CARDIORESPIRATORY FITNESS AND THE RENIN-ANGIOTENSIN SYSTEM IN TERM AND PRETERM ADOLESCENTS

BY

SEAN P. CADDIGAN

A Thesis Submitted to the Graduate Faculty of

WAKE FOREST UNIVERSITY GRADUATE SCHOOL OF ARTS AND SCIENCES

in Partial Fulfillment of the Requirements

for the Degree of

MASTER OF SCIENCE

Health and Exercise Science

May, 2018

Winston-Salem, North Carolina

Approved By:

Patricia A. Nixon, Ph.D., Advisor

Peter H. Brubaker, Ph.D., Chair

Gary D. Miller, Ph.D.

ACKNOWLEDGEMENTS

Dr. Nixon, thank you for your willingness and understanding to make it possible for me to complete my thesis from afar, your guidance and feedback regarding the content of this thesis, and the openness to help me learn about a topic that was new to both you and me.

My committee members: Dr. Brubaker and Dr. Miller for agreeing to serve on my committee and for showing the same willingness and understanding as Dr. Nixon to make it possible for me to complete my thesis from afar.

Thank you to my fellow graduate students for your camaraderie and friendship. I enjoyed getting to know all of you through our time spent in class, at HELPS, and around Winston-Salem. I wish you all the best in your future endeavors.

Lastly, thank you to all my family and friends, especially my Mom, Dad, and sister who are always there to lend their support, show me love, and let me know what truly matters.

TABLE OF CONTENTS

LIST OF TABLES, FIGURES, & FORMULAS

TABLES Page

FIGURES

FORMULAS

LIST OF ABBREVIATIONS

ACE	Angiotensin Converting Enzyme
ACE I/D	Angiotensin Converting Enzyme Insertion-Deletion Polymorphism
ANCS	Antenatal Corticosteroid
Ang1-7	Angiotensin1-7
Ang II	Angiotensin II
AngII/1-7	Angiotensin II/1-7 Ratio
AT_1	Angiotensin II type I Receptor
a-vO_2	Arteriovenous Oxygen Difference
CO	Cardiac Output
CRF	Cardiorespiratory Fitness
DNA	Deoxyribonucleic Acid
ECM	Extra-cellular Matrix
Heart Rate	HR
MAQ	Modifiable Activity Questionnaire
MET	Metabolic Equivalent
PEPC1	Prenatal Exposures Postnatal Consequences Study 1
PT	Preterm
RAS	Renin-Angiotensin System
SD	Standard Deviation
SV	Stroke Volume
T	Term
VO_2	Oxygen Consumption
VO_2Max	Maximal Oxygen Uptake
VO_2Peak	Peak Oxygen Uptake
VO_2Peak% Predicted	Percentage of VO_{2max} based on Age and Sex

ABSTRACT

PURPOSE: The angiotensin converting enzyme insertion-deletion (ACE I/D) polymorphism (rs 4340) I/I genotype has been associated with higher cardiorespiratory fitness (CRF) when compared with the I/D and D/D genotypes in some studies, and the D/D genotype has been associated with increased plasma ACE activity compared to I/I and I/D genotypes. Also, the biological actions of angiotensin II (Ang II) and angiotensin (1-7) from the renin-angiotensin system (RAS) have been associated with pathogenic and protective cardiovascular affects, respectively. Therefore, the purpose of this study is to determine if ACE genotype and plasma renin-angiotensin system (RAS) components might partially explain cardiorespiratory fitness in term (T) and preterm (PT) adolescents. **METHODS**: 133 PT (51 M) and 48 T (21 M), 14.5 ± 0.3 yrs old, participated. Peak oxygen uptake (VO_2Peak in $ml \cdot kg^{-1} \cdot min^{-1}$) was determined from expired gas collection during a progressive maximal exercise test on a cycle ergometer and ACE genotype data was determined by polymerase chain reaction (PCR) amplification and electrophoresis. Plasma Ang II and Ang (1-7) concentrations were measured via separate radioimmunoassays of completely evaporated and reconstituted eluent in an assay buffer. Main effects of birth status and ACE genotype were examined with two-way ANOVAs, and one-way ANOVAs with bonferonni post hoc tests were used to detect mean differences among term and preterm ACE genotypes. Skewed distributions were transformed with natural log transformations. Associations were determined with Pearson correlation coefficients. **RESULTS**: Two-ways ANOVAs revealed on average term subjects had significantly higher VO_2Peak and VO_2Peak% Predicted ($F=4.70$, $df=1$, $p=.03$) and ($F=6.2$, $df=1$, $p=.01$), engaged in more vigorous physical activity per week ($F=5.0$, $df=1$, $p=.03$), and had higher

Ang (1-7) plasma concentrations and a lower Ang II/(1-7) ratio ($F=18.5$, $df=1$, $p<.01$) ($F=13.9$, $df=1$, $p<.01$) compared to preterm subjects. VO_2Peak did not differ in PT subjects among ACE genotype (I/I= 37.7±8.4 vs. I/D=38.7±10.3 vs. D/D=38.7±10.5) or the T group among ACE genotype (I/I=39.4±9.9 vs. I/D=41.6±11.1 vs. D/D=47.4±9.3). There were no significant differences in plasma angiotensin (II and 1-7) values by ACE genotype. Ang II was inversely associated with VO_2Peak% predicted in T I/D subjects ($r = -0.41$, $p<.05$), and the Ang II/(1-7) ratio was inversely associated with VO_2Peak in T I/D subjects ($r = -0.34$, $p<.05$). Ang II and the Ang II/(1-7) ratio were inversely associated with VO_2Peak and VO_2Peak% predicted in PT D/D genotype subjects ($r = -0.38$ and $r = -0.37$; $p<.05$) and ($r = -0.38$ and $r= -0.43$; $p<.05$).

CONCLUSIONS: This study provides evidence that ACE I/D genotype (rs4340) is not associated with cardiorespiratory fitness, habitual physical activity, or plasma RAS Ang II and Ang (1-7) concentrations or the Ang II/(1-7) ratio in term and preterm adolescents. However, plasma Ang II concentrations and the Ang II/(1-7) ratio were inversely associated with cardiorespiratory fitness, and subjects categorized as Ang (1-7) RAS dominant had higher cardiorespiratory fitness compared to subjects categorized as Ang II RAS dominant indicating the biological actions of Ang II and Ang (1-7) along with RAS dominance may help to explain cardiorespiratory fitness in adolescents.

REVIEW OF LITERATURE

Cardiorespiratory Fitness

An important complex phenotype with health and performance implications is cardiorespiratory fitness (CRF), defined as the maximal rate of oxygen consumption per minute (VO_2max) during severe exercise[8]. cardiorespiratory fitness primarily requires the coordinated effort of an individual's pulmonary, cardiovascular, and neuromuscular systems, with every individual having an "upper limit", meaning oxygen consumption does not increase above the upper limit despite an increase in workload or changes in work rate[8]. Essentially, a plateau or peak in oxygen consumption is attained. Interestingly, intrinsic VO_2max, defined as an individual's VO_2max in the sedentary state, has been shown to aggregate within families and have a significant heritability estimate[9]. In terms of heritability, early twin[10,22,35,36,47,80] and family studies[43,45,53], most notably the HERITAGE Family Study, estimated the maximal heritability and familial aggregation of cardiorespiratory fitness. Results of the HERITAGE Family Study, a cohort of 86 nuclear Caucasian families, found intrinsic VO_2max, adjusted for age, gender, and fat-mass to be 2.6 to 2.9 times more variable between families than within families, indicating individuals within a family are more likely to have similar intrinsic VO_2max values compared to individuals in different families[9]. This amount of variation converts into a maximal heritability estimate of 51% and a maternal heritability estimate of 30% in sedentary Caucasian adults and their offspring [65]. However, this estimate is presumed to be inflated to an undetermined degree due to non-genetic environmental factors such as occupational physical activity, diet, and stress [9]. None the less, this evidence suggests genomic variants

play a significant role in determining an individual's VO_2max, and is an important topic to study because of its use as a health and performance indicator.

In terms of health, a meta-analysis of 33 observational cohort studies including 102,980 healthy men and women by Kodama et al.,[38] suggests an individual's level of cardiorespiratory fitness can be used as a predictor for all-cause mortality and cardiovascular disease, with every 1 metabolic equivalent increase in cardiorespiratory fitness resulting in a 13% risk reduction in all-cause mortality and a 15% risk reduction in cardiovascular disease in healthy individuals compared to those who died or developed cardiovascular disease during the study period. Similarly, a meta-analysis and systematic review of 160 randomized control trials by Lin et al.,[44] suggests aerobic exercise can significantly increase cardiorespiratory fitness and improve biomarkers of cardiometabolic health, such as, lowering triglycerides, glycosylated hemoglobin A1c, fibrinogen, and angiotensin II (Ang II), while increasing high-density lipoprotein when compared to non-exercising controls. These findings are important because chronic diseases have become 6 of the top ten leading causes of death worldwide in 2015, accounting for 23 million deaths, which is 41% of all deaths in 2015[81]. In particular, heart disease is the number one cause of death worldwide, accounting for 8.7 million deaths, or 15% of all reported deaths in 2015 according to the World Health Organization[81]. For the United States in 2016, 92.1 million people had some form of heart disease resulting in a total economic burden of $316 billion[81]. Therefore, to reduce the health and economic burden of chronic diseases and improve all-cause mortality and cardiometabolic health for individuals, aerobic exercise is prescribed and recommended to increase cardiorespiratory fitness in sedentary, clinical, and healthy populations.

Combining the cardiorespiratory fitness heritability and familial aggregation evidence with the health and economic burden data provides a foundation from which to build an argument that it would be beneficial to determine the underlying genomics of cardiorespiratory fitness for individuals who are non-responders to aerobic exercise. That is, non-responders are individuals who fail to improve their cardiorespiratory fitness beyond measurement error after engaging in an appropriately prescribed aerobic exercise regime. According to Bouchard et al.,[12] approximately 7% of 473 (~33 people) healthy white Caucasian adults (17-65yrs old) were classified as non-responders following twenty weeks of standardized endurance training. Using this value of 7% of Caucasians as non-responders and the 2010 United States Census population data for whites between the ages of 15 and 69, there could be approximately 10.5 million people in the United States who would fail to improve their aerobic fitness after proper endurance training[21]. Most of the 10.5 million people probably would be healthy individuals with average or above cardiorespiratory fitness because according to Bouchard et al.,[9] baseline VO_2max is a poor predictor of response to training and VO_2max is a normally distributed trait. However, there would probably be a subset of this population comprised of people with poor to very poor cardiorespiratory fitness that would not benefit from aerobic exercise in terms of improving their cardiorespiratory fitness. Therefore, to reduce their risk of all-cause mortality and development of cardiovascular disease, a possible approach is to determine the underlying genomics of cardiorespiratory fitness to establish if genetic variants or biochemical pathway components could be altered to produce a higher level of cardiorespiratory fitness, thus indicating a healthier cardiorespiratory fitness phenotype.

Components of VO_2max

The field of exercise genomics aims to determine the inter-individual genetic differences responsible for variation in human biological phenotypes. VO_2max is a human biological trait that has considerable inter-individual variation and has been a topic of study from a genomics perspective for over a decade. Candidate genes such as bradykinin receptor B_2, vascular endothelial growth factor, and the angiotensin-converting enzyme have been selected as possible contributors to the variation in VO_2max because these genes code for proteins involved processes that could affect VO_2max. There are many other candidate genes for VO_2max because this phenotype involves the coordinated function of the cardiovascular, pulmonary, and neuromuscular systems. Therefore, to build a rationale for studying a specific candidate gene an approach is to deconstruct the VO_2max phenotype into simpler components that may be directly related to a gene.

Here, the Fick equation is used to deconstruct VO_2max, in a simplified manor, into its components. A complete deconstruction is beyond the scope of this thesis, therefore, it will be deconstructed in a way to highlight the potential contribution of the genomic and biochemical products contained in the data.

According to the Fick equation, oxygen consumption (VO_2) is the product of cardiac output ($L \cdot min^{-1}$) and the arteriovenous oxygen difference (a-vO_2)[5]. CO can be further broken down to the product of stroke volume ($mL \cdot beat^{-1}$) and heart rate ($beats \cdot min^{-1}$).

A. $VO_2 = CO \cdot a - vO_2$ B. $CO = SV \cdot HR$

Formula's A-B: The Fick Equation. Cardiac Output (CO). Arteriovenous Oxygen Difference (a-vO_2). Stroke Volume (SV). Heart Rate (HR).

The left side of the Fick equation is considered central hemodynamics as depicted in Figure 1, and represents the hearts ability to pump blood[56]. The right side of the equation is considered peripheral physiology as depicted in Figure 2, and represents the ability of the human body, particularly skeletal muscle, to extract and utilize oxygen from the blood to make ATP[56].

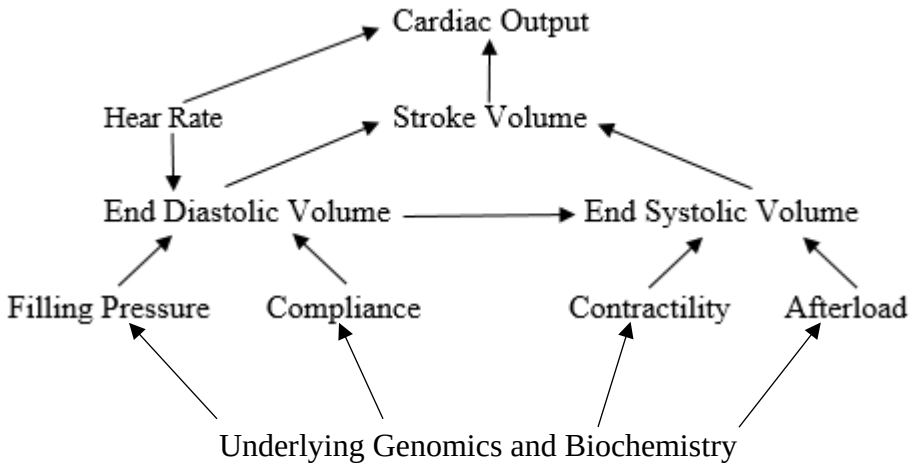


Figure 1: Central Hemodynamics of VO_2. Adapted from Myers & Froelicher[56].

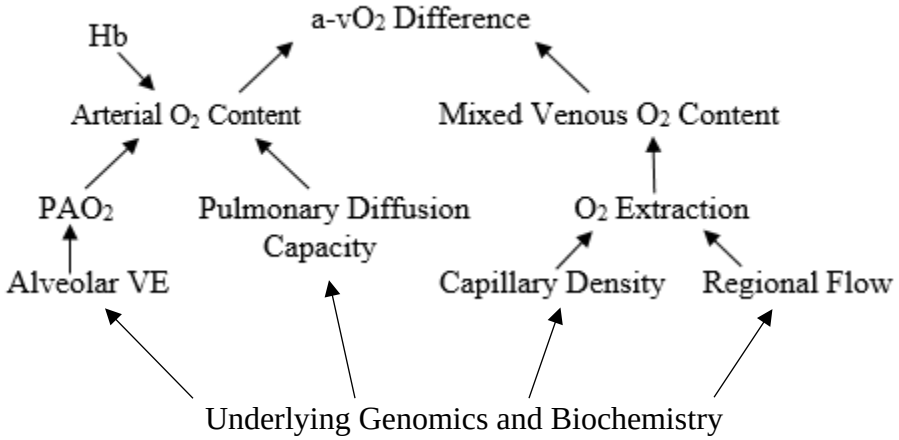


Figure 2: Peripheral Physiology of VO_2. Partial Pressure of Arterial Oxygen (PAO_2). Ventilation (VE). Adapted from Myers & Froelicher[56].

Therefore, a genomic or biochemical dysfunction which alters any of the CO or a-vO_2 components could change VO_2, and provide the components of VO_2 to investigate for underlying genomic and biochemical signatures. The data available for this thesis contains both a genomic variant and biochemical products of the renin-angiotensin system (RAS), which may impact VO_2max if the system is more or less active due to a combination of an individual's angiotensin converting enzyme (ACE ID) genotype and plasma levels of the biologically active RAS products angiotensin II (Ang II) and angiotensin 1-7 (Ang1-7).

VO_2max and RAS System Components

For the RAS to impact VO_2max it would presumably need to alter one or more of the VO_2 components depicted in Figures 1 and 2. This may be possible because research with animal and human subjects suggests the RAS plays a role in both pathologic and protective cardiovascular processes[33,49,74]. Two axes in the RAS system have been identified and are presumed to oppose one another. Figure 3 shows the components of both axes, with the cardiovascular pathogenic axis of the RAS system consisting of the ACE, Ang II, and the angiotensin II type I receptor (AT_1), and the cardiovascular protective axis of the RAS system consists of the angiotensin converting enzyme 2 (ACE2), Ang(1-7), and the Mas receptor [30,74].

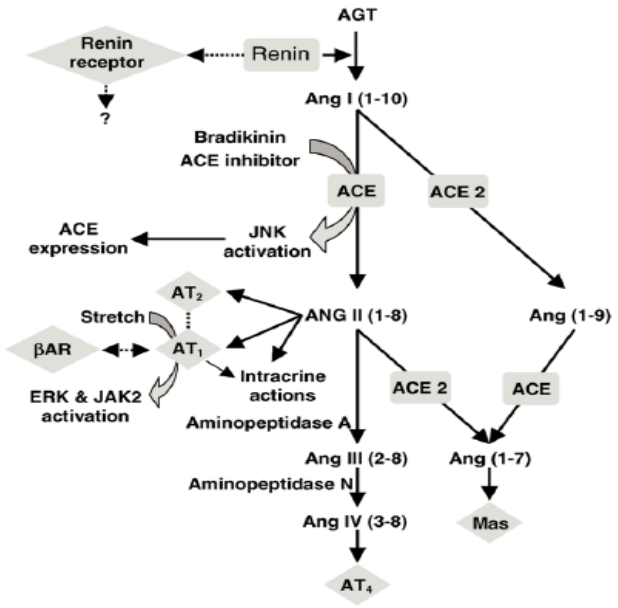


Figure 3: The RAS. Reprinted from Kurdi et al.,[42].

Normally, these axes balance each other to help maintain cardiovascular homeostasis (Figure 4), however, it seems plausible the VO_2 components of cardiac output and a-vO_2 difference, particularly, compliance and contractility of heart muscles, afterload, and regional flow could be effected if the axes are out of balance and favor the pathogenic pathway. As can been seen in Figures 3 and 4, Ang II and Ang 1-7 are the RAS components that act on either the AT_1 or Mas receptor, and the downstream processes may impact VO_2max.

.

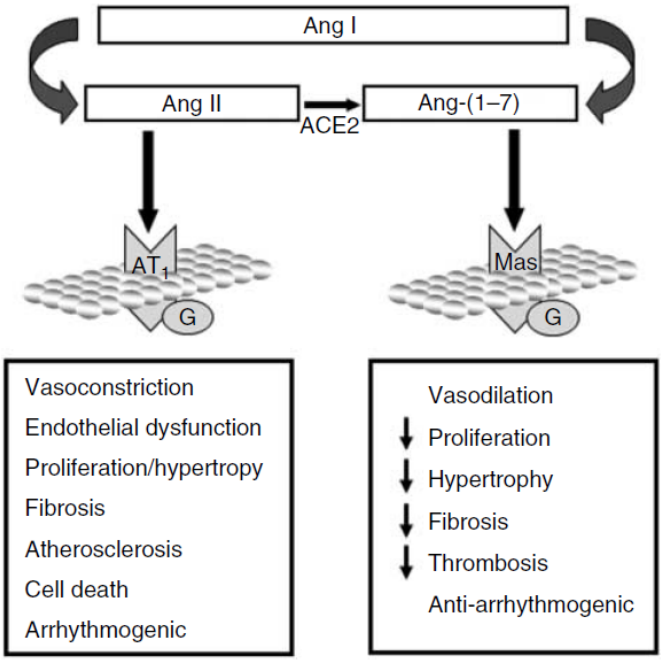


Figure 4: RAS Axis and Cardiovascular Effects.
Reprinted from Santos et al.[65].

Angiotensin II

Evidence suggests Ang II is involved in many pathological processes in cardiac tissue when cardiovascular homeostasis is disrupted, such as, myocyte hypertrophy, myocyte gene reprogramming, fibroblast proliferation, and extra-cellular matrix (ECM) protein accumulation (Figure 5)[33,49]. If so, then individuals with overactive Ang-II, meaning either higher tissue and plasma levels or increased binding affinity to AT_1Rs, may have lower VO_2max values due to a less functional cardiorespiratory fitness phenotype. However, this assertion is speculative, since most of the mechanistic evidence for the involvement of Ang II in cardiovascular pathology comes from cultured rat cells and animal models [30,43].

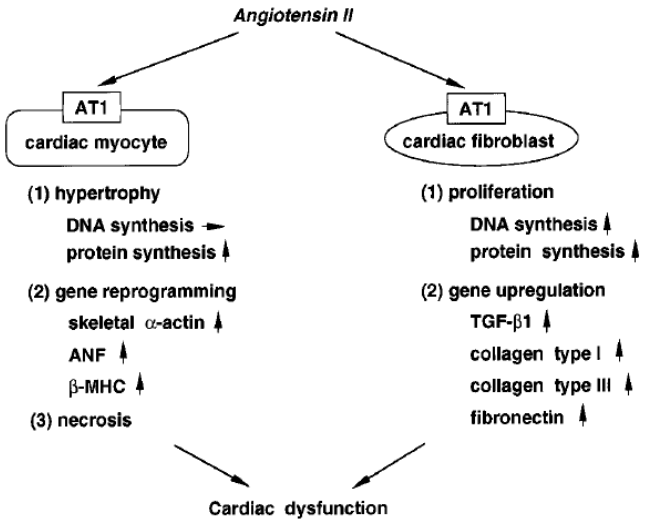


Figure 5: Proposed mechanism of AT_1R mediated pathological cardiac hypertrophy Reprinted from Kim & Iwao[33].

Angiotensin 1-7

The other biologically active component of the RAS is Ang 1-7, which appears to oppose the vasoconstrictor, proliferative, and proinflammatory actions of Ang II through Mas receptor via release of NO and prostaglandins – as seen in isolated rat heart [4,72]. Ang 1-7 levels may also effect heart contractile function as it improved post ischemic contractile function in rat hearts mediated through some Mas invoked release of bradykinin and prostaglandin mechanism, and increased cardiac output and stroke volume and decreased total peripheral resistance in anesthetized rats receiving an Ang 1-7 infusion compared to controls [23,72]. Furthermore, Ang 1-7 has been shown to counteract the profibrotic actions of Ang II by binding to isolated rat cardiac fibroblasts to inhibit the synthesis of ECM collagen proteins[32]. Lastly, in Mas receptor knockout mice cardiac function was significantly impaired and collagen type I and III and fibronectin synthesis increased significantly compared to genetically normal mice[73]. Collectively, these results suggest an

impairment in the ACE2/Ang1-7/Mas axis could lead to a lower VO_2max value suggesting impaired cardiorespiratory fitness.

Rationale for plasma RAS variables comes from their role in either the pathogenic or protective RAS axis, while, the ACE genotype point distribution rationale comes from its role in the pathogenic axis, endurance performance and VO_2max association studies, and early evidence of its association with plasma ACE activity. Table 1 provides details to many of the association studies in a review by Puthucheary et al.,[64] along with other studies from the literature.

The RAS, Birth Weight, and Epigenetics

Epidemiological evidence has established low birthweight as a risk factor for atherosclerosis, hypertension, stroke, and Type 2 diabetes in adulthood, however, the underlying pathophysiological mechanisms resulting in these outcomes are not fully understood[25]. It may be that low birth weight individuals have an altered and overactive RAS persisting into adulthood and playing a role in later cardiovascular events. Miyawaki et al.[52] found small-for-gestational age infants to have increased plasma Ang II levels compared to normal birth weight subjects, and Forsyth et al.[24] reported a significant negative association between ACE activity and birthweight at 3 months after adjustment for maternal age and placental weight. However, Washburn et al.[83] did not find any differences in circulating Ang II, Ang (1-7), and the Ang II/(1-7) ratio in male and female adolescents born with very low birth weight.

Another contributing factor to long-term health outcomes and lower fitness and physical activity levels in preterm individuals could be caused in part by epigenetic

modifications leading to changes in gene expression that are maintained throughout the lifespan[63]. Association studies on a genome-wide scale have found numerous DNA methylation differences in preterm neonates involving genes related to embryonic development, the extra-cellular matrix, and contractions and labor[37]. The epigenetic link to decreased aerobic fitness and physical activity is unclear, however, it is plausible that genes involved with VO_{2max} and physical function could have altered expression levels due to the altered developmental environment and subsequent differences in DNA methylation.

ACE I/D Polymorphism and Cardiorespiratory Fitness

Twenty-seven studies were reviewed by Puthucheary et al.,[64] with the rs4340 ACE insertion-deletion polymorphism (ACE I/D) and endurance and/or short distance performance and VO_2max. The cohorts included in the review cover a variety of sports, such as cyclists, rowers, swimmers, triathletes, track and field participants, and climbers. Of the 27 studies, the D allele or D/D genotype was associated with endurance performance in 3 studies [6,41,48], short distance performance in 7 studies [14,15,16,20,58,82,84], and VO_2max in one study[85]. The I allele or I/I genotype was associated with endurance performance in 9 studies [14,17,19,27,31,51,53,57,82] and VO_2max in 1 study[24]. Five studies did not find and an association between an ACE allele or genotype and performance of any type [26,62,66,77,78]. Not included in the review by Puthucheary et al., the HERTIAGE Family Study[64] did not detect an association between measured baseline VO_2max and ACE genotype (II, I/D, or DD) involving 476 Caucasian and 248 Black subjects, while a study by Hagberg et al.[29] found ACE I/I genotype carriers had a 6.3 and 3.3 $ml \cdot kg^{-1} \cdot min^{-1}$ higher VO_2max than individuals with the D/D and I/D genotype in 58 postmenopausal women after adjustment for physical activity levels. However, when the group of women was stratified into

sedentary, physically active, and athletes, no VO_2max differences were detected across genotypes. Interestingly, in the study by Hagberg et al.,[29] ACE genotype accounted for 17% of the variance in a-vO_2 difference, but did not affect maximal cardiac output or stroke volume indicating ACE genotype may play a role in skeletal muscles ability to uptake oxygen. Hagberg et al.[29] postulate this could be due to lower peripheral vascular tone, increased capillary perfusion, and faster red blood cell transit time in the ACE I/I group compared to ACE I/D and D/D genotypes, thus enabling the skeletal muscles to uptake oxygen at a higher rate leading to a higher measured VO_2max.

Two studies examined the relationship between ACE I/D genotype and aerobic fitness in adolescents. Mäestu et al.[50] did not find any association with ACE genotype and cardiovascular fitness in 314 twelve-year-old boys using a stepwise incremental maximal exercise test on an electronically braked cycle ergometer, and Kim et al.[34] did not find any association with ACE genotype and physical efficiency index from the Harvard step test in 856 elementary school students with a mean age of 10.3 ± 0.07 years. Mäestu et al.[50] was also the only study that examined the relationship between ACE I/D genotype and habitual physical activity. They found individuals with the ACE D/D genotype to engage in significantly more total minutes of physical activity per day compared to I/I genotype individuals (382 ± 8 vs 354 ± 9 min/day).

Comparing term and preterm individuals, a study by Clemm et al.[18] did not find any differences in VO_2Peak or VO_2Peak% Predicted in two cohorts of preterm (≤ 28 weeks gestation and birth weight ≤1000 g) and term subjects. However, Smith et al.[79] reported greater VO_2Peak in term subjects compared to preterm (<32 weeks gestation and birth weight <1000 g), and Nixon et al.[59] reported approximately half of children 8-to-11 years

old born at very low birth weight (birth weight <1501 g) had VO_2Peak values less than 80% of age and sex predicted values. Comparing physical activity participation between term and preterm individuals, Saigal et.[71] reported people born at extremely low birth weight participated less in sports/strenuous activities as young adults compared to normal birth weight subjects. Similarly, Rogers et al.[69] compared aerobic capacity and activity level in extremely low birth weight (birth weight ≤800 g) and normal birth weight adolescents approximately 17 years of age and found extremely low birth weight subjects had lower aerobic fitness and sports participation scores compared to term subjects. Therefore, this study will add to the existing literature comparing aerobic fitness and physical activity participation in preterm and term subjects.

In addition to ACE I/D performance and endurance genotype evidence, Rigat et al.[68] who demonstrated the ACE I/D accounted for approximately 47% of the variance in serum ACE levels of 80 healthy Caucasian males (n=38) and females (n=42). Individuals with D/D genotypes (n=29) had twice as much ACE activity as the I/I genotype (n=14), and ACE activity for I/D genotype (n=37) fell between the two. Therefore, it is possible that individuals with the D/D genotype could have a more active RAS system, with higher levels of Ang II.

Table 1: Studies of Human Performance And ACE insertion-deletion Polymorphism

Study	Cohort	Subjects and Ethnicity	Association with Performance/Prevalence	Allele & Genotype Associations
Amir et al.[6]	Swimmers	121 Israeli	Yes	D and Endurance
Cam et al.[14]	Sprinters	88 Caucasian	Yes	D and Short Distance
Cerit et al.[16]	Army	186 Caucasian	Yes	D and Short Duration
Lucia et al.[46]	Cyclists	50 Caucasian	Yes	D/D and Endurance
Munisea et al.[48]	Mixed	141 Mixed	Yes	D/D and Endurance Rowers
Nazarov et al.[58]	Mixed	217 Caucasian	Yes	D/D and Short Distance
Costa et al.[20]	Swimmers	72 Caucasian	Yes	D/D and Short Distance
Woods et al.[84]	Swimmers	102 Caucasian	Yes	D/D and Short Distance
Zhao et al.[85]	Army	67 Chinese	Yes	D/D and VO_2max
Gayagay et al.[27]	Rowers	64 Caucasian	Yes	I and Endurance
Myerson et al.[57]	Runners	91 Caucasian	Yes	I and Endurance
Montgomery et al. [53]	Army	78 Caucasian	Yes	I and Endurance
Collins et al.[19]	Triathletes	166 Caucasian	Yes	I and Endurance
Hruskovicova et al.[31]	Runners	445 Caucasian	Yes	I and Endurance
Cieszczyk et al.[17]	Rowers	55 Caucasian	Yes	I and Endurance
Min et al.[51]	Track/Field	277 Japanese	Yes	I and Endurance
Tsianos et al.[82]	Swimmers	35 Caucasian	Yes	I and Endurance D and Short Distance
Cam et al.[14]	Runners	55 Caucasian	Yes	I and Endurance D and Short Distance
Goh et al.[24]	Rugby	17 Singaporean	Yes	I/I and VO_2max
Rankinen et al.[66]	Mixed	192 Caucasian	No	I/I and D/D Endurance
Scott et al.[77]	Sprinters	230 African Americans	No	I/I and D/D Sprint
Scott et al.[78]	Runners	271 Africans	No	I/I and D/D Endurance
Papadimitriou et al.[62]	Track/Field	101 Greek	No	I/I and D/D in Athletes

Table 1 continued.

Study	Cohort	Subjects and Ethnicity	Association with Performance/Prevalence	Allele & Genotype Associations
Frederiksen et al.[26]	Elderly	203 Caucasian	No	I/I and D/D VO_2max
Studies not in Puthucheary Review				
Bouchard et al.[9]	Sedentary	724 Mixed	No	I/I and D/D VO_2max
Hagberg et al.[29]	Mixed	58 NR	Yes	I/I and Endurance
Bueno et al.[13]	Active	150 NR	No	I/I and D/D VO_2max
Almeida al.[2]	Active	57 Brazilian	Yes	I/I and VO_2max and Endurance
Roltsch et al.[70]	Mixed	77 Mixed	No	I/I and D/D VO_2max
Mäestu et al[50]	Adolescents	314 Estonia	Yes No	D/D and Total PA I/I and D/D CVF
Kim et al.[34]	Adolescents	856 Korean	No	I/I and D/D PEI

D = Deletion; I = Insertion; VO_2max = Maximal Oxygen Consumption

Table adapted from Puthucheary et al.,[57]. Physical Activity (PA). Cardiovascular Fitness (CVF). Harvard Step Test Physical Efficiency Index (PEI).

Overall, the results between ACE genotype, endurance performance, and cardiorespiratory fitness are mixed, and there is very little evidence examining the relationship between ACE genotype and cardiorespiratory fitness in adolescents. Collectively, the VO_2max aggregation and heritability, health, non-responder, and ACE I/D association data and rationale, as well as, the potentially pathologic actions of Ang II and protective actions of Ang 1-7 for the cardiovascular system, provides some evidence that it would be beneficial to determine the underlying genomic and biochemical components of VO_2max. To potentially develop ways to alter these components for individuals who are non-responders to aerobic training and have low cardiorespiratory

fitness, in order to possibly reduce their all-cause mortality and development of cardiovascular disease risk.

Purpose

The primary aims of this study were to determine if ACE genotype was associated with cardiorespiratory fitness and plasma RAS variables, and to establish if cardiorespiratory fitness and plasma RAS concentrations differed between term and preterm subjects. Secondary aims were to determine if cardiorespiratory fitness was associated with RAS dominance, as well as, if plasma RAS variables and habitual physical activity were correlated with cardiorespiratory fitness in term and preterm subjects.

It was hypothesized that:

1) Term subjects would have higher cardiorespiratory fitness compared to preterm subjects, and the ACE I/I genotype would have higher cardiorespiratory fitness, lower plasma Ang II and plasma Ang II/(1-7) ratio, and higher plasma Ang (1-7) compared to I/D and D/D genotypes in term and preterm subjects.

2) Plasma Ang II and Ang II/(1-7) ratio would be inversely correlated with cardiorespiratory fitness, and plasma Ang (1-7) would be positively correlated with cardiorespiratory fitness.

3) The RAS Ang 1-7 dominant subjects would have higher cardiorespiratory fitness compared to neutral and RAS Ang II dominant subjects in term and preterm individuals.

METHODS

Participants

One-hundred and eighty-one subjects, 133 preterm and 48 term, has ACE genotype data and were in genotype analysis. Of the one-hundred and eighty-one subjects, one-hundred and thirty-two, 91 preterm and 41 term, had plasma RAS data and were included in RAS analysis. All subjects were part of the first Prenatal Exposure Postnatal Consequences (PEPC1) study and born at Forsyth Medical Center in Winston-Salem, NC between 1992 and 1996. Inclusion criteria for T infants consisted of 1) 14yrs of age or in 15th year of life, 2) normal birth weight >2500 g, 3) singleton birth with no major congenital anomalies, 4) no antenatal exposure to glucocorticords, and 5) cognitive and physical ability to participate. Inclusion criteria for PT infants were 1) 14yrs of age or in 15th year of life, 2) singleton birth without a major congenital anomaly, 3) clinical evaluation at one-year adjusted age, and 4) cognitive and physical ability to participate[59].

Fitness Measurement

A cycle ergometer test, using an electronically braked Corival ergometer, and following the Godfrey protocol was used to obtain peak oxygen uptake (VO_2Peak) for all subjects[59]. VO_2Peak was determined instead of VO_2max because 50% of children can fail to reach a plateau in oxygen consumption[7]. A possible explanation for the lack of a plateau in adolescent is anaerobically they can't sustain a work rate corresponding to their VO_2max, thus they fatigue once their aerobic capacity reaches its maximal work rate[39]. For this protocol, the work load was set at "0" watts for the first minute and increased by 10, 15 or 20 watts every minute based on the child's height (<125, 125-150, and >150cm). Ten

electrodes were attached to the participant's chest for continuous monitoring of a 12 lead ECG from which heart rate was determined each minute and at peak exercise. Expired gases were collected by a CPX Med Graphic metabolic cart (St. Paul, MN), and the highest 15-second average of oxygen uptake was used to reflect their VO_2Peak ($ml \cdot kg^{1} \cdot min^{-1}$) as a measure of cardiorespiratory fitness. Verbal encouragement was provided for all participants to give a maximal effort. Three criteria were used to determine if the effort was considered maximal; 1) peak heart was >195 beats per minute, 2) a peak respiratory exchange ratio >1.05 was attained, and/or 3) agreement among two experienced testers that the subject gave a maximal effort. If two of three criteria were met, the test was considered a maximal effort. VO_2Peak% predicted values were determined from sex- and age-specific reference values from Krahenbuhl et al.[39].

Habitual Physical Activity

Habitual leisure time PA was assessed using the Modifiable Activity Questionnaire (MAQ), the validity of which, has been demonstrated in children and adolescents[1]. The MAQ questionnaire was administered by a trained tester. The participant (with parent present to assist) was read a list of activities and the adolescent reported which activities the he/she participated in at least 5 times during the past 12 months. Additional activities not listed could be added, and for all activities number of months in the past year, average number of days per month, and the average duration for each day were reported. Average total hours per week (Tot-hrs/wk), metabolic equivalent (MET) hours per week (MET-hrs/wk), and time spent in vigorous activity hours per week (Vig-hrs/wk > 6 METs) for the reported activities were determined by summing the average hours per week of all activities

for each variable[59]. MET values for reported activities were determined using the Ainsworth compendium of physical activities[3].

ACE I/D Genotyping

ACE I/D genotypes (rs4340) were determined via polymerase chain reaction amplification products using 5'-CTGGAGACCACTCCCATCCTTTCT-3'AND 5'-GATGTGG CCATCACATTCGTCA GA-3' sense and antisense primer sequences. Amplified PCR product sequences were visualized on 2% agarose gels using electrophoresis to distinguish between an I allele 490-base pair band and D allele 190-base pair band to determine ACE genotype for each subject[41].

Plasma RAS Variables

Random non-fasting venous blood samples were collected via venipuncture in an EDTA tube with protease inhibitors and was centrifuged for plasma removal. Random means no pre-sample instructions followed by the subjects. Plasma was extracted with and applied to Sep-Pak columns, then washed and eluted. Ang II and Ang (1-7) were measured via separate radioimmunoassays of completely evaporated and reconstituted eluent in an assay buffer. 2.8 pmol/L and 0.8 pmol/L were the minimum detectable levels for Ang (1-7) and Ang II. Details of the protease inhibitors, Sep-Pak columns, washing, and eluent mixtures as described by Washburn et al.[83].

RAS Score

The rational for creating a RAS score is based on the SNP score approach used in genomic studies. In these studies, researchers place a point value on each allele or genotype based on its proposed positive, neutral, or negative impact[12]. This allows researchers to

look at the overall effect of a number of gene in combination, which will mostly likely have a larger effect than a single gene. Here, a RAS score was determined for each subject to determine if the combination of RAS genomic and biochemical components resulted in differences in cardiorespiratory fitness among Ang II dominant, neutral, and Ang (1-7) dominant subjects, because of the potential cardio protective and pathogenic actions of Ang (1-7) and Ang II summarized in the literature review. The RAS score was calculated by assigning a point value to the ACE I/D genotype and plasma RAS variables based on their presumed favorable, neutral, or non-favorable role in the RAS. Low, medium, and high percentile categories were created for plasma RAS variables based on percentile rank of the total group. The assigning of points went as follows and can be seen in figure 7: one point was awarded for each of following criteria met – ACE I/I genotype, <33rd% for Ang II, >66th% for Ang (1-7), and <33rd% for the Ang II/(1-7) ratio. No points were awarded for ACE I/D genotype and being in the 33rd-66th% percentile for any of the plasma RAS variables. A point was subtracted if the subject was an ACE D/D genotype, above the 66th% for Ang II, below the 33rd% for Ang (1-7), and above the 66th% for the Ang II/(1-7) ratio. The points were summed to determine the RAS score for each subject. A maximal possible score was 4, indicating an Ang (1-7) dominant RAS, and minimum possible score was -4, indicating an Ang II dominant RAS. Subjects with RAS scores >1 were categorized as RAS Ang (1-7) dominant, -1 to 1 as neutral RAS, and < -1 as RAS Ang II dominant.

RAS Scoring

Variable, (%)	1 Point	0 Point	-1 Point
ACE Genotype	I/I	I/D	D/D
Plasma Ang II	<33rd	33-66	>66th
Plasma Ang 1-7	>66th	33-66	<33rd
Plasma Ang II/1-7	<33rd	33-66	>66th

Figure 6: RAS Scoring System.

Statistical Analysis

SPSS version 24.0 was used to perform all statistical tests. Descriptive statistics were run to determine measures of central tendency and dispersion. Measures of normalcy and equality of variances were determined by Shapiro-Wilk and Levene statistics, with the p-value set at $p<.05$. Significantly skewed distributions were transformed with natural log transformations. Chi square tests were used to determine if the observed ACE genotype proportions by gender and race differed from expected in term and preterm subjects. Participant characteristics of age, height, weight, and BMI mean differences were tested using between group (ACE genotype) one-way analysis of variance (ANOVA) and stratified by birth status (PT vs. T). Cardiorespiratory fitness, habitual physical activity, and plasma RAS variables main effects for birth status and ACE genotype were tested using 2-way Analysis of Variance (ANOVA), and bonferroni post hoc tests were carried out to test for differences between ACE genotypes. RAS score mean differences in all, term, and preterm groups were tested using between groups (RAS Score) one-way ANOVA's. The alpha level was set at $p<.05$ for all tests. Plasma RAS variable and cardiorespiratory fitness associations were determined with Pearson correlation coefficients. Significance was set at a p-value of $p<.05$.

RESULTS

As shown in Figure 7, of the initial 240 participants 40 were excluded due to a symptom limited, technical problem, or did not perform the maximal exercise test. Of the remaining 200 participants, 19 were excluded because they did not have ACE genotype data leaving 181 participants available for ACE genotype association analysis. Of the remaining 181 participants, 49 were excluded from plasma RAS analysis because they did not have data, leaving 132 participants available plasma RAS analysis.

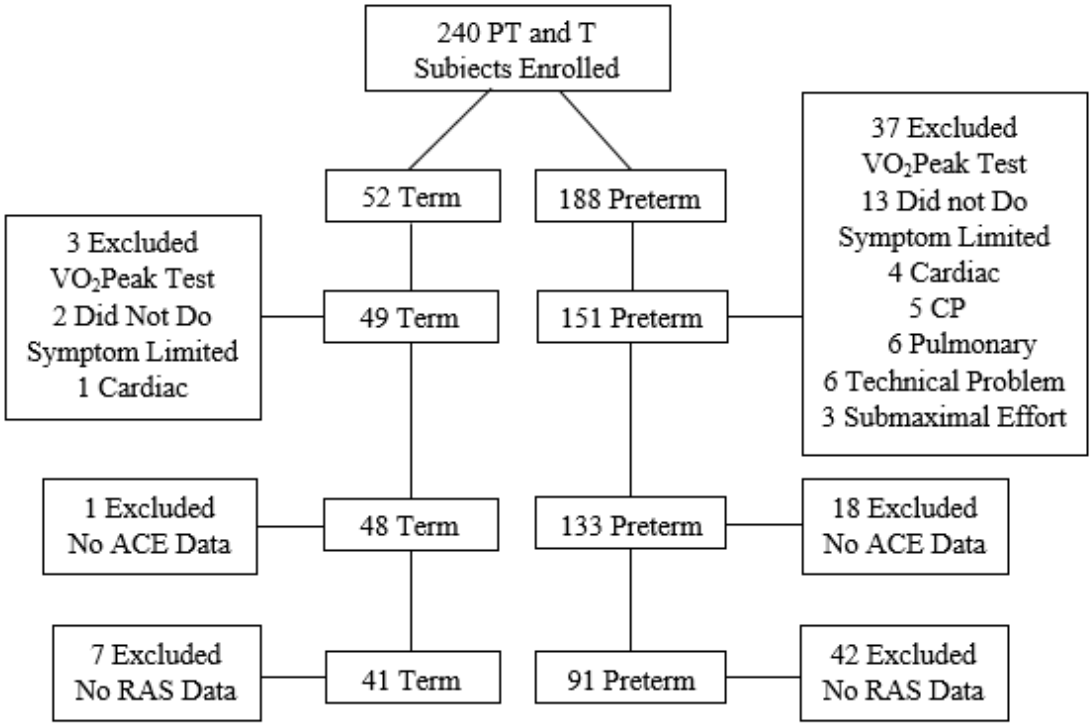


Figure 7. Flow Chart of Subject Inclusion adapted from Nixon et al.,[53].

Participant Characteristics

Table 2 provides the participant characteristics by term and ACE genotype for the 48 T and 133 PT subjects. ACE genotype proportions were statically different for term (p=.02 and p=.01), but not preterm, gender and race groups compared to expected. For gender, fewer than expected I/I genotype males and more than expected I/I genotype females contributed the most to the chi square value. For race, fewer than expected I/I genotype non-blacks and more than expected I/I genotype blacks contributed most to the chi square value. There were no significant mean differences in age, height, weight, or BMI among term and preterm subjects by ACE genotype.

Table 2. Participant Characteristics by preterm and ACE genotype expressed as Mean ± SD or n (%).

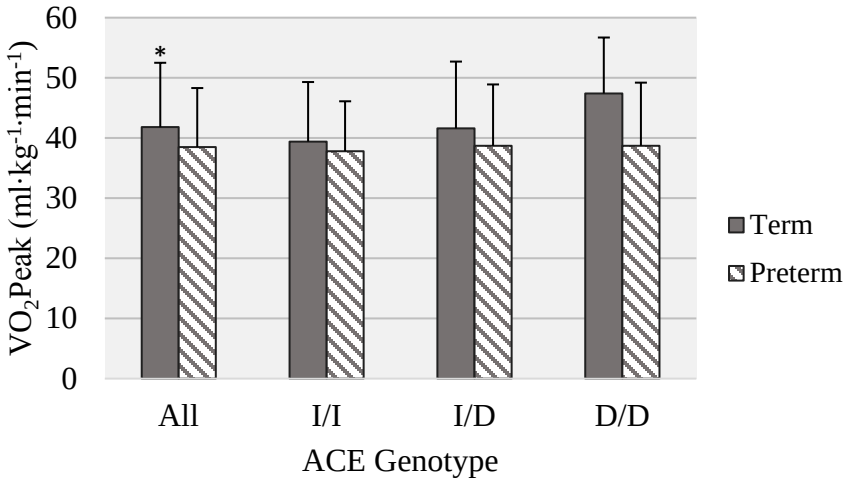
	Term ACE Genotype			
Variables	I/I (n=11)	I/D (n=31)	D/D (n=6)	p-value
Male, n (%)	1 (9)	16 (52)	4 (67)	.02*
Non-Black, n (%)	3 (27)	20 (65)	6 (100)	.01*
Age, yrs	14.5 ± 0.3	14.6 ± 0.3	14.5 ± 0.3	.43
Height, cm	165 ± 5.5	169 ± 6.7	165 ± 10.8	.24
Weight, kg	59.7 ± 7.5	66.8 ± 17.8	58.3 ± 5.3	.25
BMI, $kg \cdot m^{-2}$	21.9 ± 2.7	23.3 ± 6.0	21.4 ± 3.1	.57
	Preterm ACE Genotype			
Variables	I/I (n=32)	I/D (n=61)	D/D (n=40)	p-value
Male, n (%)	14 (45)	19 (31)	17 (43)	.33
Non-Black, n (%)	19 (61)	34 (56)	24 (60)	.85
Age, yrs	14.5 ± 0.3	14.5 ± 0.3	14.5 ± 0.3	.12
Height, cm	163 ± 8.5	160 ± 7.9	162 ± 9.1	.25
Weight, kg	59.7 ± 12.8	60.1 ± 18.6	57.1 ± 15.4	.65
BMI, $kg \cdot m^{-2}$	22.5 ± 5.1	23.2 ± 6.3	21.4 ± 5.0	.32

*p<.05. Percentages are within ACE genotype (Term or Preterm).

Cardiorespiratory fitness and Physical Activity

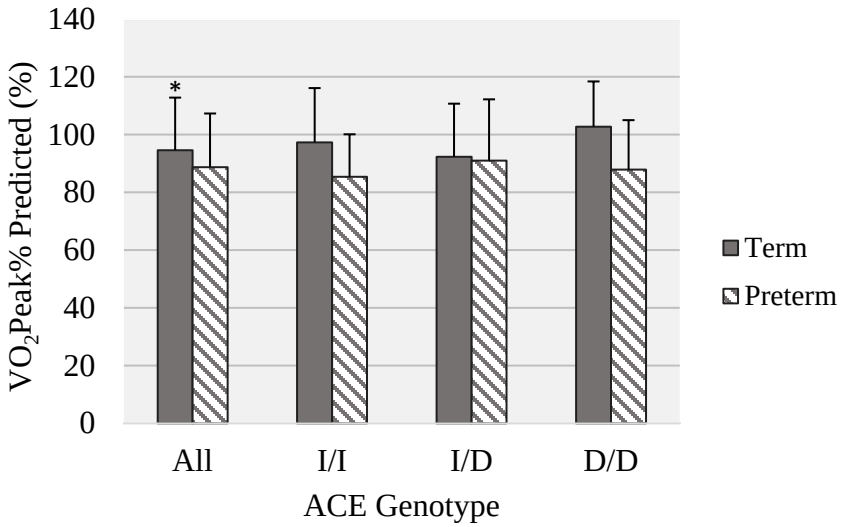
Table 3 and Figure 7 present the cardiorespiratory fitness and physical activity by ACE genotype data, stratified into term and preterm groups. All of the cardiorespiratory fitness variables were normally distributed according to Shapiro-Wilk tests, p>.05. A two-way ANOVA found a significant main effect for birth status, with the term group having significantly higher VO_2Peak (41.8 ± 10.7 vs 38.5 ± 9.8 $ml \cdot kg^{-1} \cdot min^{-1}$) and VO_2Peak% predicted (94.8 ± 18.2 vs 88.7 ± 18.6) compared to the preterm group (F= 4.70, df=1, p=.03) and (F=6.2, df=1, p=.01), but did not find a significant main effect for ACE genotype or an interaction between birth status and ACE genotype.

Physical activity data were not normally distributed according to Shapiro-Wilk tests, p<.05, therefore natural log transformation were performed. Two-way ANOVAs found no significant main effects for birth status or ACE genotype for average total weekly physical activity or average MET hours per week, but did find a main effect for average vigorous activity perk week, with the term group engaging in more vigorous hours of PA per week on average (4.4 ± 4.6 vs 3.0 ± 4.8) compared to the preterm group (F=5.0, df=1, p=.03)



*p<.05 for Term vs. Preterm comparison.

Figure 8. VO_2Peak and ACE Genotype Expressed as Mean ± SD



*p<.05 for Term vs. Preterm comparison.

Figure 9. VO_2Peak% Predicted and ACE Genotype Expressed as Mean ± SD

Table 3. Cardiorespiratory Fitness and Habitual Physical Activity expressed as Mean ± SD

	Term ACE Genotype			
Variables	I/I (n=11)	I/D (n=31)	D/D (n=6)	p-value
VO_2Peak, $ml \cdot kg^{-1} \cdot min^{-1}$	39.4 ± 9.9	41.6 ± 11.1	47.4 ± 9.3	.34
VO_2Peak% Predicted	97.3 ± 18.8	92.3 ± 18.4	102.7±15.7	.39
Tot-hrs/wk	10.5 ± 6.1	10.4 ± 6.6	7.6 ± 3.8	.71
MET-hrs/wk	65.4 ± 40.3	61.6 ± 44.3	44.1 ± 26.0	.64
Vig-hrs/wk	5.3 ± 5.0	4.4 ± 4.7	2.9 ± 3.0	.67
	Preterm ACE Genotype			
Variables	I/I (n=32)	I/D (n=61)	D/D (n=40)	p-value
VO_2Peak, $ml \cdot kg^{-1} \cdot min^{-1}$	37.7 ± 8.4	38.7 ± 10.3	38.7 ± 10.5	.90
VO_2Peak% Predicted*	85.6 ± 14.9	91.0 ± 21.1	87.9 ± 17.1	.40
Tot-hrs/wk	8.2 ± 8.6	12.1 ± 9.7	13.6 ± 17.3	.05
MET-hrs/wk	44.5 ± 51.0	60.9 ± 50.2	73.7 ± 95.6	.06
Vig-hrs/wk	2.4 ± 4.1	2.6 ± 4.1	3.8 ± 5.5	.34

*VO_2Peak% predicted values are based on sex- and age-specific reference values from Krahenbuhl, Skinner, & Kohrt[34]. Physical activity p-values are for log transformed one-way ANOVAs.

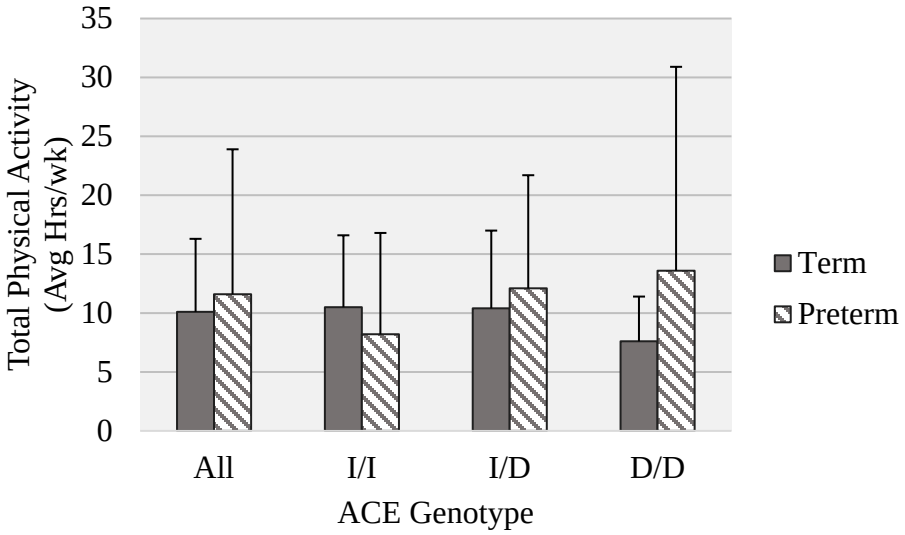


Figure 10. Average Weekly Physical Activity and ACE Genotype Expressed as Mean ± SD

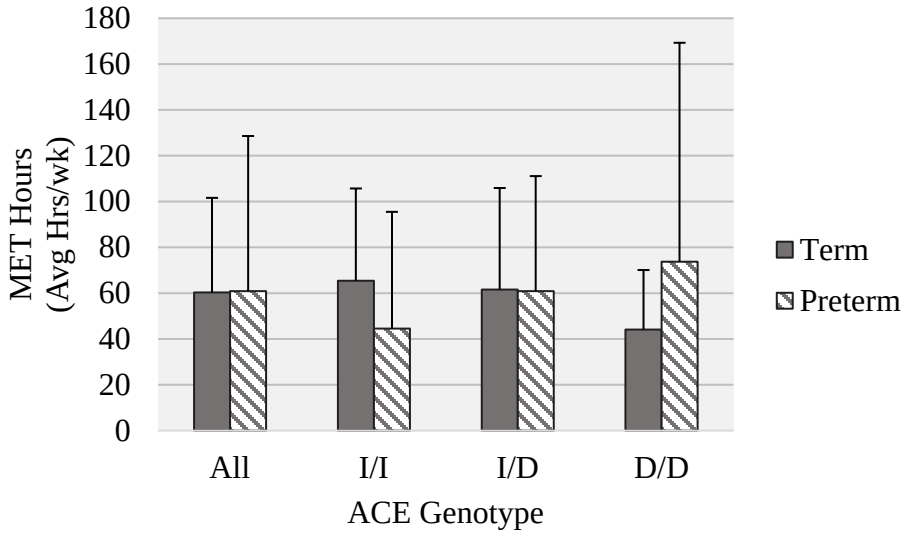
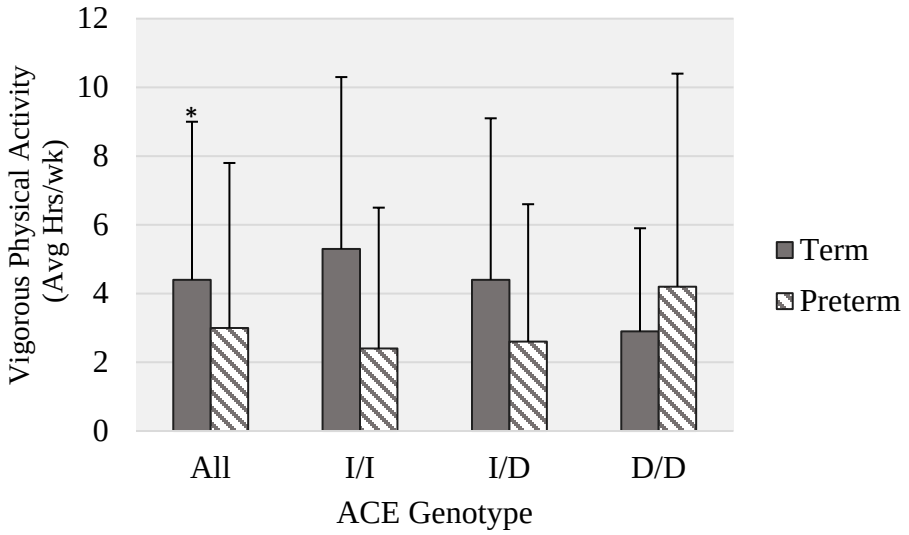


Figure 11. Average MET Hours per Week of Physical Activity and ACE Genotype Expressed as Mean ± SD



*p<.05 for Term vs. Preterm comparison.

Figure 12. Average Vigorous Hours per Week of Physical Activity and ACE Genotype Expressed as Mean ± SD

Plasma Angiotensin (II and 1-7)

Table 4 and Figure 9 present the plasma RAS variables by ACE genotype data stratified into term and preterm groups. All of the plasma RAS variables were not normally distributed according to Shapiro-Wilk tests, $p<.05$, therefore natural log transformations were performed. Two-way ANOVAs found no significant main effects for plasma RAS variables Ang II or ACE genotype for Ang II, Ang (1-7), and the Ang II/(1-7) ratio, as well as, no interaction between birth status and ACE genotype for any of the plasma RAS variables - indicating ACE genotype does not seem to play a significant role in plasma Ang II and Ang (1-7) concentrations and birth status does not affect plasma Ang II concentrations. However, significant main effects were found for Ang (1-7) and the Ang II/(1-7) ratio between term and preterm groups, ($F=18.5$, $df=1$, $p<.01$) and ($F=13.9$, $df=1$, $p<.01$), indicating on average term individuals have higher plasma Ang (1-7) concentrations and lower Ang II/(1-7) ratios.

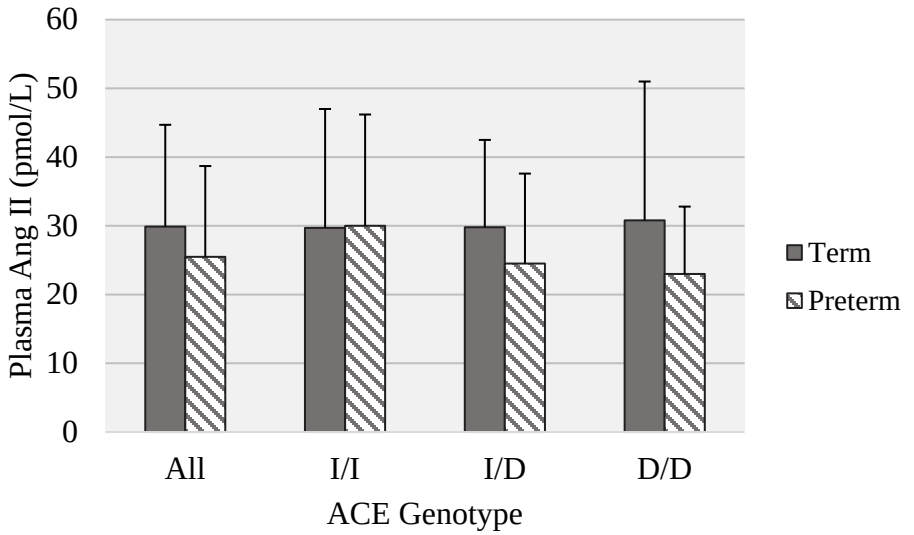
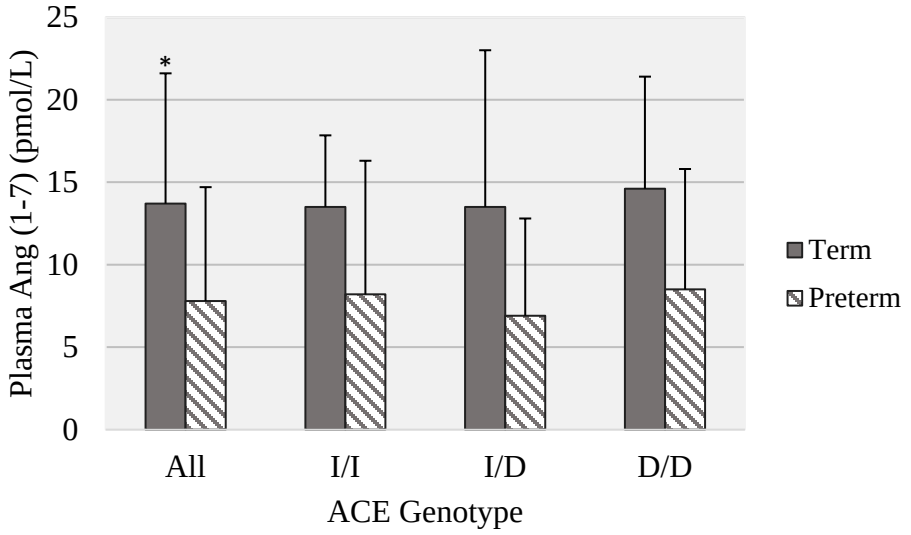
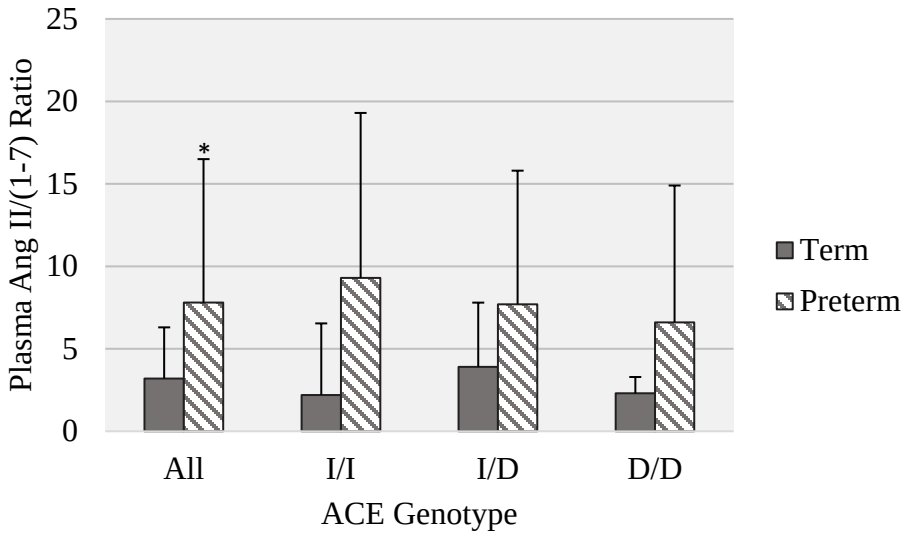


Figure 13. Plasma Angiotensin II and ACE Genotype Expressed as Mean ± SD



*p<.05 for Term vs. Preterm comparison.

Figure 14. Plasma Angiotensin II and ACE Genotype Expressed as Mean ± SD



*p<.05 for Term vs. Preterm comparison.

Figure 15. Plasma Angiotensin II/(1-7) Ratio and ACE Genotype Expressed as Mean ± SD

Table 4. Plasma angiotensin (II and 1-7) values presented as Mean ± SD

	Term ACE Genotype			
Variables	I/I (n=11)	I/D (n=24)	D/D (n=6)	p-value*
Ang II, pmol/L	29.7 ± 17.3	29.8 ± 12.7	30.8 ± 20.2	.95
Ang (1-7) pmol/L	13.5 ± 4.3	13.5 ± 9.5	14.6 ± 6.8	.55
Ang II/(1-7)	2.2 ± 1.3	3.9 ± 3.7	2.3 ± 0.99	.38
	Preterm ACE Genotype			
Variables	I/I (n=24)	I/D (n=37)	D/D (n=30)	p-value*
Ang II, pmol/L	29.4 ± 16.1	24.5 ± 13.1	23.0 ± 9.9	.21
Ang (1-7), pmol/L	8.1 ± 8.0	6.9 ± 5.9	8.5 ± 7.3	.64
Ang II/(1-7)	9.1 ± 9.9	7.7 ± 8.1	6.6 ± 8.3	.32

*p-values are for log transformed one-way ANOVAs.

Plasma Angiotensin (II and 1-7) Correlations

Table V presents the Pearson correlations for plasma RAS and cardiorespiratory fitness variables by ACE genotype. As shown in Table V higher levels of Ang II were associated with lower VO2peak and VO2peak % predicted in the term I/D subjects. In the preterm subjects, lower Ang II and Ang II/1-7 were significantly associated with higher

VO2peak in the DD subjects, and lower Ang II/(1-7) was significantly associated with higher VO_2peak in preterm I/D subjects.

Table 5. Pearson Correlation Coefficients among plasma Angiotensin and cardio-respiratory fitness by preterm and ACE I/D genotype.

	Term ACE Genotype			Preterm ACE Genotype		
VO_2Peak, $ml \cdot kg^{-1} \cdot min^{-1}$	I/I (n=11)	I/D (n=24)	D/D (n=6)	I/I (n=25)	I/D (n=37)	D/D (n=30)
Ang II, pmol/L	-.16	-.41*	-.09	-.13	-.25	-.38*
Ang (1-7) pmol/L	-.41	-.30	.19	-.17	.21	.23
Ang II/(1-7)	.07	.09	-.29	.13	-.34*	-.37*
VO_2Peak, % Predicted						
Ang II, pmol/L	-.14	-.45*	.11	-.05	-.10	-.38*
Ang (1-7), pmol/L	-.22	-.22	.22	-.01	.22	.29
Ang II/(1-7)	-.03	-.01	-.11	.01	-.27	-.43*

*p<.05 p-values are for log transformed RAS values.

Cardiorespiratory Fitness and RAS Score

Table VI presents cardiorespiratory fitness values by RAS score. A significant mean difference was detected for VO_2Peak and VO_2Peak% Predicted in all subjects. Bonferroni post hoc analysis showed significantly higher cardiorespiratory fitness for the Ang (1-7) dominant group compared to the Ang II dominant group. In the term group, a one-way ANOVA did not detect any significant mean differences in cardiorespiratory fitness among RAS groups. Significant mean differences were detected VO_2Peak and VO_2Peak% Predicted in PT subjects. Bonferroni post hoc analysis showed significantly higher cardiorespiratory fitness for the Ang I-7 dominant group compared to the Ang II dominant group.

Table 6. Cardiorespiratory fitness values among RAS score groups expressed as Mean ± SD.

	RAS Score All Subjects			
	Ang (1-7) Dominant n=32	Neutral n=71	Ang II Dominant n=30	p-value
VO_2Peak, $ml \cdot kg^{-1} \cdot min^{-1}$	41.8 ± 10.2*	40.3 ± 10.2	35.5 ± 9.1	.03
VO_2Peak% Predicted	95.8 ± 18.4*	91.3 ± 17.6	83.6 ± 18.4	.03
	RAS Score Term Subjects			
	Ang (1-7) Dominant n=14	Neutral n=23	Ang II Dominant n=4	p-value
VO_2Peak, $ml \cdot kg^{-1} \cdot min^{-1}$	40.2 ± 10.5	43.7 ± 10.6	42.3 ± 13.1	.64
VO_2Peak% Predicted	94.8 ± 20.0	96.1 ± 16.7	100.2 ± 20.8	.87
	RAS Score Preterm Subjects			
	Ang (1-7) Dominant n=18	Neutral n=48	Ang II Dominant n=26	p-value
VO_2Peak, $ml \cdot kg^{-1} \cdot min^{-1}$	43.1 ± 10.1*	38.6 ± 9.7	34.5 ± 8.2	.01
VO_2Peak% Predicted	96.6 ± 17.5*	89.0 ± 17.7	81.1 ± 17.0	.02

VO_2Peak% predicted values are based on sex- and age-specific reference values from Krahenbuhl, Skinner, & Kohrt[34]. *p<.05 for Ang (1-7) vs Ang II.

DISCUSSION

Cardiorespiratory fitness in the untrained state is a heritable complex phenotype with health and performance implications[9]. This means, genomic variants make up some of the inter-individual variation in the phenotype. These variations could lead to alterations in gene expression and protein function able to alter cardiorespiratory fitness in the untrained state. Additionally, genomic variants leading to alterations in gene expression and protein function could impact the biochemical pathways they are a part of. For cardiorespiratory fitness, altered biochemical pathways within the pulmonary, cardiovascular, or neuromuscular systems could impact VO_2max. Therefore, investigating genomic variants and biochemical pathways in these systems, such as the ACE I/D polymorphism and the RAS, is an approach to help explain the inter-individual variation in cardiorespiratory fitness. The following discussion provides hypothesis test results with possible explanations, strengths and limitations of this study, and a conclusion with future directions.

ACE Genotype, Cardiorespiratory Fitness, and Plasma RAS Components

In regards to the first hypothesis, T subjects had greater cardiorespiratory fitness compared to PT subjects, but within T or PT groups, ACE I/I genotype had no effect on cardiorespiratory fitness values, and therefore the null hypothesis was retained. This finding is similar to other studies that found preterm individuals to have lower cardiorespiratory fitness compared to term individuals[59,69,79]. ACE genotype was not associated with cardiorespiratory fitness, thus adding to existing literature reporting similar findings[9,13,26,70]. A possible explanation is that cardiorespiratory fitness is a complex phenotype, meaning it can be broken down into simpler phenotypes such as cardiac output,

the a-vO_2 difference, and all the other components displayed in Figures 1 and 2. Therefore, many genes are involved and each gene most likely accounts for a small percentage of the variance in cardiorespiratory fitness which, according to Sarzynski et al.,[66] is about 1-3%. Consequently, it would require a very large sample size to detect any differences. Since the maximal heritably estimate of cardiorespiratory fitness is 51% in the untrained state for healthy individuals, the remaining 49% is composed of environmental differences, such as occupational physical activity, diet, and stress, and measurement error[9].

Term individuals did have higher plasma Ang (1-7) concentrations and lower Ang II/(1-7) ratios compared to preterms, but Ang II concentrations were not different. These findings differ from Miyawaki et al.[52] who found preterm individuals to have higher plasma Ang II concentrations compared to terms. Additionally, Washburn et al.[83] did not find any differences in plasma Ang II, Ang (1-7), or the Ang II/(1-7) ratio, however, their sample consisted of only very low birth weight adolescents.

Also part of the first hypothesis was ACE genotype and plasma RAS comparisons of which there were no significant differences among genotype, and thus, the null hypothesis was retained. Similarly, one other study failed to find associations between plasma Ang II and Ang (1-7) concentrations[67]. However, Reyes-Engel et al.[67] did find men and women with the ACE I/D genotype to have higher Ang (1-7)/Ang II ratios compared to I/I and D/D genotypes. Overall, the findings remain mixed as to whether or not the ACE I/D polymorphism influences cardiorespiratory fitness, and more studies are needed to determine the relationship between ACE I/D genotype and plasma RAS Ang II and Ang (1-7) concentrations.

Cardiorespiratory Fitness and Plasma RAS Correlations

In regards to the second hypothesis, statistically significant effects between plasma Ang II and cardiorespiratory fitness in term I/D and preterm D/D genotypes were observed, and thus, the null hypothesis was rejected in those instances. The expected inverse association between Ang II and cardiorespiratory fitness could result from Ang II's participation in the pathogenic RAS axis as a contributor to pro inflammatory, fibrotic, trophic, and gene expression and reprogramming processes observed in animal cell cultures and models [32,49,74]. Moderate effects were found between cardiorespiratory fitness and the Ang II/(1-7) ratio in preterm D/D genotypes, and thus, the null hypothesis was rejected in these instances. The expected inverse association between cardiorespiratory fitness and the Ang II/(1-7) ratio follows the same rationale as Ang II alone, in that, larger ratios could indicate an imbalance in the RAS system potentially more prone to pathogenic outcomes of the cardiovascular system such as increased inflammation, fibrosis, cardiac hypertrophy, and fetal gene reprogramming, thus, leading to lower cardiorespiratory fitness[32,49,74]. A moderate effect was also found between cardiorespiratory fitness and Ang (1-7) in the term I/I genotype, however it wasn't statistically significant at the $p<-.05$ level, most likely due to the relatively small number of subjects in this group. The different associations in the term and preterm subjects could also reflect differences in epigenetic regulation. Premature birth may disrupt normal development and alter DNA methylation, which may affect gene expression and be linked to long term health outcomes[37,63].

Cardiorespiratory Fitness and RAS Score

Lastly, the third hypothesis regarding cardiorespiratory fitness and RAS score group was retained for the term subjects and rejected for the preterm subjects. There were

no significant differences in cardiorespiratory fitness among ACE genotype groups in term subjects, probably due to the fact that they were all healthy adolescents. Therefore, differences in ACE genotype and plasma RAS variables do not result in large enough alternations in cardiorespiratory fitness to be noticeable, as those categorized as Ang II dominant aren't experiencing any of the pathologic actions of Ang II noted in the literature [32,49]. There were statistically significant differences between cardiorespiratory fitness and RAS Score in the preterm group. The detected differences could be explained by the rationale that the Ang II dominant group would presumably have a more active pathogenic RAS axis, since the point distribution was created such that, subjects with D/D genotypes, higher levels of Ang II, lower levels of Ang 1-7, and higher Ang II/1-7 ratios would fall into the Ang II dominant axis group. Whereas the Ang (1-7) dominant axis group would have a more cardiovasculary protective RAS axis indicated by an I/I genotype, lower Ang II levels, higher Ang 1-7 levels, and a lower Ang II/1-7 ratio.

Strengths and Limitations

The cross-sectional study design does not allow for the determination of cause and effect, therefore it cannot be said that an ACE genotype or plasma RAS association causes a difference in cardiorespiratory fitness. Experimental studies able to manipulate the levels of plasma RAS variables or knockout ACE genotypes would be needed for determination of cause and effect. A strength of this study is that all data were collected by trained study personnel and the habitual physical activity, cardiorespiratory fitness, and ACE genotype data collection methods had been previously validated. Another strength is that only maximal exercise tests rated as a "good effort" were included in the analysis, as well as, having a large sample of preterm adolescents and including a term group for comparison.

A limitation of the cross-sectional study design is no temporal sequence, therefore there is not a dynamic perspective of changes over time. Another limitation is that plasma RAS concentrations likely reflect many tissues, including fat and renal. Therefore, plasma RAS concentrations don't provide a clear picture of muscle or cardiac RAS concentrations. Additionally, dietary sodium intake, gender, and race could play a role in plasma RAS Ang II, Ang (1-7), and Ang II/(1-7) ratio concentrations and differences between these groups were not tested for in the analysis. In a small sample of male and female adolescents, Mahler et al.,[48] did find plasma Ang II concentrations to be steady during the day and increase at night. However, the sample of Mahler's et al.[48] is presumably all Caucasian since the study was carried out in Denmark whereas the current sample is 41% black and 74% born preterm, so their results may not apply. Furthermore, diurnal variations in Ang II and (1-7) may occur, and a single blood sample may not accurately represent average plasma levels. Moreover, potential epigenetic differences between term and preterm individuals were not determined or accounted for. Another limitation is the rs4340 ACE I/D polymorphism is not presumed to be the true causal variant for differences in serum ACE activity because it is located in an intron segment of the DNA; however, it is thought to be in linkage disequilibrium with the true causal variant and is why it is commonly used[76]. In other words, the rs4340 polymorphism acts as a marker for the true causal variant, which may be located nearby within the ACE gene and travels with the rs4340 polymorphism when recombination events occur during meiosis. Also, cardiorespiratory fitness is a complex phenotype with many genes contributing to the trait, therefore, many more gene association tests are needed to gain a better understanding of the genomic contribution to VO_2max. Lastly, the RAS scoring system used to classify an individual as

having a dominant Ang (1-7) or dominant Ang II RAS axis is not a validated classification method, and the point distributions based on percentiles were arbitrarily determined. Also, the points assigned to each classification are the same indicating they all have the same effect. When in reality ACE genotypes and plasma RAS concentrations may have varying effect sizes and could be weighted to reflect their actual impact.

Conclusions and Future Directions

This study provides evidence that ACE I/D genotype (rs4340) is not associated with cardiorespiratory fitness, habitual physical activity, or plasma RAS Ang II and Ang (1-7) concentrations or the Ang II/(1-7) ratio in this sample of term and preterm adolescents. However, plasma Ang II concentrations and the Ang II/(1-7) ratio were inversely associated with cardiorespiratory fitness, and subjects categorized as Ang (1-7) RAS dominant had higher cardiorespiratory fitness compared to subjects categorized as Ang II RAS dominant indicating the biological actions of Ang II and Ang (1-7) along with RAS dominance may help to explain cardiorespiratory fitness in adolescents.

Cardiorespiratory fitness is a heritable trait and is used as an indicator of cardiovascular morbidity and mortality risk. Therefore, it seems logical to put forth the effort to determine the underlying genomic and biochemical mechanisms of VO_2max, in order to understand how they could be manipulated to promote health and prevent disease. Future research directed at identifying the underlying mechanisms using animal and human models would provide a foundation from which to develop therapeutic alternatives to aerobic exercise with the intention to positively change cardiorespiratory fitness and ultimately cardiometabolic health

REFERENCES

1. Aaron, D. J., Kriska, A. M., Dearwater, S. R., Cauley, J. A., Metz, K. F., & LaPorte, R. E. (1995). Reproducibility and validity of an epidemiologic questionnaire to assess past year physical activity in adolescents. American journal of epidemiology, 142(2), 191-201.

2. Almeida, J. A., Boullosa, D. A., Pardono, E., Lima, R. M., Morais, P. K., Denadai, B. S., & Simões, H. G. (2012). The influence of ACE genotype on cardiovascular fitness of moderately active young men. Arquivos brasileiros de cardiologia, 98(4), 315-320.

3. Ainsworth, B. E., Haskell, W. L., Whitt, M. C., Irwin, M. L., Swartz, A. M., Strath, S. J., & Jacobs, D. R. (2000). Compendium of physical activities: an update of activity codes and MET intensities. Medicine and science in sports and exercise, 32(9; SUPP/1), S498-S504.

4. Almeida, A. P., Frabregas, B. C., Madureira, M. M., Santos, R. J. S., Campagnole-Santos, M. J., & Santos, R. A. S. (2000). Angiotensin-(1-7) potentiates the coronary vasodilatatory effect of bradykinin in the isolated rat heart. Brazilian Journal of Medical and Biological Research, 33(6), 709-713.

5. American College of Sports Medicine. (2013). ACSM's guidelines for exercise testing and prescription. Lippincott Williams & Wilkins.

6. Amir, O., Amir, R., Yamin, C., Attias, E., Eynon, N., Sagiv, M., … Meckel, Y. (2007). The ACE deletion allele is associated with Israeli elite endurance athletes. *Experimental Physiology*, 92(5), 881–886.

7. Åstrand, P. O. (1952). Experimental studies of physical working capacity in relation to sex and age. E. Munksgaard.

8. Bassett Jr, D. R., & Howley, E. T. (2000). Limiting factors for maximum oxygen uptake and determinants of endurance performance. *Medicine & Science in Sports & Exercise*, 32(1), 70.

9. Bouchard, C., Daw, E. W., Rice, T., Pérusse, L., Gagnon, J., Province, M. A., Wilmore, J. H. (1998). Familial resemblance for VO2max in the sedentary state: the HERITAGE family study. *Medicine and Science in Sports and Exercise*, 30(2), 252–258.

10. Bouchard, C., Lesage, R, Lortie, G., Simoneau, J. A., Hamel, P., Boulay, M. R., & Leblanc, C. (1986). Aerobic performance in brothers, dizygotic and monozygotic twins. Med Sci Sports Exerc, 18(6), 639-46.

11. Bouchard, C., Rankinen, T., & Timmons, J. A. (2011). Genomics and Genetics in the Biology of Adaptation to Exercise. Comprehensive Physiology, 1, 1603–1648.

12. Bouchard, C., Sarzynski, M. A., Rice, T. K., Kraus, W. E., Church, T. S., Sung, Y. J. Rankinen, T. (2011). Genomic predictors of the maximal O2 uptake response to standardized exercise training programs. Journal of Applied Physiology, 110(5), 1160–1170.

13. Bueno, S., Pasqua, L. A., de Araújo, G., Lima-Silva, A. E., & Bertuzzi, R. (2016). The Association of ACE Genotypes on Cardiorespiratory Variables Related to Physical Fitness in Healthy Men. PloS one, 11(11), e0165310.

14. Cam, F. S., Colakoglu, M., Sekuri, C., Colakoglu, S., Sahan, Ç., & Berdeli, A. (2005). Association Between the ACE I/D) Gene Polymorphism and Physical Performance in a Homogeneous Non-Elite Cohort. Canadian journal of applied physiology, 30(1), 74-86.

15. Cam, S., Colakoglu, M., Colakoglu, S., Sekuri, C., & Berdeli, A. (2007). ACE I/D gene polymorphism and aerobic endurance development in response to training in a non-elite female cohort. Journal of sports medicine and physical fitness, 47(2), 234.

16. Cerit, M., Colakoglu, M., Erdogan, M., Berdeli, A., & Cam, F. S. (2006). Relationship between ace genotype and short duration aerobic performance development. European journal of applied physiology, 98(5), 461-465.

17. Cieszczyk, P., Krupecki, K., Maciejewska, A., & Sawczuk, M. (2009). The angiotensin converting enzyme gene I/D polymorphism in Polish rowers. International journal of sports medicine, 30(08), 624-627.

18. Clemm, H., Røksund, O., Thorsen, E., Eide, G. E., Markestad, T., & Halvorsen, T. (2012). Aerobic capacity and exercise performance in young people born extremely preterm. Pediatrics, 129(1), e97-e105.

19. Collins, M., Xenophontos, S. L., Cariolou, M. A., Mokone, G. G., Hudson, D. E., Anastasiades, L., & Noakes, T. D. (2004). The ACE gene and endurance

performance during the South African Ironman Triathlons. Medicine and science in sports and exercise, 36(8), 1314-1320.

20. Costa, A. M., Silva, A. J., Garrido, N. D., Louro, H., de Oliveira, R. J., & Breitenfeld, L. (2009). Association between ACE D allele and elite short distance swimming. European journal of applied physiology, 106(6), 785-790.

21. Data Access and Dissemination Systems (DADS). (2010, October 05). American FactFinder - Community Facts. Retrieved November 09, 2017, from https://factfinder.census.gov/faces/nav/jsf/pages/community_facts.xhtml.

22. Fagard, R., Bielen, E., & Amery, A. (1991). Heritability of aerobic power and anaerobic energy generation during exercise. Journal of Applied Physiology, 70(1), 357-362.

23. Ferreira, A. J., Santos, R. A. S., & Almeida, A. P. (2002). Angiotensin-(1-7) improves the post-ischemic function in isolated perfused rat hearts. Brazilian Journal of Medical and Biological Research, 35(9), 1083-1090.

24. Forsyth, J. S., Reilly, J., Fraser, C., & Struthers, A. D. (2004). Angiotensin converting enzyme activity in infancy is related to birth weight. Archives of Disease in Childhood-Fetal and Neonatal Edition, 89(5), F442-F444.

25. Franco, M. C., Casarini, D. E., Carneiro-Ramos, M. S., Sawaya, A. L., Barreto-Chaves, M. L., & Sesso, R. (2008). Circulating renin–angiotensin system and catecholamines in childhood: is there a role for birthweight?. Clinical Science, 114(5), 375-380.

26. Frederiksen, H., Bathum, L., Worm, C., Christensen, K., & Puggaard, L. (2003). ACE genotype and physical training effects: a randomized study among elderly Danes. Aging clinical and experimental research, 15(4), 284-291.

27. Gayagay, G., Yu, B., Hambly, B., Boston, T., Hahn, A., Celermajer, D. S., & Trent, R. J. (1998). Elite endurance athletes and the ACE I allele–the role of genes in athletic performance. Human genetics, 103(1), 48-50.

28. Goh, K. P., Chew, K., Koh, A., Guan, M., Wong, Y. S., & Sum, C. F. (2009). The relationship between ACE gene ID polymorphism and aerobic capacity in Asian rugby players. Singapore medical journal, 50(10), 997.

29. Hagberg, J. M., Ferrell, R. E., McCole, S. D., Wilund, K. R., & Moore, G. E. (1998). V̇ o 2 max is associated with ACE genotype in postmenopausal women. Journal of Applied Physiology, 85(5), 1842–1846.

30. Hunyady, L., & Catt, K. J. (2006). Pleiotropic AT1 receptor signaling pathways mediating physiological and pathogenic actions of angiotensin II. Molecular endocrinology, 20(5), 953-970.

31. Hruskovicova, H., Dzurenkova, D., Selingerova, M., & Bohus, B. (2006). The angiotensin converting enzyme I/D polymorphism in long distance runners. Journal of sports medicine and physical fitness, 46(3), 509.

32. Iwata, M., Cowling, R. T., Gurantz, D., Moore, C., Zhang, S., Yuan, J. X. J., & Greenberg, B. H. (2005). Angiotensin-(1–7) binds to specific receptors on cardiac fibroblasts to initiate antifibrotic and antitrophic effects. American Journal of Physiology-Heart and Circulatory Physiology, 289(6), H2356-H2363.

33. Kim, S., & Iwao, H. (2000). Molecular and cellular mechanisms of angiotensin II-mediated cardiovascular and renal diseases. Pharmacological reviews, 52(1), 11-34.

34. Kim, K., Ahn, N., Cheun, W., Byun, J., & Joo, Y. (2015). Association of Angiotensin Converting Enzyme I/D and α-actinin-3 R577X Genotypes with Growth Factors and Physical Fitness in Korean Children. The Korean Journal of Physiology & Pharmacology, 19(2), 131.

35. Klissouras, V. (1971). Heritability of adaptive variation. Journal of applied physiology, 31(3), 338-344.

36. Klissouras, V., Pirnay, F., & Petit, J. M. (1973). Adaptation to maximal effort: genetics and age. Journal of Applied Physiology, 35(2), 288-293.

37. Knight, A. K., & Smith, A. K. (2016). Epigenetic biomarkers of preterm birth and its risk factors. Genes, 7(4), 15.

38. Kodama, S., Saito, K., Tanaka, S., Maki, M., Yachi, Y., Asumi, M. (2009). Cardiorespiratory Fitness as a quantitative predictor of all-cause mortality and cardiovascular events in healthy men and women: a meta-analysis. *Jama*, *301*(19), 2024–2035.

39. Krahenbuhl, G. S., Skinner, J. S., & Kohrt, W. M. (1985). Developmental aspects of maximal aerobic power in children. Exercise and sport sciences reviews, 13(1), 503-538.

40. Kriska, A. M. Development of questionnaire to examine relationship of physical activity and diabetes in Pima Indians. Diabetes Care 13, 401–411 (1990).

41. Kritchevsky, S. B., Nicklas, B. J., Visser, M., Simonsick, E. M., Newman, A. B., Harris, T. B., & Colbert, L. H. (2005). Angiotensin-converting enzyme insertion/deletion genotype, exercise, and physical decline. Jama, 294(6), 691-698.

42. Kurdi, M., De Mello, W. C., & Booz, G. W. (2005). Working outside the system: an update on the unconventional behavior of the renin–angiotensin system components. *The international journal of biochemistry & cell biology*, *37*(7), 1357-1367.

43. Lesage, R., Simoneau, J. A., Jobin, J., Leblanc, J., & Bouchard, C. (1985). Familial resemblance in maximal heart rate, blood lactate and aerobic power. Human heredity, 35(3), 182-189.

44. Lin, X., Zhang, X., Guo, J., Roberts, C. K., McKenzie, S., Wu, W.-C., Song, Y. (2015). Effects of Exercise Training on Cardiorespiratory Fitness and Biomarkers of Cardiometabolic Health: A Systematic Review and Meta-Analysis of Randomized Controlled Trials. Journal of the American Heart Association: Cardiovascular and Cerebrovascular Disease, 4(7).

45. Lortie, G., Bouchard, C., Leblanc, C., Tremblay, A., Simoneau, J. A., Thériault, G., & Savoie, J. P. (1982). Familial similarity in aerobic power. Human biology, 801-812.

46. Lucia, A., Gómez-Gallego, F., Chicharro, J. L., Hoyos, J., Celaya, K., Córdova, A., & Earnest, C. P. (2005). Is there an association between ACE and CKMM polymorphisms and cycling performance status during 3-week races?. International journal of sports medicine, 26(06), 442-447.

47. Maes, H. H., Beunen, G. P., Vlietinck, R. F., Neale, M. C., Thomis, M., Vanden, E. B., & Derom, R. (1996). Inheritance of physical fitness in 10-yr-old twins and their parents. Medicine and science in sports and exercise, 28(12), 1479-1491.

48. Mahler, B., Kamperis, K., Ankarberg-Lindgren, C., Djurhuus, J. C., & Rittig, S. (2015). The effect of puberty on diurnal sodium regulation. American Journal of Physiology-Renal Physiology, 309(10), F873-F879.

49. Mehta, P. K., & Griendling, K. K. (2007). Angiotensin II cell signaling: physiological and pathological effects in the cardiovascular system. American Journal of Physiology-Cell Physiology, 292(1), C82-C97.

50. Mäestu, J., Lätt, E., Rääsk, T., Sak, K., Laas, K., Jürimäe, J., & Jürimäe, T. (2013). Ace I/D polymorphism is associated with habitual physical activity in pubertal boys. The Journal of Physiological Sciences, 63(6), 427-434[PAN1].

51. Min, S. K., Takahashi, K., Ishigami, H., Hiranuma, K., Mizuno, M., Ishii, T., & Nakazato, K. (2009). Is there a gender difference between ACE gene and race distance?. Applied Physiology, Nutrition, and Metabolism, 34(5), 926-932.

52. Miyawaki, M., Okutani, T., Higuchi, R., & Yoshikawa, N. (2006). Plasma angiotensin II concentrations in the early neonatal period. Archives of Disease in Childhood-Fetal and Neonatal Edition, 91(5), F359-F362.

53. Montgomery, H. E., Marshall, R., Hemingway, H., Myerson, S., Clarkson, P., Dollery, C., & Brynes, A. E. (1998). Human gene for physical performance. Nature, 393(6682), 221-222.

54. Montoye, H. J., & Gayle, R. (1978). Familial relationships in maximal oxygen uptake. Human biology, 241-249.

55. Muniesa, C. A., González-Freire, M., Santiago, C., Lao, J. I., Buxens, A., Rubio, J. C., & Lucia, A. (2010). World-class performance in lightweight rowing: is it genetically influenced? A comparison with cyclists, runners and non-athletes. British journal of sports medicine, 44(12), 898-901.

56. Myers, J., & Froelicher, V. F. (1991). Hemodynamic determinants of exercise capacity in chronic heart failure. Annals of internal medicine, 115(5), 377-386.

57. Myerson, S., Heminway, H., Budget, R., Martin, J., Humphries, S., & Montgomery, H. (1999). Human angiotensin I-converting enzyme gene and endurance performance. J *Applied Physiology*, 87(4), 1313–1316.

58. Nazarov, I. B., Woods, D. R., Montgomery, H. E., Shneider, O. V., Kazakov, V. I., Tomilin, N. V., & Rogozkin, V. A. (2001). The angiotensin converting enzyme I/D polymorphism in Russian athletes. European journal of human genetics: EJHG, 9(10), 797.

59. Nixon, P. A., Washburn, L. K., Mudd, L. M., Webb, H. H., & O'Shea, T. M. (2011). Aerobic fitness and physical activity levels of children born prematurely following randomization to postnatal dexamethasone. The Journal of Pediatrics, 158(1), 65–70.

60. Nixon, P. A., Washburn, L. K., O'Shea, T. M., Shaltout, H. A., Russell, G. B., Snively, B. M., & Rose, J. C. (2017). Antenatal steroid exposure and heart rate variability in adolescents born with very low birth weight. Pediatric Research, 81(1–1), 57.

61. Oh, S. D. (2007). The distribution of I/D polymorphism in the ACE gene among Korean male elite athletes. Journal of sports medicine and physical fitness, 47(2), 250.

62. Papadimitriou, I. D., Papadopoulos, C., Kouvatsi, A., & Triantaphyllidis, C. (2009). The ACE I/D polymorphism in elite Greek track and field athletes. Journal of Sports Medicine and Physical Fitness, 49(4), 459.

63. Parets, S. E., Bedient, C. E., Menon, R., & Smith, A. K. (2014). Preterm birth and its long-term effects: methylation to mechanisms. Biology, 3(3), 498-513.

64. Puthucheary, Z., Skipworth, J. R., Rawal, J., Loosemore, M., Van Someren, K., & Montgomery, H. E. (2011). The ACE Gene and Human Performance. *Sports Med*, 41(6), 433–448.

65. Rankinen, T., Russe, L. P., Gagnon, J., Chagnon, Y. C., Leon, A. S., Skinner, J. S., Bouchard, C. (2000). Angiotensin-converting enzyme ID polymorphism and fitness phenotype in the HERITAGE Family Study. *J. Appl. Physiol*, *88*, 1029–1035.

66. Rankinen, T., Wolfarth, B., Simoneau, J. A., Maier-Lenz, D., Rauramaa, R., Rivera, M. A., & Bouchard, C. (2000). No association between the angiotensin-converting enzyme ID polymorphism and elite endurance athlete status. Journal of Applied Physiology, 88(5), 1571-1575.

67. Reyes-Engel, A., Morcillo, L., Aranda, F. J., Ruiz, M., Gaitan, M. J., Mayor-Olea, Á., & Ferrario, C. M. (2006). Influence of gender and genetic variability on plasma angiotensin peptides. Journal of the Renin-Angiotensin-Aldosterone System, 7(2), 92-97.

68. Rigat, B., Hubert, C., Alhenc-Gelas, F., Cambien, F., Corvol, P., & Soubrier, F. (1990). Gene Accounting for Half the Variance of Serum Enzyme Levels. *Age*, 44(7.4), 44–3.

69. Rogers, M., Whitfield, M. F., Grunau, R. E., Williams, L., Fay, T. B., & Tomlinson, J. (2003). Aerobic capacity, strength, flexibility and activity level in unimpaired ELBW survivors compared to term born controls at 17 years of age. Pediatric Research, 53(4), 458A-459A.

70. Roltsch, M. H., Brown, M. D., Hand, B. D., Kostek, M. C., Phares, D. A., Huberty, A., & Hagberg, J. M. (2005). No association between ACE I/D polymorphism and cardiovascular hemodynamics during exercise in young women. International journal of sports medicine, 26(08), 638-644.

71. Saigal, S., Stoskopf, B., Boyle, M., Paneth, N., Pinelli, J., Streiner, D., & Goddeeris, J. (2007). Comparison of current health, functional limitations, and health care use of young adults who were born with extremely low birth weight and normal birth weight. Pediatrics, 119(3), e562-e573.

72. Sampaio, W. O., Nascimento, A. A., & Santos, R. A. (2003). Systemic and regional hemodynamic effects of angiotensin-(1–7) in rats. American Journal of Physiology-Heart and Circulatory Physiology, 284(6), H1985-H1994.

73. Santos, R. A., Castro, C. H., Gava, E., Pinheiro, S. V., Almeida, A. P., de Paula, R. D., & Bader, M. (2006). Impairment of in vitro and in vivo heart function in angiotensin-(1-7) receptor MAS knockout mice. Hypertension, 47(5), 996-1002.

74. Santos, R. A., Ferreira, A. J., Verano-Braga, T., & Bader, M. (2013). Angiotensin-converting enzyme 2, angiotensin-(1–7) and Mas: new players of the renin–angiotensin system. Journal of Endocrinology, 216(2), R1-R17.

75. Sarzynski, M., loos, R. J. F., Lucia, A., & Perusse, L. (2015). Advances in Exercise, Fitness, and Performance Genomics in 2015. Medicine & Science in Sports & Exercise, 48(10), 1906–1916.

76. Sayed-Tabatabaei, F. A., Oostra, B. A., Isaacs, A., Van Duijn, C. M., & Witteman, J. C. M. (2006). ACE polymorphisms. Circulation Research, 98(9), 1123–1133.

77. Scott, R. A., Irving, R., Irwin, L., Morrison, E., Charlton, V., Austin, K., & Yang, N. (2010). ACTN3 and ACE genotypes in elite Jamaican and US sprinters. Medicine & Science in Sports & Exercise, 42(1), 107-112.

78. Scott, R. A., Moran, C., Wilson, R. H., Onywera, V., Boit, M. K., Goodwin, W. H., & Pitsiladis, Y. P. (2005). No association between Angiotensin Converting Enzyme (ACE) gene variation and endurance athlete status in Kenyans. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology, 141(2), 169-175.

79. Smith, L. J., van Asperen, P. P., McKay, K. O., Selvadurai, H., & Fitzgerald, D. A. (2008). Reduced exercise capacity in children born very preterm. Pediatrics, 122(2), e287-e293.

80. Sundet, J. M., Magnus, P., & Tambs, K. (1994). The heritability of maximal aerobic power: a study of Norwegian twins. Scandinavian journal of medicine & science in sports, 4(3), 181-185.

81. The top 10 causes of death. (n.d.). Retrieved November 08, 2017, from http://www.who.int/mediacentre/factsheets/fs310/en/.

82. Tsianos, G., Sanders, J., Dhamrait, S., Humphries, S., Grant, S., & Montgomery, H. (2004). The ACE gene insertion/deletion polymorphism and elite endurance swimming. *European Journal of Applied Physiology*, 92(3), 360–362.

83. Washburn, L. K., Brosnihan, K. B., Chappell, M. C., Diz, D. I., Gwathmey, T. M., Nixon, P. A., & O'Shea, T. M. (2015). The renin–angiotensin–aldosterone system in adolescent offspring born prematurely to mothers with preeclampsia. Journal of the Renin-Angiotensin-Aldosterone System, 16(3), 529-538.

84. Woods, D., Hickman, M., Jamshidi, Y., Brull, D., Vassiliou, V., Jones, A., ... & Montgomery, H. (2001). Elite swimmers and the D allele of the ACE I/D polymorphism. Human genetics, 108(3), 230-232.

85. Zhao, B., Moochhala, S. M., Tham, S. Y., Lu, J., Chia, M., Byrne, C., & Lee, L. K. (2003). Relationship between angiotensin-converting enzyme ID polymorphism and VO 2max of Chinese males. Life sciences, 73(20), 2625-2630.

CURRICULUM VITAE

SEAN CADDIGAN

Education

Master of Science in Health and Exercise Science May 2018
Wake Forest University, Winston-Salem, NC
3.8 *GPA*

Bachelor of Science in Exercise Science – Health Fitness May 2016
University of Southern Maine (USM), Gorham, ME
3.8 GPA; Outstanding Exercise Science Student Award

Research Experience

Undergraduate Research Assistant, September 2013 – May 2016
Department of Biology, USM

Publications

Walker, J.A., & Caddigan, S.P. (2015). Performance trade-offs and individual quality in decathletes. *Journal of Experimental Biology*, 218(22), 3647-3657.

Professional Affiliations

National Strength and Conditioning Association 2015 - Present

Certifications

SilverSneakers Classic Fitness Instructor November 2016

American Heart Association CPR and AED for Health Care Providers August 2016

Certified Strength and Conditioning Specialist 2016 - Present