{2max} (ml/kg/min) = 3.5 + 483/(time in minutes)

Appendix D: Health assessment data sheet

5-DIGIT TICKET NUMBER _____

Please use your results from the OLIN Health Center General Fitness Assessment test to answer the following questions. If you do not have a result for a certain question, please leave it blank.

1. What was your weight in pounds? _____

2. What was your height (example: **5'4**") _____

3. What was your PERCENT BODY FAT according to the results of the fitness test? (example: PERCENT BODY FAT (%BF) = **28.8**)

   _____

4. What was your dominant hand grip strength? (example: DOMINANT HAND GRIP = **27**) _____

5. What were the results for the SIT & REACH test (inches)? (example: SIT & REACH = **23**)

   _____

6. What was your resting blood pressure? (example: RESTING BP = **132/74**) _____

7. What was your VO2 max? (example: VO2 MAX ml/kg/min = **41**)

   _____

8. How many push-ups did you complete in one minute? (example: PUSH UPS (60sec) = **38**)

   _____

9. How many sit-ups did you complete in one minute? (example: SIT UPS (60sec) = **50**)

_____

10. What was your total cholesterol? (example: chol.= **208**)

_____

11. What was your HDL cholesterol? (example: HDL= **58**)

_____

12. What was your FVC? (example FVC = **3.6**) _____

13. What was your FEV1? (example FEV1 = **3.4**) _____

Appendix E: Description of Variables

Description of Main Variables		
MAIN EXPOSURE VARIABLES		
Variable Name	**Variable Type**	**Analyses**
Angiotensin Converting Enzyme Polymorphism • Allele • Genotype	• D or I • II, ID, DD	• Binary • Categories
Physical Activity • Physical Activity (PA)	• $kcals \cdot kg \cdot wk^{-1}$	• Quartiles
MAIN OUTCOME VARIABLES		
Variable Name	**Variable Type**	**Analyses**
Blood pressure • Blood pressure	• mmHg • Normal (<120/<80 mmHg), pre-hypertensive (120-139/80-89 mmHg), hypertensive (≥140/≥90 mmHg)	• Continuous • 3 Categories
Cholesterol • Total • HDL Cholesterol	• mg/dL • Desirable (<200 mg/dL), Borderline (200-239 mg/dL), High (≥240 mg/dL) • mg/dL • Low (<40 mg/dL), High (≥ 60 mg/dL	• Continuous • 3 Categories • Continuous • 3 Categories
Overweight/Obesity • Skinfolds	• $kg \cdot m^{-2}$	• 3 Categories

• Body Mass Index (BMI)	• Normal (18.6–24.9 $kg \cdot m^{-2}$), Overweight (25-29.9 $kg \cdot m^{-2}$), Obese (≥30 $kg \cdot m^{-2}$)	• Continuous • 3 Categories
Description of Covariates		
POSSIBLE THIRD VARIABLES		
Variable Name	**Variable Type**	**Analyses**
• Gender	• Male/Female	• Dichotomous
• Age	• 18 years (yrs), 19 yrs, 20 yrs, 21 yrs, ≥22 yrs	• Categorical
• Race	• Caucasian, African American, Other (Hispanic, Asian, Pacific Islander, Native American)	• Categorical
• Height	• inches	• Continuous
• Weight	• Pounds	• Continuous
• Computer/TV time	• Hour of use per day • Low (<2 hrs/day), High (≥2 hrs/day)	• Continuous • Dichotomous
• Estimated VO^2max	• $ml \cdot kg^{-1} \cdot m^{-1}$	• Continuous

REFERENCES

1. Relationship of atherosclerosis in young men to serum lipoprotein cholesterol concentrations and smoking. A preliminary report from the Pathobiological Determinants of Atherosclerosis in Youth (PDAY) Research Group. *JAMA* 264, 3018-3024 (1990).

2. *The Renin-Angiotensin system*, (Mosby, London, 1993).

3. Youth Risk Behavior Surveillance: National College Health Risk Behavior Survey--United States, 1995. *Morbidity and mortality weekly report. CDC surveillance summaries / Centers for Disease Control* 46, 1-54 (1997).

4. US Department of Health and Human Services. Prevalence of physical activity, including lifestyle activities among adults - US, 2000-2001. *Morbidity and Mortality Weekly Report* 52, 764-769 (2002).

5. Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) Final Report. *Circulation* 106, 3143- (2002).

6. (2005). Digest of Education Statistics. http://nces.ed.gov/programs/digest/d05/.

7. (2005). YRBSS-United States 2005: Percentage of students who were physically active for a total of 60 minutes or more per day on five or more of the past seven days.

8. *ACSM's guidelines for exercise testing and prescription*, (Lippincott, Williams, and Wilkins, New York, NY, 2006).

9. (2007). American Heart Association. AHA Scientific Position: Risk factors and coronary heart disease. http://www.americanheart.org/presenter.jhtml?identifier=4726.

10. (2007). Whatman plc. Applying and preparing buccal cell samples on FTA cards for DNA analysis. http://www.whatman.com/UserFiles/File/Protocols/Bioscience/BD03%20%20Applying%20and%20Preparing%20Buccal%20Cell%20Samples%20on%20FTA%20Cards.pdf.

11. (2008). American Heart Association: Heart Disease and Stroke Statistics -- 2008 Update (At-a-Glance). http://www.americanheart.org/presenter.jhtml?identifier=3037327.

12. Centers for Disease Control and Prevention (CDC). *Behavioral Risk Factor Surveillance System Survey Data*. Atlanta, Georgia: U.S. Department of Health and Human Services. (2009).

13. (2010). World Health Organization: Obesity and Overweight. http://www.who.int/dietphysicalactivity/publications/facts/obesity/en/.

14. *American College of Sports Medicine's Guidelines for Exercise Testing and Prescription*, 8th ed (Lippincott Williams & Wilkins, 2010).

15. National Center for Health Statistcs. Health, United States, 2009: With Special Feature on Medical Technology. . (2010).

16. Aadahl, M. & Jorgensen, T. Validation of a new self-report instrument for measuring physical activity. *Medicine and Science in Sports and Exercise* 35, 1196-1202 (2003).

17. Agerholm-Larsen, B., Nordestgaard, B.G. & Tybjarg-Hansen, A. ACE Gene Polymorphism in Cardiovascular Disease : Meta-Analyses of Small and Large Studies in Whites. *Arterioscler Thromb Vasc Biol* 20, 484-492 (2000).

18. Ainslie, P., Reilly, T., Maclaren, D. & Campbell, I. Changes in plasma lipids and lipoproteins following 10-days of prologed walking: influence of age and relationship to physical activity level. *Ergonomics* 48, 1352-1364 (2005).

19. Ainsworth, B., Haskell, W., Whitt, M., Irwin, M., Swartz, A., Strath, S., O'Brien, W., Bassett, D.J., Schmitz, K., Emplaincourt, P., Jacobs, D.J. & Leon, A. Compendium of physical activities: an update of activity codes and MET intensities. *Medicine and Science in Sports and Exercise* 32, S498-S504 (2000).

20. Ainsworth, B.E., Keenan, N.L., Strogatz, D.S., Garrett, J.M. & James, S.A. Physical activity and hypertension in black adults: the Pitt County Study. *Am J Public Health* 81, 1477-1479 (1991).

21. Alhenc-Gelas, F., Soubrier, F., Hubert, C., Allefrini, J., Lattion, A.L. & Corvol, P. The angiotensin I-converting enzyme (Kininase II) progress in molecular adn genetic structure. *Journal of Cardiovascular Pharmacology* 5, S25-S29 (1990).

22. Alhenc-Gelas, F., Richard, J., Courbon, J., Warnet, J. & Corvol, P. Distribution of plasma angiotensin I-converting enzyme levels in healthy men: Relationship to environmental and hormonal parameters. *Journal of Laboratory and Clinical Medicine* 117, 33-39 (1991).

23. Alsaeid, M., Moussa, M.A.A., Haider, M.Z., Refai, T.M.K., Abdella, N., Al-Sheikh, N. & Gomez, J.E. Angiotensin-converting enzyme gene polymorphism and lipid profiles in Kuwaiti children with type 1 diabetes. *Pediatric Diabetes* 5, 87-94 (2004).

24. Alvarez, R., Terrados, N., Ortolano, R., Iglesias-Cubero, G., Reguero, J., Batalla, A., Cortina, A., Fernández-García, B., Rodríguez, C., Braga, S., Alvarez, V. & Coto, E. Genetic variation in the renin-angiotensin system and athletic performance. *European Journal of Applied Physiology* 82, 117-120 (2000).

25. Amir, O., Amir, R., Yamin, C., Attias, E., Eynon, N., Sagiv, M., Sagiv, M. & Meckel, Y. The ACE deletion allele is associated with Israeli elite endurance athletes. *Experimental Physiology* 92, 881-886 (2007).

26. Andersen, L.B., Hasselstrom, H., Gronfeldt, V., Hansen, S. & Karsten, F. The relationship between physical fitness and clustered risk, and tracking of clustered risk from adolescence to young adulthood: eight years follow-up in the Danish Youth and Sport Study. *International Journal of Behavioral Nutrition and Physical Activity* 1, 6 (2004).

27. Barbalic, M., karic'-Juric, T., Cambien, F., Barbaux, S., Poirier, O., Turek, S., Vrhovski-Hebrang, D., ubrilo-Turek, M., Rudan, I., Rudan, P., Naranc & ic, N.S. Gene Polymorphisms of the Renin-Angiotensin System and Early Development of Hypertension. *American Journal of Hypertension* 19, 837 (2006).

28. Barley, J., Blackwood, A., Miller, M., Markandu, N., Carter, N., Jeffery, S., Cappuccio, F.P., MacGregor, G. & Sagnella, G. Angiotensin converting enzyme gene I/D polymorphism, blood pressure and the renin-angiotensin system in Caucasian and Afro-Caribbean peoples. *Journal of Human Hypertension* 10, 31-35 (1996).

29. Barron, J.T., Sasse, M.F. & Nair, A. Effect of angiotensin II on energetics, glucose metabolism and cytosolic NADH/NAD and NADPH/NADP redox in vascular smooth muscle. *Molecular and Cellular Biochemistry* 262, 91-99 (2004).

30. Bell, C.G., Meyre, D., Petretto, E., Levy-Marchal, C., Hercberg, S., Charles, M.A., Boyle, C., Weill, J., Tauber, M., Mein, C.A., Aitman, T.J., Froguel, P. & Walley, A.J. No contribution of angiotensin-converting

enzyme (ACE) gene variants to severe obesity: a model for comprehensive case//control and quantitative cladistic analysis of ACE in human diseases. *Eur J Hum Genet* 15, 320 (2007).

31. Berenson, G.S., Srinivasan, S.R., Bao, W., Newman, W., Tracy, R. & Watigney, W. Association between multiple cardiovascular risk factors and atherosclerosis in children and young adults. *New England Journal of Medicine* 338, 1650-1656 (1998).

32. Blair, S.N., Goodyear, N., Gibbons, L. & Cooper, K. Physical fitness and incidence of hypertension in healthy normotensive men and women. *Journal of American Medical Association* 252, 487-490 (1984).

33. Boks, M.P.M., Schipper, M., Schubart, C.D., Sommer, I.E., Kahn, R.S. & Ophoff, R.A. Investigating gene environment interaction in complex diseases: increasing power by selective sampling for environmental exposure. *Int. J. Epidemiol.* 36, 1363-1369 (2007).

34. Bonner, G., Preis, S., Schunk, U., Toussaint, C. & Kaufmann, W. Hemodynamic effects of bradykinin on systemic and pulmonary circulation in healthy and hypertensive humans. *Journal of Cardiovascular Pharmacology* 15 (Suppl. 9), S74-S82 (1990).

35. Boomsma, D., Kempen, H., Gevers Leuven, J., Havekes, L., de Knijff, P. & Frant, R. Genetic analysis of sex and generation differences in plasma lipid, lipoprotein, and apolipoprotein levels in adolescent twins and their parents. *Genetic Epidemiology* 13, 49-60 (1996).

36. Booth, F.W., Chakravarthy, M.V., Gordon, S.E. & Spangenburg, E.E. Waging war on physical inactivity: using modern molecular ammunition against an ancient enemy. *J Appl Physiol* 93, 3-30 (2002).

37. Bossé, Y., Vohl, M.-C., Dumont, M., Brochu, M., Bergeron, J., Després, J.-P. & Prud'homme, D. Influence of the angiotensin-converting enzyme gene insertion/deletion polymorphism on lipoprotein/lipid response to gemfibrozil. *Clinical Genetics* 62, 45-52 (2002).

38. Bouchard, C., Perusse, L., Leblanc, C., Tremblay, A. & Theriault, G. Inheritance of the amount and distribution of human body fat. *Int J Obes* 12, 205-215 (1988).

39. Bray, M.S. Genomics, genes, and environmental interaction: the role of exercise. *Journal of Applied Physiology* 88, 788-792 (2000).

40. Brown, N., Gainer, J.V., Murphey, L.J. & Vaughan, D.E. Bradykinin stiumlates tissue plasminogen activator release from human forearm

vasculature through B(2) receptor-dependent, NO synthase-independent, and cyclooxygenase-independent pathway. *Circulation* 102, 2190-2196 (2000).

41. Cambien, F., Alhenc-Gelas, F., Herbeth, B., Andre, J., Rakotovao, R., Gonzales, M., Allefrini, J. & Bloch, C. Familial resemblance of plasma angiotensin-converting enzyme levle: The Nancy study. *American Journal of Human Genetics* 43, 774-780 (1988).

42. Cambien, F., Poirier, O., Lecerf, L., Evans, A., Cambou, J.-P., Arveiler, D., Luc, G., Bard, J.-M., Bara, L., Ricard, S., Tiret, L., Amouyel, P., Alhenc-Gelas, F. & Soubrier, F. Deletion polymorphism in the gene for angiotensin-converting enzyme is a potent risk factor for myocardial infarction. *Nature* 359, 641-644 (1992).

43. Carluccio, M., Soccio, M. & De Caterina, R. Aspects of gene polymorphisms in cardiovascular disease: the renin-angiotensin system. *European Journal of Clinical Investigation* 31, 476-488 (2001).

44. Carnethon, M.R., Gidding, S.S., Nehgme, R., Sidney, S., Jacobs, D.R., Jr. & Liu, K. Cardiorespiratory Fitness in Young Adulthood and the Development of Cardiovascular Disease Risk Factors. *JAMA* 290, 3092-3100 (2003).

45. Carter, C.S., Onder, G., Kritchevsky, S.B. & Pahor, M. Angiotensin-Converting Enzyme Inhibition Intervention in Elderly Persons: Effects on Body Composition and Physical Performance. *J Gerontol A Biol Sci Med Sci* 60, 1437-1446 (2005).

46. Caspersen, C., Pereira, M. & Curran, K. Changes in phyical activity patterns in the United States by sex cross-sectional age. . *Medicine and Science in Sports and Exercise* 32, 1601-1609 (2000).

47. Catto, A., Carter, A., Barrett, J., Stickland, M., Bamford, J., Davies, J. & Grant, P. Angiotensin-converting enzyme insertion/deletion polymorphism and cerebrovascular diease. *Stroke* 27, 435-440 (1996).

48. Chase, N.L., Sui, X., Lee, D.-C. & Blair, S.N. The Association of Cardiorespiratory Fitness and Physical Activity With Incidence of Hypertension in Men. *Am J Hypertens* 22, 417-424 (2009).

49. Chiang, F.-T., Chern, T.-H., Lai, Z.-P., Tseng, C.-D., Hsu, K.-L., Lo, H.-M. & Tseng, Y. Age- and gender-dependent association of the angiotensin-converting enzyme gene with essential hypertension in a Chinese population. *Journal of Human Hypertension* 10, 823-826 (1996).

50. Choi, Y. & Lee, W. ACE and ApoE genotypes and their influences on plasma lipids in Korean men. *J Korean Geriatr Soc* 2, 18-26 (1998).

51. Collins, M., Xenophontos, S.L., Cariolou, M.A., Mokone, G.G., Hudson, D.E., Anastasiades, L. & Noakes, T.D. The ACE Gene and Endurance Performance during the South African Ironman Triathlons. *Medicine & Science in Sports & Exercise* 36, 1314-1320 (2004).

52. Cooper, R., McFarlane-Anderson, N., Bennett, F., Wilks, R., Puras, A., Tewksbury, D., Ward, R. & Forrester, T. ACE, angiotensinogen and obesity: a potential pathway leading to hypertension. *Journal of Human Hypertension* 11, 107-111 (1997).

53. Corbo, R., Vilardo, T., Mantuano, E., Ruggeri, M., Gemma, A. & Scacchi, R. Apolipoproteins B and E, and angiotensin I-converting enzyme (ACE) genetic polymorphisms in Italian women with coronary artery disease (CAD) and their relationships with plasma lipid and apolipoprotein levels. *Clinical Genetics* 52, 77-82 (1997).

54. Costa, A.M., Silva, A.J., Garrido, N.D., Louro, H., de Oliveira, R.J. & Breitenfeld, L. Association between ACE D allele and elite short distance swimming. *European journal of applied physiology* 106, 785-790 (2009).

55. Crews, D.E., Fitton, L.J., Kottke, B.A. & Kamboh, M.I. Population genetics of apolipoproteins A-IV, E, and H, and the angiotensin converting enzyme (ACE): Associations with lipids, and apolipoprotein levels in American Samoans. *American Journal of Physical Anthropology* 124, 364-372 (2004).

56. Crisan, D. & Carr, J. Angiotensin I-converting enzyme: Genotype and disease association. *Journal of Molecular Diagnostics* 2, 105-115 (2000).

57. Daniels, S.R., Greer, F.R. & and the Committee on, N. Lipid Screening and Cardiovascular Health in Childhood. *Pediatrics* 122, 198-208 (2008).

58. Das, U.N. Is angiotensin II an endogenous pro-inflammatory molecule? *Medical Science Monitor* 11, RA155-162 (2005).

59. Day, S.H., Gohlke, P., Dhamrait, S.S. & Williams, A.G. No correlation between circulating ACE activity and VO2max or mechanical efficiency in women. *European Journal of Applied Physiology* 99, 11-18 (2007).

60. Deckelbaum, R.J. & Williams, C.L. Childhood obesity: The health issue. *Obesity research* 9, 239S-243S (2001).

61. Dempfle, A., Scherag, A., Hein, R., Beckmann, L., Chang-Claude, J. & Schafer, H. Gene-environment interactions for complex traits: definitions, methodological requirements and challenges. *Eur J Hum Genet* 16, 1164-1172 (2008).

62. Després, J., Bouchard, C. & Malina, R.M. Physical activity and coronary heart disease risk factors during childhood and adolescents. *Exercise Sports Science Review* 18, 243-261 (1990).

63. Dishman, R.K., Washburn, R.A. & Heath, G.W. *Physical Activity Epidemiology*, (Human Kinetics, 2004).

64. Duru, K., Farrow, S., Wang, J.-M., Lockette, W. & Kurtz, T. Frequency of a deletion polymorphism in the gene for angiotensin converting enzyme is increases in African-Americans with hypertension. *American Journal of Hypertension* 7, 759-762 (1994).

65. Eaton, D.K., Kann, L., Kinchen, S., Ross, J., Hawkins, J., Harris, W.A., Lowry, R., McManus, T., Chyen, D., Shanklin, S., Lim, C., Grunbaum, J.A. & Wechsler, H. US Department of Health and Human Services. Youth risk behavior surveillance - United States, 2005. *Morbidity and Mortality Weekly Report* 55, 1-112 (2006).

66. Ehlers, M.R.W. & Riordan, J. Angiotensin-convertin enzyme : New concepts concerning its biological role. *Biochemistry* 28, 5311-5318 (1989).

67. El-Hazmi, M. & Warsy, A. Increased frequency of angiotensin-converting enzyme DD genotype in Saudi overweight and obese patients. *Annals of Saudi Medicine* 23, 24-27 (2003).

68. Engeli, S., Negrel, R. & Sharma, A.M. Physiology and Pathophysiology of the Adipose Tissue Renin-Angiotensin System. *Hypertension* 35, 1270-1277 (2000).

69. Erdös, E.G. The angiotensin I-converting enzyme. *Laboratory Investigation* 56, 345-348 (1987).

70. Eriksson, U., Danilczyk, U. & Penninger, J.M. Just the Beginning: Novel Functions for Angiotensin-Converting Enzymes. *Current Biology* 12, R745 (2002).

71. Eyre, H., Kahn, R., Robertson, R.M., the, A.C.S.A.D.A.A.H.A.C.W.C., Members, A.A.A.C.W.C., Clark, N.G., Doyle, C., Hong, Y., Gansler, T., Glynn, T., Smith, R.A., Taubert, K. & Thun, M.J. Preventing Cancer, Cardiovascular Disease, and Diabetes: A Common Agenda for the American Cancer Society, the American Diabetes Association, and the American Heart Association. *Circulation* 109, 3244-3255 (2004).

72. Ferrario, C.M., Smith, R., Levy, P. & Strawn, W. The Hypertension-Lipid Connection:: Insights into the Relation between Angiotensin II and Cholesterol in Atherogenesis. *The American Journal of the Medical Sciences* 323, 17-24 (2002).

73. Fornage, M., Amos, C.I., Kardia, S., Sing, C.F., Turner, S.T. & Boerwinkle, E. Variation in the Region of the Angiotensin-Converting Enzyme Gene Influences Interindividual Differences in Blood Pressure Levels in Young White Males. *Circulation* 97, 1773-1779 (1998).

74. Freedman, D., Dietz, W., Srinivasan, S.R. & Berenson, G.S. The relation of overweight to cardiovascular risk factors among children and adolescents: the Bogalusa heart study. *Pediatrics* 103, 1175-1182 (1999).

75. Fuentes, R.M., Perola, M., Nissinen, A. & Tuomilehto, J. ACE gene and physical activity, blood pressure, and hypertension: a population study in Finland. *J Appl Physiol* 92, 2508-2512 (2002).

76. Gardemann, A., Fink, M., Stricker, J., Nguyen, Q.D., Humme, J., Katz, N., Tillmanns, H., Hehrlein, F.W., Rau, M. & Haberbosch, W. ACE I/D gene polymorphism: presence of the ACE D allele increases the risk of coronary artery disease in younger individuals. *Atherosclerosis* 139, 153 (1998).

77. Gaya, A., Alves, A., Aires, L., Martins, C., Ribeiro, J. & Mota, J. Association between time spent in sedentary, moderate to vigorous physical activity, body mass index, cardiorespiratory fitness and blood pressure. *Annals of Human Biology* 36, 379-387 (2009).

78. Gayagay, G., Yu, B., Hambly, B., Boston, T., Hahn, A., Celermajer, D.S. & Trent, R.J. Elite endurance athletes and the ACE I allele – the role of genes in athletic performance *Human Genetics* 103, 48-50 (1998).

79. Geer, E.B. & Shen, W. Gender differences in insulin resistance, body composition, and energy balance. *Gender Medicine* 6, 60-75 (2009).

80. Getchell, L., Kirkendall, D. & Robbins, G. Prediction of maximal oxygen uptake in young adult women joggers. *The Research Quarterly* 48, 61-67 (1977).

81. Goossens, G.H., Blaak, E.E., Saris, W.H.M. & van Baak, M.A. Angiotensin II-Induced Effects on Adipose and Skeletal Muscle Tissue Blood Flow and Lipolysis in Normal-Weight and Obese Subjects. *J Clin Endocrinol Metab* 89, 2690-2696 (2004).

82. Gregory, C.O., Martorell, R., Narayan, K.V., Ramirez-Zea, M. & Stein, A.D. Five year changes in adiposity and cardio-metabolic risk factors among Guatemalan young adults. *Public Health Nutrition* 12, 228-235 (2008).

83. Gui-yan, W., Yan-hua, W., Qun, X., Wei-jun, T., Ming-ling, G., Jian, W., Ming-wu, F. & Yong-hong, Z. Associations between RAS Gene Polymorphisms, Environmental Factors and Hypertension in Mongolian People. *European Journal of Epidemiology* 21, 287-292 (2006).

84. Gulati, S. Childhood hypertension. *Indian Pediatrics* 43, 326-333 (2006).

85. Harrap, S.B., Davidson, H.R., Connor, J.M., Soubrier, F., Corvol, P., Fraser, R., Foy, C.J. & Watt, G.C. The angiotensin I converting enzyme gene and predisposition to high blood pressure. *Hypertension* 21, 455-460 (1993).

86. Haskell, W.L., Lee, I.M., Pate, R.R., Powell, K.E., Blair, S.N., Franklin, B.A., Macera, C.A., Heath, G.W., Thompson, P.D. & Bauman, A. Physical activity and public health: Updated recommendation for adults from the ACSM and AHA. *Medicine and Science in Sports and Exercise* 39, 1423-1434 (2007).

87. Hayek, T., Pavlotzky, E., Hamoud, S., Coleman, R., Keidar, S., Aviram, M. & Kaplan, M. Tissue Angiotensin-Converting-Enzyme (ACE) Deficiency Leads to a Reduction in Oxidative Stress and in Atherosclerosis: Studies in ACE-Knockout Mice Type 2. *Arterioscler Thromb Vasc Biol* 23, 2090-2096 (2003).

88. Hayman, L.L. Abnormal Blood Lipids: Is It Environment or Is It Genes? *Journal of Cardiovascular Nursing* 14, 39-49 (2000).

89. Heimann, A.S., Favarato, M.H., Gozzo, F.C., Rioli, V., Carreno, F.R., Eberlin, M.N., Ferro, E.S., Krege, J.H. & Krieger, J.E. ACE gene titration in mice uncovers a new mechanism for ACE on the control of body weight. *Physiol. Genomics* 20, 173-182 (2005).

90. Hunter, D.J. Gene-environment interactions in human diseases. *Nat Rev Genet* 6, 287-298 (2005).

91. Islam, M.S., Lehtimaki, T., Juonala, M., Kahonen, M., Hutri-Kahonen, N., Kainulainen, K., Miettinen, H., Taittonen, L., Kontula, K., Viikari, J.S.A. & Raitakari, O.T. Polymorphism of the angiotensin-converting enzyme (ACE) and angiotesinogen (AGT) genes and their associations with blood pressure and carotid artery intima media thickness among healthy Finnish young adults--the Cardiovascular Risk in Young Finns Study. *Atherosclerosis* 188, 316 (2006).

92. Jacobs, D.R., Jr., Ainsworth, B., Hartman, T. & Leon, A.S. A simultaneous evaluation of 10 commonly used physical activity questionnaires. *Medicine and Science in Sports and Exercise* 25, 81-91 (1993).

93. Janssen, I., Katzmarzyk, P.T., Ross, R., Leon, A.S., Skinner, J.S., Rao, D.C., Wilmore, J.H., Rankinen, T. & Bouchard, C. Fitness Alters the Associations of BMI and Waist Circumference with Total and Abdominal Fat[ast][ast]. *Obesity* 12, 525-537 (2004).

94. Jones, A., Montgomery, H.E. & Woods, D.R. Human Performance: A Role for the ACE Genotype? *Exercise and Sport Sciences Reviews* 30, 184-190 (2002).

95. Katzmarzyk, P.T., Malina, R.M. & Bouchard, C. Physical activity, physical fitness, and coronary heart disease risk factors in youth: The Quebec Family Study. *Preventive Medicine* 29, 555-562 (1999).

96. Keidar, S., Kaplan, M. & Aviram, M. Angiotensin II–Modified LDL Is Taken Up by Macrophages Via the Scavenger Receptor, Leading to Cellular Cholesterol Accumulation. *Arterioscler Thromb Vasc Biol* 16, 97-105 (1996).

97. Keidar, S., Attias, J., Heinrich, R., Coleman, R. & Aviram, M. Angiotensin II atherogenicity in apolipoprotein E deficient mice is associated with increased cellular cholesterol biosynthesis. *Atherosclerosis* 146, 249-257 (1999).

98. Kim, D.-K., Kim, J.-W., Kim, S., Gwon, H.-C., Ryu, J.-C., Huh, J.-E., Choo, J.-A., Choi, Y., Rhee, C.-H. & Lee, W.-R. Polymorphism of Angiotensin Converting Enzyme Gene Is Associated With Circulating Levels of Plasminogen Activator Inhibitor-1. *Arterioscler Thromb Vasc Biol* 17, 3242-3247 (1997).

99. Kobayashi, K., Amemiya, S., Mochizuki, M., Matsushita, K., Sawanobori, E., Ishihara, T., Higashida, K., Shimura, Y., Kato, K. & Nakazawa, S. Association of Angiotensin-Converting Enzyme Gene Polymorphism with Lipid Profiles in Children and Adolescents with Insulin-Dependent Diabetes mellitus. *Hormone Research in Paediatrics* 51, 201-204 (1999).

100. Kramkowski, K., Mogielnicki, A. & Buczko, W. The physiological significance of the alternative pathways of angiotensin II production. *Journal of Physiology and Pharmacology* 57, 529-539 (2006).

101. Lakka, T.A. & Bouchard, C. Genetics, physical activity, fitness, and health: What does the future hold? *The journal of the Royal Society for the Promotion of Health* 124, 14-15 (2004).

102. Laragh, J., Angers, M., Kelly, W. & Lieberman, S. Hypotensive agents and pressor substances. The effects of epinephrine, norepinephrine, angiotensin II and others on the secretory rate of aldosterone in man. *JAMA* 174, 234-240 (1960).

103. Larsen, G.E., George, J.D., Alexander, J.L., Fellingham, G.W., Aldana, S.G. & Parcell, A.C. Prediction of maximum oxygen consumption from walking, jogging, or running. *Research Quarterly for Exercise and Sport* 73, 66-72 (2002).

104. Lasky-Su, J., Lyon, H.N., Emilsson, V., Heid, I.M., Molony, C., Raby, B.A., Lazarus, R., Klanderman, B., Soto-Quiros, M.E., Avila, L., Silverman, E.K., Thorleifsson, G., Thorsteinsdottir, U., Kronenberg, F., Vollmert, C., Illig, T., Fox, C.S., Levy, D., Laird, N., Ding, X., McQueen, M.B., Butler, J., Ardlie, K., Papoutsakis, C., Dedoussis, G., O'Donnell, C.J., Wichmann, H.E., Celedón, J.C., Schadt, E., Hirschhorn, J., Weiss, S.T., Stefansson, K. & Lange, C. On the Replication of Genetic Associations: Timing Can Be Everything! *The American Journal of Human Genetics* 82, 849-858 (2008).

105. Leary, S.D., Ness, A.R., Smith, G.D., Mattocks, C., Deere, K., Blair, S.N. & Riddoch, C. Physical Activity and Blood Pressure in Childhood: Findings From a Population-Based Study. *Hypertension* 51, 92-98 (2008).

106. Leslie, E., Owen, N., Salmon, J., Bauman, A., Sallis, J. & Lo, S. Insufficiently active Austrailian college students: Perceived personal, social, and environmental influences. *Preventive Medicine.* 28, 20-27 (1999).

107. Li, D., Singh, R.M., Liu, L., Chen, H., Singh, B.M., Kazzaz, N. & Mehta, J.L. Oxidized-LDL through LOX-1 increases the expression of angiotensin converting enzyme in human coronary artery endothelial cells. *Cardiovasc Res* 57, 238-243 (2003).

108. Lindpaintner, K., Pfeffer, M.A., Kreutz, R., Stampfer, M.J., Grodstein, F., LaMotte, F., Buring, J. & Hennekens, C.H. A Prospective Evaluation of an Angiotensin-Converting-Enzyme Gene Polymorphism and the Risk of Ischemic Heart Disease. *N Engl J Med* 332, 706-712 (1995).

109. Lloyd-Jones, D., Adams, R.J., Brown, T.M., Carnethon, M., Dai, S., De Simone, G., Ferguson, T.B., Ford, E., Furie, K., Gillespie, C., Go, A., Greenlund, K., Haase, N., Hailpern, S., Ho, P.M., Howard, V., Kissela, B., Kittner, S., Lackland, D., Lisabeth, L., Marelli, A., McDermott, M.M., Meigs, J., Mozaffarian, D., Mussolino, M., Nichol, G., Roger, V.L., Rosamond, W., Sacco, R., Sorlie, P., Stafford, R., Thom, T., Wasserthiel-Smoller, S., Wong, N.D., Wylie-Rosett, J., Committee, o.b.o.t.A.H.A.S. & Stroke Statistics Subcommittee. Heart Disease and Stroke Statistics--2010 Update: A Report From the American Heart Association. *Circulation* 121, e46-215 (2010).

110. Lorenzo Bermejo, J. & Hemminki, K. Gene-environment studies: any advantage over environmental studies? *Carcinogenesis* 28, 1526-1532 (2007).

111. Luma, G.B. & Spiotta, R.T. Hypertension in children and adolescents. *American Family Physician* 73, 1158-1168 (2006).

112. Maia, J.A.R., Thomis, M. & Beunen, G. Genetic factors in physical activity levels: A twin study. *American Journal of Preventive Medicine* 23, 87-91 (2002).

113. Manly, K.F. Reliability of statistical associations between genes and disease. *Immunogenetics* 57, 549-558 (2005).

114. Margaglione, M., Celentano, E., Grandone, E., Vecchione, G., Cappucci, G., Giuliani, N., Colaizzo, D., Panico, S., Mancini, F.P. & Di Minno, G. Deletion Polymorphism in the Angiotensin-Converting Enzyme Gene in Patients With a History of Ischemic Stroke. *Arterioscler Thromb Vasc Biol* 16, 304-309 (1996).

115. Marian, A.J., Safavi, F., Ferlic, L., Dunn, J.K., Gotto, A.M. & Ballantyne, C.M. Interactions between angiotensin-I converting enzyme insertion/deletion polymorphism and response of plasma lipids and coronary atherosclerosis to treatment with fluvastatin : The lipoprotein

and coronary atherosclerosis study. *Journal of the American College of Cardiology* 35, 89-95 (2000).

116. Matsubara, M., Suzuki, M., Fujiwara, T., Kikuya, M., Metoki, H., Michimata, M., Araki, T., Kazama, I., Satoh, T., Hashimoto, J., Hozawa, A., Ohkubo, T., Tsuji, I., Katsuya, T., Higaki, J., Ogihara, T., Satoh, H. & Imai, Y. Angiotensin-converting enzyme I/D polymorphism and hypertension: The Ohasama study. *Journal of Hypertension* 20, 1121-1126 (2002).

117. McGill, H.C., Jr., McMahan, C.A., Herderick, E.E., Malcom, G.T., Tracy, R.E. & Strong, J.P. Origin of atherosclerosis in childhood and adolescence. *Am J Clin Nutr* 72, 1307S-1315 (2000).

118. McGuire, K., Janssen, I. & Ross, R. Ability of Physical activity to predict Cardiovascular Disease Beyond Commonly Evaluated Cardiometabolic Risk Factors. *The American Journal of Cardiology* 104, 1522-1526 (2009).

119. McNaughton, L., Hall, P. & Cooley, D. Validation of several methods of estimating maximal oxygen uptake in young men. *Perceptual and Motor Skills* 87, 575-584 (1998).

120. McWilliams, J.M., Meara, E., Zaslavsky, A.M. & Ayanian, J.Z. Differences in Control of Cardiovascular Disease and Diabetes by Race, Ethnicity, and Education: U.S. Trends From 1999 to 2006 and Effects of Medicare Coverage. *Annals of Internal Medicine* 150, 505-515 (2009).

121. Meloni, F. & Schmaier, A. Low molecular weight kininogen binds to platelets to modulate thrombin-induced platelet activation. *Journal of Biological Chemistry* 266, 6786-7694 (2001).

122. Milne, E., van Bockxmeer, F.M., Robertson, L., Brisbane, J.M., Ashton, L.J., Scott, R.J. & Armstrong, B.K. Buccal DNA Collection: Comparison of Buccal Swabs with FTA Cards. *Cancer Epidemiol Biomarkers Prev* 15, 816-819 (2006).

123. Miyama, N., Hasegawa, Y., Suzuki, M., Hida, W., Kazama, I., Hatano, R., Sanada, S., Arata, T., Michimata, M., Sato, A., Satomi, S. & Matsubara, M. Investigation of Major Genetic Polymorphisms in the Renin-Angiotensin-Aldosterone System in Subjects with Young-Onset Hypertension Selected by a Targeted-Screening System at University. *Clinical and Experimental Hypertension* 29, 61-67 (2007).

124. Mombouli, J.-V. & Vanhoutte, P.M. Kinins and enothelial control of vascular smooth muscle. *Annual Review of Pharmacolical Toxicology* 35, 679-705 (1995).

125. Monda, K.L., Ballantyne, C.M. & North, K.E. Longitudinal impact of physical activity on lipid profiles in middle-aged adults: the Atherosclerosis Risk in Communities Study. *J. Lipid Res.* 50, 1685-1691 (2009).

126. Mondorf, U.F., Russ, A., Wiesemann, A., Herrero, M., Oremek, G. & Lenz, T. Contribution of Angiotensin I Converting Enzyme Gene Polymorphism and Angiotensinogen Gene Polymorphism to Blood Pressure Regulation in Essential Hypertension. *American Journal of Hypertension* 11, 174 (1998).

127. Mondry, A., Loh, M., Liu, P., Zhu, A.-L. & Nagel, M. Polymorphisms of the insertion / deletion ACE and M235T AGT genes and hypertension: surprising new findings and meta-analysis of data. *BMC Nephrology* 6, 1 (2005).

128. Montgomery, H., Clarkson, P., Barnard, M., Bell, J., Brynes, A., Dollery, C., Hajnal, J., Hemingway, H., Mercer, D., Jarman, P., Marshall, R., Prasad, K., Rayson, M., Saeed, N., Talmud, P., Thomas, L., Jubb, M., World, M. & Humphries, S. Angiotensin-converting-enzyme gene insertion/deletion polymorphism and response to physical training. *The Lancet* 353, 541-545 (1999).

129. Montgomery, H.E., Clarkson, P., Dollery, C.M., Prasad, K., Losi, M.-A., Hemingway, H., Statters, D., Jubb, M., Girvain, M., Varnava, A., World, M., Deanfield, J., Talmud, P., McEwan, J.R., McKenna, W.J. & Humphries, S. Association of Angiotensin-Converting Enzyme Gene I/D Polymorphism With Change in Left Ventricular Mass in Response to Physical Training. *Circulation* 96, 741-747 (1997).

130. Montgomery, H.E., Marshall, R., Hemingway, H., Myerson, S., Clarkson, P., Dollery, C., Hayward, M., Holliman, D.E., Jubb, M., World, M., Thomas, E.L., Brynes, A.E., Saeed, N., Barnard, M., Bell, J.D., Prasad, K., Rayson, M., Talmud, P.J. & Humphries, S.E. Human gene for physical performance. *Nature* 393, 221 (1998).

131. Moran, C.N., Vassilopoulos, C., Tsiokanos, A., Jamurtas, A.Z., Bailey, M.E.S., Wilson, R.H. & Pitsiladis, Y.P. Effects of Interaction between Angiotensin I-Converting Enzyme Polymorphisms and Lifestyle on Adiposity in Adolescent Greeks. *Obesity Res* 13, 1499-1504 (2005).

132. Morrow, J.R., Jackson, A., Disch, J.G. & Mood, D.P. Physical fitness ans activity assesment in adults. in *Measurement and Evaluation in Human Performance* (Human Kinetics, Champaign, IL, 2005).

133. Mudd, L.M., Rafferty, A.P., Reeves, M.J. & Pivarnik, J.M. Physical Activity Recommendations: An Alternative Approach Using Energy Expenditure. *Medicine & Science in Sports & Exercise* 40, 1757-1763 1710.1249/MSS.1750b1013e31817bb31818a31812 (2008).

134. Murphey, L.J., Gainer, J.V., Vaughan, D.E. & Brown, N.J. Angiotensin-Converting Enzyme Insertion/Deletion Polymorphism Modulates the Human In Vivo Metabolism of Bradykinin. *Circulation* 102, 829-832 (2000).

135. Myerson, S., Hemingway, H., Budget, R., Martin, J., Humphries, S. & Montgomery, H. Human angiotensin I-converting enzyme gene and endurance performance. *J Appl Physiol* 87, 1313-1316 (1999).

136. Nelson, M.C., Story, M., Larson, N.I., Neumark-Sztainer, D. & Lytle, L.A. Emerging adulthood and college-aged youth: An overlooked age for weight-related behavior change. *Obesity* 16, 2205-2211 (2008).

137. Niemiec, P., Zak, I. & Wita, K. Modification of the Coronary Artery Disease Risk Associated with the Presence of Traditional Risk Factors by Insertion/Deletion Polymorphism of the ACE Gene. *Genetic Testing* 11, 353-360 (2007).

138. O'Donnell, C.J., Lindpaintner, K., Larson, M.G., Rao, V.S., Ordovas, J.M., Schaefer, E.J., Myers, R.H. & Levy, D. Evidence for Association and Genetic Linkage of the Angiotensin-Converting Enzyme Locus With Hypertension and Blood Pressure in Men but Not Women in the Framingham Heart Study. *Circulation* 97, 1766-1772 (1998).

139. Ode, J.J. Assessing Physical Activity Behaviors in College Students. *Dissertation for the Degree of Ph. D. Michigan State University* (2007).

140. Ode, J.J., Pivarnik, J.M., Reeves, M. & Knous, J.L. Body mass index as a predictor of percent fat in college athletes and nonathletes. *Med Sci Sports Exerc* 39, 403-409 (2007).

141. Okumura, K., Matsui, H., Kawakami, K., Numaguchi, Y., Kaneko, S., Morishima, I., Mokuno, S., Toki, Y. & Hayakawa, T. Relationship between the apolipoprotein E and angiotensin-converting enzyme genotypes and LDL particle size in Japanese subjects. *Clinica Chimica Acta* 285, 91-103 (1999).

142. Oren, I., Brook, J.G., Gershoni-Baruch, R., Kepten, I., Tamir, A., Linn, S. & Wolfovitz, E. The D allele of the angiotensin-converting enzyme gene contributes towards blood LDL-cholesterol levels and the presence of hypertension. *Atherosclerosis* 145, 267 (1999).

143. Paffenbarger, R.S., Jr., Wing, A.L., Hyde, R.T. & Jung, D.L. Physical activity and incidence of hypertension in college alumni. *Am. J. Epidemiol.* 117, 245-257 (1983).

144. Pall, D., Settakis, G., Katona, E., Zatik, J., Kollar, J., Limburg, M. & Fulesdi, B. Angiotensin-converting enzyme gene polymorphism, carotid intima-media thickness, and left ventricular mass index in adolescent hypertension. *Journal of clinical ultrasound* 32, 129-135 (2004).

145. Pallaud, C., Gueguen, R., Sass, C., Grow, M., Cheng, S., Siest, G. & Visvikis, S. Genetic influences on lipid metabolism trait variability within the Stanislas Cohort. *J. Lipid Res.* 42, 1879-1890 (2001).

146. Pate, R.R., Pratt, M., Blair, S.N., Haskell, W.L., Macera, C.A., Bouchard, C., Buchner, D., Ettinger, W., Heath, G.W., King, A.C. & et, a. Physical activity and public health. A recommendation from the Centers for Disease Control and Prevention and the American College of Sports Medicine. Vol. 273 402-407 (1995).

147. Prasad, A., Narayanan, S., Waclawiw, M.A., Epstein, N. & Quyyumi, A.A. The insertion/deletion polymorphism of the angiotensin-converting enzyme gene determines coronary vascular tone and nitric oxide activity. *J Am Coll Cardiol* 36, 1579-1586 (2000).

148. Raitakari, O.T., Porkka, K.V.K., Rasanen, L., Ronnemaa, T. & Viikari, J.S.A. Clustering and six year cluster tracking of serum total cholesterol, HDL-cholesterol and diastolic blood pressure in children and young adults: The Cardiovascular risk in young Finns study. *J Clin Epidemiol* 47, 1085-1093 (1994).

149. Raitakari, O.T., Juolala, M., Kähönen, M., Taittonen, L., Laitinen, L., Mäki-Torkko, N., Järvisalo, M.J., Uhari, M., Jokinen, E., Rönnemaa, T., Åkerblom, H.K. & Viikari, J.S. Cardiovascular risk factors in childhood and carotid artery intima-media thickness in adulthood: the cardiovascular risk in young Finns study. *Journal of American Medical Association* 290, 2277-2283 (2003).

150. Rankinen, T., Perusse, L., Gagnon, J., Chagnon, Y.C., Leon, A.S., Skinner, J.S., Wilmore, J.H., Rao, D.C. & Bouchard, C. Angiotensin-converting enzyme ID polymorphism and fitness phenotype in the HERITAGE Family Study. *J Appl Physiol* 88, 1029-1035 (2000).

151. Rankinen, T., Wolfarth, B., Simoneau, J.-A., Maier-Lenz, D., Rauramaa, R., Rivera, M.A., Boulay, M.R., Chagnon, Y.C., Perusse, L., Keul, J. & Bouchard, C. No association between the angiotensin-converting enzyme ID polymorphism and elite endurance athlete status. *J Appl Physiol* 88, 1571-1575 (2000).

152. Rankinen, T. & Bouchard, C. Invited commentary: Physical activity, mortality, and genetics. *Am J Epidemiol* 166, 260-262 (2007).

153. Re, R.N. Mechanisms of disease: local renin-angiotensin aldosterone system and the pathogenesis and treatment of cardiovascular disease. *Nature Clinical Practice* 1, 42-47 (2004).

154. Reaven, P., Barrett-Connor, E. & Edelstein, S. Relation between leisure-time physical activity and blood pressure in older women. *Circulation* 83, 559-565 (1991).

155. Reilly, J.J. Obesity in childhood and adolescence: evidence based clinical and public health perspectives. *Postgrad Med J* 82, 429-437 (2006).

156. Ribeiro, J.C., Guerra, S., Oliveira, J., Andersen, L., Durarte, J.A. & Mota, J. Body fatness and clustering of cardiovascular disease risk factors in Portuguese children and adolescents. *American Journal of Human Biology* 16, 556-562 (2004).

157. Riera-Fortuny, C., Real, J.T., Chaves, F.J., Morales-Suarez-Varela, M., Martinez-Triguero, M.L., Morillas-Arino, C. & Hernandez-Mijares, A. The relation between obesity, abdominal fat deposit and the angiotensin-converting enzyme gene I//D polymorphism and its association with coronary heart disease. *Int J Obes Relat Metab Disord* 29, 78 (2004).

158. Rigat, B., Hubert, C., Alhenc-Gelas, F., Cambien, F., Corvol, P. & Soubrier, F. An insertion/deletion polymorphism in the angiotensin I-converting enzyme gene accounting for half the variance of serum enzyme levels. *Journal of Clinical Investigation* 86, 1343-1346 (1990).

159. Roberts, C.K. & Barnard, R.J. Effects of exercise and diet on chronic disease. *J Appl Physiol* 98, 3-30 (2005).

160. Rosamond, W., Flegal, K., Furie, K., Go, A., Greenlund, K., Haase, N., Hailpern, S.M., Ho, M., Howard, V., Kissela, B., Kittner, S., Lloyd-Jones, D., McDermott, M., Meigs, J., Moy, C., Nichol, G., O'Donnell, C., Roger, V., Sorlie, P., Steinberger, J., Thom, T., Wilson, M. & Hong, Y. Heart Disease and Stroke Statistics--2008 Update: A Report From the

American Heart Association Statistics Committee and Stroke Statistics Subcommittee. Vol. 117 e25-146 (2008).

161. Ross, R., Dagnone, D., Jones, P.J.H., Smith, H., Paddags, A., Hudson, R. & Janssen, I. Reduction in Obesity and Related Comorbid Conditions after Diet-Induced Weight Loss or Exercise-Induced Weight Loss in Men. *Annals of Internal Medicine* 133, 92-103 (2000).

162. Ross, R., Janssen, I., Dawson, J., Kungl, A.-M., Kuk, J.L., Wong, S.L., Nguyen-Duy, T.-B., Lee, S., Kilpatrick, K. & Hudson, R. Exercise-Induced Reduction in Obesity and Insulin Resistance in Women: a Randomized Controlled Trial[ast][ast]. *Obesity* 12, 789-798 (2004).

163. Ross, R. & Janssen, I. *Physical Activity and Health*, (Human Kinetics, Champaign, IL, 2007).

164. Sadoshima, J. & Izumo, S. Molecular characterization of angiotensin II--induced hypertrophy of cardiac myocytes and hyperplasia of cardiac fibroblasts. Critical role of the AT1 receptor subtype. *Circ Res* 73, 413-423 (1993).

165. Sallis, J. Age-related decline in physical activity: a synthesis of human and animal studies. *Medicine and Science in Sports and Exercise* 32, 1598-1600 (2000).

166. Sallis, J. & Saelens, B. Assessment of physical activity by self-report: status, limitations, and future directions. *Research Quarterly for Exercise and Sport* 71, S1-14 (2000).

167. Samani, N.J., Thompson, J.R., O'Toole, L., Channer, K. & Woods, K.L. A Meta-analysis of the Association of the Deletion Allele of the Angiotensin-Converting Enzyme Gene With Myocardial Infarction. *Circulation* 94, 708-712 (1996).

168. Sayed-Tabatabaei, F.A., Houwing-Duistermaat, J.J., van Duijn, C.M. & Witteman, J.C.M. Angiotensin-Converting Enzyme Gene Polymorphism and Carotid Artery Wall Thickness: A Meta-Analysis. *Stroke* 34, 1634-1639 (2003).

169. Sayed-Tabatabaei, F.A., Oostra, B.A., Isaacs, A., van Duijn, C.M. & Witteman, J.C.M. ACE polymorphism. *Circulation Research* 98, 1123-1133 (2006).

170. Seaward, B. *Managing Stress: Principles and strategies for health and well-being.*, (Jones and Bartlett Publishers, Boston, MA, 2004).

171. Sekuri, C., Cam, F.S., Ercan, E., Tengiz, I., Sagcan, A., Eser, E., Berdeli, A. & Akin, M. Renin-angiotensin system gene polymorphisms and premature coronary heart disease. *Journal of the Renin-Angiotensin-Aldosterone System* 6, 38-42 (2005).

172. Shanmugam, V., Sell, K.W. & Saha, B.K. Mistyping ACE heterozygotes. *PCR Methods Appl.* 3, 120-121 (1993).

173. Sharma, J.N. Role of Tissue Kallikrein-Kininogen-Kinin Pathways in the Cardiovascular System. *Archives of Medical Research* 37, 299 (2006).

174. Sonna, L.A., Sharp, M.A., Knapik, J.J., Cullivan, M., Angel, K.C., Patton, J.F. & Lilly, C.M. Angiotensin-converting enzyme genotype and physical performance during US Army basic training. *J Appl Physiol* 91, 1355-1363 (2001).

175. Sorof, J. & Daniels, S.R. Obesity hypertension in children: a problem of epidemic proportions. *Hypertension* 40, 441-447 (2002).

176. Sparling, P.B. & Snow, T.K. Physical activity patterns in recent college alumni. *Research Quarterly for Exercise and Sport* 73, 200-205 (2002).

177. Staessen, J.A., Wang, J.G., Ginocchio, G., Petrov, V., Saavedra, A.P., Soubrier, F., Vlietinck, R. & Fagard, R. The deletion/insertion polymorphism of the angiotensin converting enzyme gene and cardiovascular-renal risk. *Journal of Hypertension* 15, 1579-1592 (1997).

178. Stoops, N. Educational Attainment in the United States: 2003. (US Census Bureau, Washington DC, 2004).

179. Strazzullo, P., Iacone, R., Iacoviello, L., Russo, O., Barba, G., Russo, P., D'Orazio, A., Barbato, A., Cappuccio, F.P., Farinaro, E. & Siani, A. Genetic Variation in the Renin-Angiotensin System and Abdominal Adiposity in Men: The Olivetti Prospective Heart Study. *Ann Intern Med* 138, 17-23 (2003).

180. Strong, J.P., Zieske, A. & Malcom, G. Lipoprotiens and atherosclerosis in children: an early marriage? *Nutrition, Metabolism, and Cardiovascular Diseases* 11, 16-22 (2001).

181. Strong, W.B., Malina, R.M., Blimkie, C.J., Daniels, S.R., Dishman, R.K., Gutin, B., Hergenroeder, A.C., Must, A., Nixion, P.A., Pivarnik, J.M., Rowland, T., Trost, S. & Trudeau, F. Evidence based physical activity for school-age youth. *The Journal of Pediatrics* 146, 732-737 (2005).

182. Su, J.B. Kinins and Cardiovascular Disease. *Current Pharmaceutical Design* 12, 3423-3435 (2006).

183. Suchanek, P., Hubacek, J.A., Lesna, I.K., Pinekerova, V. & Adamkova, V. Actigenetic of ACE gene polymorphism in Czech obese sedentary females. *Physiological Research* 58, S47-S52 (2009).

184. Taittonen, L., Uhari, M., Kontula, K., Kainulainen, K., Miettinen, H., Turtinen, J. & Nuutinen, M. Angiotensin converting enzyme gene insertion/deletion polymorphism, angiotensinogen gene polymorphisms, family history of hypertension, and childhood blood pressure. *American Journal of Hypertension* 12, 858 (1999).

185. Tambalis, K., Panagiotakos, D., Kavouras, S. & Sidossis, L. Response of blood lipids to aerobic, resistance, and combined aerobic with resistance exercise training: a systematic review of current evidence. *Angiology* 60, 614-632 (2008).

186. Taylor, R.R., Mamotte, C.D.S., Fallon, K. & van Bockxmeer, F.M. Elite athletes and the gene for angiotensin-converting enzyme. *J Appl Physiol* 87, 1035-1037 (1999).

187. Telama, R. & Yang, X. Decline of physical activity from youth to young adulthood in Finland. *Medicine and Science in Sports and Exercise* 32, 1617-1622 (2000).

188. Thomas, G.N., Tomlinson, B., Chan, J.C.N., Sanderson, J.E., Cockram, C.S. & Critchley, J.A.J.H. Renin-Angiotensin System Gene Polymorphisms, Blood Pressure, Dyslipidemia, and Diabetes in Hong Kong Chinese: A significant association of the ACE insertion/deletion polymorphism with type 2 diabetes. *Diabetes Care* 24, 356-361 (2001).

189. Thompson, J., Raitt, J., Hutchings, L., Drenos, F., Bjargo, E., Loset, A., Grocott, M. & Montgomery, H. Angiotensin-Converting Enzyme Genotype and Successful Ascent to Extreme High Altitude. *High Altitude Medicine & Biology* 8, 278-285 (2007).

190. Thompson, W.R. & Binder-Macleod, S.A. Association of Genetic Factors With Selected Measures of Physical Performance. *Phys Ther* 86, 585-591 (2006).

191. Tiret, L., Rigat, B., Visvikis, S., Breda, C., Corvol, P., Cambien, F. & Soubrier, F. Evidence, from combined segregation and linkage analysis, that a variant of the angiotensin I-converting enzyme (ACE) gene controls plasma ACE levels. *American Journal of Human Genetics* 51, 197-205 (1992).

192. Tiret, L. Gene-enviromental interaction: a central concept in multifactorial diseases. *Proceedings of the Nutrition Society* 61, 457-463 (2002).

193. Tsianos, G., Sanders, J., Dhamrait, S., Humphries, S., Grant, S. & Montgomery, H. The ACE gene insertion/deletion polymorphism and elite endurance swimming *European Journal of Applied Physiology* 92, 360-362 (2004).

194. Turner, S.T., Boerwinkle, E. & Sing, C.F. Context-dependent association of ACE I/D polymorphism with blood pressure. *Hypertension* 34, 773-778 (1999).

195. Uemura, K., Nakura, J., Kohara, K. & Miki, T. Association of ACE I/D polymorphism with cardiovascular risk factors. *Human Genetics* 107, 239-242 (2000).

196. Vallejo, M., Martinez-Palomino, G., Ines-Real, S., Perez-Hernandez, N., Juarez-Rojas, J. & Vargas-Alarcón, G. Relationship between the angiotensin I-converting enzyme insertion/deletion (I/D) polymorphism and cardiovascular risk factors in healthy young Mexican women. *Genetic Testing and Molecular Biomarkers* 13, 237-242 (2009).

197. Varda, N.M., Borut, P., Bradač, S.U., Gregorič, A. & Milanez, T. Angiotensin-converting enzyme gene polymorphism as a cardiovascular risk factor in children. *European Journal of Physiology* Suppl 1(2000).

198. Vargas-Alarcón, G., Zamora, J., Sánchez-García, S., Rodríguez-Pérez, J.M., Cardoso, G. & Posadas-Romero, C. Angiotensin-I-converting enzyme (ACE) insertion/deletion polymorphism in Mexican patients with coronary artery disease. Association with the disease but not with lipid levels. *Experimental and Molecular Pathology* 81, 131-135 (2006).

199. Vassilikioti, S., Doumas, M., Douma, S., Petidis, K., Karagiannis, A., Balaska, K., Vyzantiadis, A. & Zamboulis, C. Angiotensin Converting Enzyme Gene Polymorphism Is Not Related to Essential Hypertension in a Greek Population. *American Journal of Hypertension* 9, 700 (1996).

200. Volzke, H., Engel, J., Kleine, V., Schwahn, C., Dahm, J.B., Eckel, L. & Rettig, R. Angiotensin I-Converting Enzyme Insertion/Deletion Polymorphism and Cardiac Mortality and Morbidity After Coronary Artery Bypass Graft Surgery*. *Chest* 122, 31-36 (2002).

201. Wacker, M.J., Godard, M.P., McCabe, E.H., Donnelly, J.E. & Kelly, J.K. Sex difference in the association of the Angiotensin Converting Enzyme I/D polymorphism and Body Mass Index. *Medical Science Monitor: International Medical Journal of Experimental and Clinical Research* 14, CR353-357 (2008).

202. Williams, A.G., Rayson, M.P., Jubb, M., World, M., Woods, D.R., Hayward, M., Martin, J., Humphries, S.E. & Montgomery, H.E. Physiology: The ACE gene and muscle performance. *Nature* 403, 614 (2000).

203. Williams, C.L., Hyaman, L.L., Daniels, S.R., Robinson, T.N., Steinberger, J., Paridon, S. & Bazzarre, T. Cardiovascular health in childhood: A statement for health professionals from the Committee on atherosclerosis, hypertension, and obesity in the young (AHOY) of the council on cardiovascular disease in the young, American Heart Association. *Circulation* 106, 143-160 (2002).

204. Winkleby, M.A., Robinson, T.N., Sundquist, J. & Kraemer, H.C. Ethnic Variation in Cardiovascular Disease Risk Factors Among Children and Young Adults: Findings From the Third National Health and Nutrition Examination Survey, 1988-1994. *Journal of American Medical Association* 281, 1006-1013 (1999).

205. Winnicki, M., Accurso, V., Hoffmann, M., Pawlowski, R., Dorigatti, F., Santonastaso, M., Longo, D., Krupa-Wojciechowska, B., Jeunemaitre, X., Pessina, A.C., Somers, V.K. & Palatini, P. Physical activity and angiotensin-converting enzyme gene polymophism in mild hypertensives. *American Journal of Medical Genetics* 125A, 38-44 (2004).

206. Wong, M.Y., Day, N.E., Luan, J.A. & Wareham, N.J. Estimation of magnitude in gene-environment interactions in the presence of measurement error. *Statistics in Medicine* 23, 987-998 (2004).

207. Yang, Q. & Khoury, M.J. Evolving Methods in Genetic Epidemiology. III. Gene-Environment Interaction in Epidemiologic Research. *Epidemiol Rev* 19, 33-43 (1997).

208. Zanovec, M., Johnson, L., Marx, B., Keenan, M. & Tuuri, G. Self-reported physical activity improves prediction of body fatness in young adults. . *Med Sci Sports Exerc* 41, 328-335 (2009).

209. Zeno, S.A., Kim-Dorner, S.-J., Deuster, P.A., Davis, J.L., Remaley, A.T. & Poth, M. Cardiovascular fitness and risk factors of healthy African

American and Causcasians. *Journal of the National Medical Association* 102, 28-35 (2010).

210. Zhang, B., Tanaka, H., Shono, H., Miura, S., Kiyonaga, A., Shindo, M. & Saku, K. The I allele of the angiotensin-converting enzyme is associated with an increased percentage of slow twitch type i fibers in human skeletal muscle. *Clinical Genetics* 63, 139-144 (2003).

211. Zhao, B., Moochhala, S.M., Tham, S.-y., Lu, J., Chia, M., Byrne, C., Hu, Q. & Lee, L.K.H. Relationship between angiotensin-converting enzyme ID polymorphism and VO2max of Chinese males. *Life Sciences* 73, 2625-2630 (2003).

212. Zwiren, L.D., Freedson, P.S., Ward, A., Wilke, S. & Rippe, J.M. Estimation of VO_2max: A comparative analysis of five exercise tests. *Research Quarterly for Exercise and Sport* 62, 73-78 (1991).