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The Association of Genotype, and the Gene-Physical Activity Interaction Effect on Aerobic Fitness in Prepubertal, African American, Obese Children

Jennifer Irene Flynn
jflynn6@utk.edu

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To the Graduate Council:

I am submitting herewith a thesis written by Jennifer Irene Flynn entitled "The Association of Genotype, and the Gene-Physical Activity Interaction Effect on Aerobic Fitness in Prepubertal, African American, Obese Children." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Kinesiology.

Dawn P. Coe, Major Professor

We have read this thesis and recommend its acceptance:

Dixie L. Thompson, David R. Bassett, Cheryl J. Kojima

Accepted for the Council:
Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

THE ASSOCIATION OF GENOTYPE, AND THE GENE-PHYSICAL ACTIVITY INTERACTION EFFECT ON AEROBIC FITNESS IN PREPUBERTAL, AFRICAN AMERICAN, OBESE CHILDREN

A Thesis
Presented for the
Master of Science
Degree
The University of Tennessee, Knoxville

Jennifer Irene Flynn
August 2011

DEDICATION

To my parents, Mike and Nancy, and my brother Jim. Thank you for all of your love, encouragement and understanding. I so am blessed to have you in my life.

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ABSTRACT

Purpose: To determine the association of certain aerobic fitness and physical activity genotypes and the gene-physical activity interaction effect on aerobic fitness in pre-pubertal, African American, obese children. **Methods:** Subjects were 30 pre-pubertal, African American, obese children (9.5 ± 1.7 years) who were free of clinical disease. Height and weight were measured according to standard procedures. Body fat was assessed using dual energy x-ray absorptiometry, and DNA samples were collected using buccal swabs. Aerobic fitness was assessed using a cycle ergometer and the McMaster cycle protocol. ANOVAs were used to determine associations and interaction effects of the ACE, ADRB2, NOS3, IL6, IGF-1, and APO-E genes, physical activity and aerobic fitness. **Results:** Age, height, weight, body mass index, and waist circumference were significantly lower in girls compared to boys. Subjects averaged approximately 51 minutes of moderate-to-vigorous intensity activity per day, and girls were significantly more active than boys. There were no significant associations between the candidate genes and aerobic fitness level. $P > 0.05$). There were trends towards significance for the IL6 rs2069845 gene for absolute and relative VO_{2peak} measures ($P = 0.078$, and $P = 0.094$, respectively). There was also a trend toward significance for the ADRβ2 rs1042717 gene for leanVO_{2peak} ($P = 0.092$). **Conclusions:** In children, further research is needed that includes diverse populations and large sample sizes in order to more accurately assess the association and interaction effects of the candidate genes, physical activity and fitness.

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CHAPTER 1:
INTRODUCTION

Low aerobic fitness has been identified as an independent risk factor for cardiovascular disease in both adults and children.[1-2] Specifically, associations between low aerobic fitness, referred to as fitness for the remaining sections, elevated blood pressure and lipid values, and obesity have all been shown in the pediatric population, and may play a significant role in the development of chronic disease later in adulthood.[3] In addition, aerobic fitness levels may affect how physically active a child is by influencing his/her activity intensity. Poor fitness levels may then translate into lower accumulations of moderate-to-vigorous physical activity during the day because they are physiologically not able to achieve this intensity. Aerobic fitness is an important aspect of health, and various factors play an important role in the development and maintenance of fitness levels including weight status, physical activity levels and genetics.

The prevalence of overweight and obesity is 35.5% of children, ages 6 to 11 years, in the United States.[4] Furthermore, in the African American population, 37.6% of 6 to 11 year old children are at or above the 85th percentile.[4] Not only is high weight status related to increased number of cardiovascular disease risk factors, but the presence of obesity has also been found to be inversely related to fitness levels.[5] Prior research has shown that weight status and other factors such as physical inactivity may also negatively impact fitness levels. Therefore, it is important to better understand variables contributing to fitness within the pediatric population.[6]

High levels of physical activity have been previously associated with reducing the risk of all-cause mortality, diabetes, cardiovascular disease in the adult population, and it is possible that a similar relationship exists within children.[7-8] Physical activity has been found to have an impact on cardiovascular, muscular, and metabolic systems in adults. However, children's systems are immature and underdeveloped compared to adults, and therefore may not respond to physical

activity in the same ways that adults respond. Previous research has shown that physical activity has a positive influence on fitness levels in children, however, these studies have only shown a moderate/weak correlation.[9-10]

Genetic individuality is another contributing factor to fitness levels. Genetic studies conducted over the previous years in adults have found that an individual's genotype may contribute anywhere from 25 to 66% of the variation in fitness levels,[11-16] and because of this contribution, it is important to assess the associations of certain genes in order to better understand the individuality contributing to fitness. Unfortunately, none of these studies have assessed the interaction between habitual physical activity and genotype and the role these two variables may play on fitness.

Within the integration of genetic and exercise physiology research, the primary physical trait, or the phenotype, being assessed has typically been a performance-related variable such as race time. In the case of this study, the phenotype being assessed is aerobic fitness level. Aerobic fitness is a multi-factorial trait, meaning that an individual's physical traits being expressed are influenced by a combination of inherited factors such as the genotype, as well as lifestyle and environmental interactions.[17] As previously mentioned, variations in genotype have been shown to make up a significant percentage of the variance in fitness levels among adults, however these relationships have been studied in very little detail in children. Candidate genes have been identified relating to both fitness and physical activity. The Angiotensin Converting Enzyme (ACE) gene is related to physical activity and fitness in adults, and β2 Adrenergic Receptor (ADRβ2), Endothelial Nitric Oxide Synthase 3 (NOS3), Interleukin 6 (IL6), Insulin-like Growth Factor 1 (IGF-1), and Apolipoprotein-E (APOE) are all related to fitness in adults[18] and it is possible that these same relationships exist in children.

Therefore, there are two main objectives of this study. First, the association of aerobic fitness genes and fitness will be assessed in a specified population. A better understanding of the associations will aid in identifying those predisposed to having lower fitness levels. However, in an attempt to control for the effect of race, maturation status and body composition on fitness levels and genetic variation, only African American, pre-pubertal, obese children will be included in the study as the specified population of interest.

The second objective of this study will assess the relationship of physical activity on the expression of genes and the fitness phenotype, defined as peak oxygen uptake. Since fitness is a multi-factorial trait that typically involves both an environmental and genetic components, it is important to assess the interaction that occurs between the two factors in order to better understand fitness levels of an individual. This concept of a multi-factorial phenotype is the basis for the assessment of a gene-environment interaction, specifically using physical activity level as the environment in which the child is exposed. The research questions that will be investigated in this study are as follows:

Research Question 1:

Is there an association between the aerobic fitness candidate genes and fitness levels in children?

Hypothesis 1:

Specific genotypes will be significantly associated with the level of fitness.

Research Question 2:

Is there a gene-environment interaction that exists between physical activity levels and the candidate genes on fitness in children?

Hypothesis 2:

There will be a significant interaction between physical activity levels and the specific genotypes on fitness levels in children.

CHAPTER 2
REVIEW OF LITERATURE

Introduction

A variety of factors contribute to the development of fitness including sex, race, maturity status, body composition, physical activity and genetics. The interaction between physical activity, genetics, and fitness has been assessed in the adult population; however research is limited in the pediatric population. It is essential to assess the relationship between genetics and fitness in order to understand the impact that interventions and physical activity will play on the fitness phenotype, with a phenotype being defined as the physical manifestation of the genotype in an individual. Unfortunately, the influence of physical activity on fitness is equivocal in previous pediatric literature, and even less is known as to how genetic profile influences the fitness of children. This study reduces the variation of multiple factors by controlling using a homogeneous group of pre-pubertal, African American, obese subjects.

The focus of this literature review will be on the contributions of the various factors to the development of fitness. Additionally, this review will identify candidate genes associated with fitness and the potential environmental interaction of physical activity and these genotypes.

Aerobic Fitness

Maximal aerobic fitness can be defined as the highest rate at which the skeletal muscles can utilize the oxygen being delivered during exercise, and is the gold standard for the measurement of fitness in adults and youth.[19] In children and adolescents, the term "peak" oxygen uptake is becoming more widely used.[20] The criteria for VO_{2max} in children includes: VO_2 plateau ($\leq$ 2.0 $ml \cdot kg^{-1} \cdot min^{-1}$ change), RER ($\geq$1.05), maximal heart rate ($\geq$ 190beats min^{-1}), and a subjective measure of volitional exhaustion. Since children do no often meet these criteria,

the peak value attained during the test might be a more reasonable term. Using peak oxygen uptake as a measure of fitness has been validated in youth.[21] The current study will focus on peak oxygen uptake values. The literature is inconsistent with the use of the terms "maximal" or "peak" values to define fitness in children. The following section will discuss studies that have used both maximal and peak values, however findings are generally consistent that higher fitness levels are associated with positive health outcomes in children.

Poor fitness levels have been associated with increased risk of cardiovascular disease,[22-23] metabolic complications, hyperlipidemia, and elevated blood pressure[24] in children. [25] An early study by Fripp et al.[22] assessed 37 adolescent boys and found that when divided into three fitness groups (low, moderate, and high), the high fitness level subjects had fewer cardiovascular disease risk factors compared to those in the low and moderate fitness level groups. This trend has been shown repeatedly in the literature, and demonstrates the importance of fitness to improve health profiles. Ultimately, the research has led to the promotion of fitness for increased cardiovascular health. However, when comparing fitness levels, it is important to consider the fact that children experience a change and development in fitness as they age chronologically and approach maturity. Children's physiological systems are immature compared to that of an adult and because adaptations in aerobic fitness differ in children, factors including weight and pubertal status should be considered when assessing peak fitness.

Aerobic Fitness and Body Composition

As stated earlier, approximately 35.5% of school-aged children in the United States are categorized as overweight or obese based on body mass index (BMI).[4] Due to increasing trends in high weight status, it is important that the effect of body composition on fitness be considered. Having a high percentage of body fat has been shown to have detrimental effects on submaximal

and maximal fitness in youth. Therefore, high weight status does need to be accounted for when assessing fitness.

A study by Goran et al.[26] assessed 129 pre-pubertal lean and obese children (ages 8.6 ± 1.6 years and 8.9 ± 1.2 years, respectively). Aerobic fitness was assessed using a VO_{2max} treadmill test to volitional exhaustion, and body composition was assessed using dual energy x-ray absorptiometry (DXA). It was found that compared to lean children, overweight and obese children do have a higher absolute VO_{2max} (1.24 ± 0.27 L· min^{-1}vs 1.56 ± 0.40 L· min^{-1}), mainly because of their larger body size.[26] When normalized to relative total body weight, the lean children had higher VO_{2max} values compared to overweight/obese children (44.2 ± 3.2 ml·kg^{-1}· min^{-1} vs. 32.0 ± 4.1 ml·kg^{-1}· min^{-1}). This study demonstrates the inverse relationship between excess weight and fitness in children. Specifically, overweight and obese children demonstrate a lower submaximal exercise capacity, as demonstrated by their higher submaximal heart rates, compared to normal weight children. In addition, the subjects had higher respiratory exchange ratios, and were working at a higher percent of VO_{2max} during the submaximal stages of the test.[26]

Bovet et al.[27] assessed 4,599 secondary school children, ages 12-14 years, and found a significant, inverse relationship between body mass index and fitness level as measured by the multistage shuttle run. A high fitness level was found in 29.6% of normal weight children compared to 7.9% of overweight children and 1.2% of the obese children.[27] Although fitness variables were not measured using indirect calorimetry like the previous study, this study warrants mentioning because it does display dramatic differences in fitness level between children of different weight status. These results reiterate the importance of accounting for

weight status, and the negative impact that poor body composition has elicit on fitness levels in children.

Aerobic Fitness and Racial Differences

It is possible that there are racial differences in aerobic fitness. It has been suggested that race contributes significantly to the variation in fitness levels.

A study by Trowbridge et al.[28] assessed African American and Caucasian children in order to determine whether or not differences in VO_{2max} existed across racial groups. Results showed that the African American children had lower VO_{2max} values than Caucasian children (1.21 ± 0.03 vs. 1.430 ± 0.03 $L \cdot min^{-1}$, $P < 0.01$) and remained significant even after controlling for body weight, lean body mass, total energy expenditure, and activity energy expenditure.

An additional study by Shaibi et al.[29] assessed African American, Latino, and Caucasian children and found that African American children had significantly lower absolute VO_{2peak} values compared to Latino and Caucasian children (1.57 ± 0.05 vs. 1.68 ± 0.05 vs. 1.84 ± 0.04 $L \cdot min,^{-1}$ $P < 0.05$) while controlling for pubertal status, gender and body mass.

These studies clearly demonstrate the association between race and aerobic fitness, and suggest that racial differences may be in important factor to consider when assessing fitness levels. These variations could be explained by physiological differences in African Americans compared to Caucasian and Latino children. For example, African Americans may have lower hemoglobin levels,[30] which may influence the oxygen carrying capacity to the working muscles during exercise. In addition, differences in skeletal muscle fibers have also been suggested. Research had suggested that African Americans have a greater percentage of type II fibers.[28, 31] Racial differences may also influence physical activity levels[28], which may in turn affect the fitness level of a child.

Aerobic Fitness and Sex and Maturity Differences

The sex differences in VO_{2peak} among boys and girls are still somewhat unclear despite the number of studies that have addressed the issue. Traditionally, pre-pubertal boys exhibit higher peak VO_2 values than girls, and it is estimated that this difference may be 4 to 18% higher in some cases.[32] However, once puberty begins, boys' relative VO_{2max} tends to maintain, while girls VO_{2max} declines.[33] This trend is evidence that maturation may play an influential role in aerobic fitness; however, there is little research supporting the relationship. To date, only a small influence of maturation has been shown to contribute to aerobic fitness (5% in boys and 8% in girls). More research is needed to better establish the influence of the maturation process on aerobic fitness. In order to control for the potential effect of maturation on aerobic fitness and physical activity, only pre-pubertal children were recruited and assessed for this study.

Aerobic Fitness and Scaling of VO_{2peak}

One aspect of aerobic fitness that needs to be considered when assessing fitness in children is allometric scaling. Instead of reporting O_2 uptake in absolute or relative measures, it may be more appropriate to scale per kilograms of lean body mass. Previous research has questioned the detrimental effect of excess body fat on aerobic fitness.[26, 34] Therefore, it might be more reasonable to assess oxygen uptake in terms of the lean mass that physiologically contributes to exercise performance. This lean mass scaling technique has been explored, and results are equivocal. It is especially important to use scaling when working with obese subjects, since the contributing factors of aerobic fitness such as lean body mass may be masked by the high percent fat. Therefore, a better physiological picture might be seen when oxygen uptake is expressed relative to lean body mass.[33]

Davies et al.[35] measured body composition and fitness of 17 obese and 17 normal weight girls and young women in order to assess differences in VO_{2max} using different scaling methods. There were no significant differences in VO_{2max} ($L \cdot min^{-1}$) in obese subjects compared to normal weight subjects. However, when subjects were normalized for lean body mass, the VO_{2max} decreased by approximately 16% in obese subjects compared to normal weight subjects (44.4 ± 6.5 $ml \cdot kg_{FFM}^{-1} \cdot min^{-1}$ vs. 53.0 ± 6.9 $ml \cdot kg_{FFM}^{-1} \cdot min^{-1}$).[35] This is an important finding because it demonstrates the use of normalizing for lean body mass for teasing out differences between groups. For children, this may be a more beneficial mode of making comparisons.

Normalization is important because it takes into account body composition and calculates the peak oxygen uptake during a maximal exercise test relative to the lean mass of the subject. Due to a lack of normalization and varying methods of reporting relative VO_{2peak} values, it is unclear to what extent body composition influences aerobic fitness within children.

Physical Activity

By definition, physical activity is "any bodily movement produced by skeletal muscle that results in caloric expenditure."[36] Exercise is defined as "planned, structured, and repetitive bodily movement done to improve or maintain one or more components of physical fitness."[36] It is important to note that the primary mode of physical activity in adults is exercise, but physical activity is much more sporadic and intermittent in children.[37] Therefore the benefits that planned exercise elicits in adults may not apply to children. However, as often seen in adult literature, it is likely that physical activity positively influences health-related fitness, and therefore influences cardiovascular health. In addition, it is possible that physical activity may act as a preventative measure against obesity, as well as function in the treatment for individuals with increased weight status.[38] Based on established evidence supporting this notion, guidelines for

physical activity have been established by Strong et al.[39] and the US Department of Health and Human Services.[40] These organizations recommend that all youth accumulate 60 or more minutes of moderate to vigorous physical activity daily.[39, 40]

Physical Activity Measurements Using Accelerometry

The assessment of habitual physical activity in children can be difficult. However, advances in devices and methods allow for more accurate physical activity assessment. Activity monitors, specifically accelerometers, are one way to objectively assess physical activity. These devices are typically small, computer interfaced monitors that detect bodily movements to quantify a time and intensity of activity.[41] In addition, accelerometers require little burden on the subject in that they record and store the data, making them ideal for use in youth. It has been established that in order to capture an accurate representation of habitual physical activity in children, at least three days with three consecutive hours of wear time must be fulfilled.[42] Ideally, children will wear the monitor for a full seven days with hopes that at least three days, or more, are valid and usable.[42]

Evenson et al.[43] has validated cutpoints that are used to calculate physical activity based the number of activity counts accumulated during a 15 second epoch. The study included 33 children who wore the Actigraph and the Actical, as well as a portable metabolic system in order to determine the validity and reliability of the intensities measured by the accelerometers in 5 to 8 year old children. ROC curves were used to create cutpoints. The cutpoints used for analysis are as follows: sedentary (0-25 counts), light (26-573 counts), moderate (574-1002 counts), vigorous ($\geq$ 1003 counts). The establishment of these cutpoints allows for an accurate calculation of time spent in various intensities of activity based in the 5 – 8 year old population.

One of the limitations of accelerometry that needs to be considered is that the devices are unable to measure static activities such as strength training, and also increases in intensity due to carrying loads or walking up an incline.[38] Even with this limitation, the accelerometer still allows for an accurate, objective measure of physical activity in children.

Physical Activity and Aerobic Fitness

In adults, there is a direct relationship between physical activity and aerobic fitness. However, this relationship has not been well established in children. Inactivity is an independent risk factor of cardiovascular disease, and it has been suggested that physical activity tracks though childhood, into adolescence and adulthood. Therefore, it is important to determine the role that physical activity plays in the aerobic fitness level of a child in order to help with prevention of higher weight status and chronic disease.

A potential complication to the establishment of a clear relationship between physical activity and fitness in children is the wide variety of methods used to assess children's physical activity levels, as well as different modes of assessing and reporting fitness. [44] In a review paper by Morrow and Freedson,[20] the authors discuss numerous studies reporting either no association or positive associations between activity and fitness. These studies have determined that there may be a weak correlation between the two variables. Included in the review is a study by Seliger et al.[45] Physical activity was assessed in 12 year old boys using an interview questionnaire and heart rate monitoring as measures of physical activity, and assessed VO_{2max} using a cycle ergometer. There were no significant correlations found between fitness and physical activity. A similar study by Mirwald and colleagues[46] assessed physical activity and VO_{2max} on a treadmill and also did not find any significant associations. LaPorte et al.[47] used questionnaires to assess physical activity and three different tests (mile run, treadmill time to

exhaustion, and VO_{2max}) in order to estimate fitness; and similar to the other studies, found no significant associations.

Despite a number of studies that found no relation between the two variables, encouraging results linking physical activity and fitness have also been reported. A study by Rowlands et al.[48] found results supporting the positive influence of a child's physical activity level on their fitness. Thirty-four subjects, ages 8 to 10 years, were followed for six days using the Tritrac-R3D activity monitor, and their aerobic fitness was measured using the Bruce treadmill protocol. Although this study does not include direct measures of oxygen uptake, authors did use time to exhaustion as an indicator of aerobic fitness. Authors concluded that there was a positive correlation between the both boys' and girls' aerobic fitness and their activity levels measured in minutes of activity by the Tritrac ($r=0.66$, $P< 0.05$).[48] This significant correlation is noteworthy because it demonstrates the importance of physical activity on overall aerobic fitness. An understanding of how physical activity translates into improved fitness is equivocal; however these studies may be used to justify the need for physical activity promotion in both normal weight and high weight status children to improve fitness.

A more recent study by Dencker and collegues[44] found similar results to Rowlands in a group of 248 Swedish children, ages 8 to 11 years old. Aerobic fitness, measured as VO_{2peak}, was assessed using a cycle ergometer. Results showed that VO_{2peak} was significantly correlated with activity levels, as measured using an accelerometer, for boys and girls ($r = 0.23$ and $r = 0.23$, $P < 0.05$, respectively).

Overall, research has demonstrated a weak-to-moderate correlation between physical activity and aerobic fitness within the pediatric population.[20] Research with longitudinal,

objectively monitored physical activity is still needed to assess the dose needed to benefit aerobic fitness in youth.

Genetic Individuality and Aerobic Fitness

A reasonable argument that may arise when discussing cardiovascular health is the influence of genetic individuality on our ability to develop, improve and maintain fitness levels. At the basis of our genetic variation is the allele. Alleles are defined as alternative forms of a gene, and each different combination of two alleles can then be defined as a genotype. These different genotypes are responsible for the determination of genetic traits. Observable traits are then manifested into physical features known as phenotypes. A phenotype is specifically defined as an observable, physical attribute or property that is genetically controlled, such as hair and eye color. For this study, the aerobic fitness of an individual is the phenotype of interest.[49]

Genetic contributions to aerobic fitness were not assessed until the early 1990's with the first studies assessing blood cell antigens and enzyme markers and the effects of the markers on skeletal muscle and endurance phenotypes. In an effort to help establish future research directions within the fields of exercise physiology and genetics, *Medicine and Science in Sports and Exercise* published a series of reports entitled The Human Gene Map for Performance and Health-Related Fitness Phenotypes[18, 50-56] and more recently renamed Advances in Exercise, Fitness and Performance Genomics.[57-58] These review articles provide annual updates of additions made to the literature. Since its original publication in 2001, hundreds of articles examined the relationships found between performance and genetics. The published studies have aided in identifying key candidate genes contributing to health-related fitness which include hemodynamics such as heart rate, blood pressure, and heart morphology, as well as body composition, anthropometry, insulin, glucose metabolism, blood lipids, lipoproteins, and

hemostatic factors during exercise.[50] The following sections will discuss the relationships found between six of the determined aerobic fitness candidate genes and aerobic fitness.

Angiotensin Converting Enzyme Gene and Aerobic Fitness

To date, the Angiotensin Converting Enzyme (ACE) gene is the most widely studied candidate gene in the field of exercise physiology. The ACE gene contributes significantly to the function of the renin-angiotensin system and is primarily responsible for converting Angiotensin I to Angiotensin II, as well as for its role in facilitating the degradation of vasodilatory substances in order facilitate an increase in blood pressure.[59] Since ACE plays a primary role in the renin-angiotensin system, down-regulation of ACE may be beneficial, since high levels of ACE may predispose an individual to high blood pressure. Higher circulating levels of ACE are most commonly associated with specific genotypes, whereas lower circulating levels have been found within different genotypes.

The relationship between ACE and aerobic fitness is unclear and requires further investigation in order to determine if the relationship holds true. The gene's association to fitness has shown equivocal results due to the large number of studies done in which both significant and non-significant results have been determined.

Previous case-control studies have found consistent results demonstrating that the ACE I/I genotype is more prevalent in athletes than in the controls. The first article known to date assessing the relationship between ACE and performance was published in 1998 by Gayagay et al.[60] This study included 64 Australian national rowers, and found that compared to a normal population, the rowers exhibited a higher distribution of the ACE I allele and ACE I/I genotype. Myerson et al.[61] published results demonstrating that within a group of 1,086 elite runners, there was an increasing frequency of the I allele as the distance of the runner's event increased, when

compared with a non-athlete control group (0.35, 0.53, 0.62 for ≤ 200 m, 400-3000 m and ≥ 5000 m, respectively).[61] Previous studies have also demonstrated findings similar to these within marathon runners.[62]

The HERITAGE study is one of the largest data sets of genetic and health-related outcomes. A study by Rankinen et al.[63] of the HERITAGE group reported insignificant differences between the ACE genotype and VO_{2max} after a training program. The training program does show how individuals with differing genotypes may respond to exercise, and mimics, to an extent, the influence habitual physical activity may play. The study included 724 adults who were healthy and sedentary, and participated in a 20-week endurance training program.[63] Across genotypes, VO_{2max} values were not different, however parents and children with the DD genotype did show increases in VO_{2max} following the training program. Although findings were not significant, the results do indicate the potential relationship between ACE and cardiorespiratory fitness in both adults and children.

These studies demonstrate that there may be a potential association between the I allele of the ACE gene and improved endurance performance. The ACE gene is an important candidate gene not only because of the extent to which is has been studied, but also because of the role the renin-angiotensin system plays in exercise response. It is important that we better understand individual blood pressure responses and how that affects fitness levels and responses to exercise. If varying responses are present among genotypes, then individuals may respond differently to training programs.

β-2 Adrenergic Receptor Gene and Aerobic Fitness

The β-2 adrenergic receptor (ADRβ2) is involved in many different critical pathways related to aerobic fitness. These receptors are located on the plasma membrane of the cell, and are

responsible for binding with primarily epinephrine in order to initiate the adenylate cyclase cascade. This adenylate cyclase cascade then goes on to stimulate cyclic adenosine monophosphate (AMP) in order to alter the rate at which cellular processes, such as fat metabolism, occur.[64]

The primary effect of the β-2 adrenergic receptor is to increase both bronchodialation and vasodilation,[65] and although lung function is not a limiting factor in most aerobic activities, peripheral vasodilation is an important aspect of aerobic performance and ultimately fitness levels. Therefore, the ADRβ2 gene is a potential candidate gene because of its function in cardiovascular responses to exercise such as vasodilation[21] as well as its regulation in lipid mobilization from adipose tissue. [66] Obesity, activity, and the ADRβ2 gene have been previously assessed in a study by Meirhaeghe et al.,[67] which found that sedentary men with the Gln27Glu polymorphism of the ADRβ2 gene had a nearly 3.5 times greater risk of developing obesity compared to active men. However, the relationship between activity, aerobic fitness and ADRβ2 has not been assessed in detail. Since fitness is a moderator in the development of obesity, it is important that we explore this relationship as well.

ADRβ2 has two commonly studied alleles, Gln and Glu, located at the ADRβ2 codon 27, which can be combined to form three different genotypes: Gln27Glu, Glu27Gln, and Glu27Glu. A study by Moore et al.[68] assessed the Gln27Glu polymorphism, and its association with aerobic fitness in a group of 63 non-obese, postmenopausal women who were categorized as either sedentary or athletic. Results of the study showed that women with the ADRβ2 Glu27Glu genotype had a higher weight, higher BMI, and lower VO_{2max} when compared with the ADRβ2 Glu27Gln and ADRβ2 Gln27Gln. The study concluded that Glu27Gln and Gln27Gln were

significantly associated with aerobic performance within older women compared with the Glu27Glu genotype.

A similar study by Macho-Azcarate et al.[69] determined that in obese women Glu27Glu genotype, there were significantly lower rates of fat oxidation compared to obese women with the Gln27Gln genotype. It has been previously shown that the ADRβ2 gene may be linked with a higher BMI measurement and higher fat mass.[68, 70] These studies demonstrate that there is a relationship between a ADRβ2 polymorphism, specifically the Glu27Glu genotype, and lower oxygen consumption.

Another instrumental study on the role of ADRβ2 and aerobic performance is that of Wolfarth et al.[66] This case-control study included 297 sedentary controls and 313 male elite endurance athletes, and found that a different genotype, the Arg16Gly genotype, was associated with increased aerobic performance. In addition, when compared to carriers of the Gly allele, it was found that the Gly allele elicits an unfavorable effect regarding endurance performance and that increases in oxygen consumption may be partially attributed to the possession of the Arg allele within the ADRβ2 gene.

Although there have been significant relationships found between the ADRβ2 gene and aerobic fitness, research is still needed to determine the extent to which the gene plays a role in lipid metabolism during aerobic activity and how different polymorphism may or may not determine how aerobically fit an individual may become. Currently, there is no research on the relation of the ADRβ2 gene and fitness in children, and it is critical that we explore how this gene influences fitness in children since their lipid metabolism may function differently compared to adults.

Endothelial Nitric Oxide Synthase Gene and Aerobic Fitness

The Endothelial Nitric Oxide Synthase Gene (NOS3) is one of three genes primarily involved in the mediation of nitric oxide. The release of nitric oxide causes the relaxation of blood vessels in order to facilitate a reduction in blood pressure.[71]

As previously mentioned, most genetics research in relation to exercise response deals with the area of sport performance. One of the first studies done assessing NOS3 and aerobic performance was done by Saunders et al.[72] This was a case-control study and included Ironman triathlon finishers and a group of controls. Specifically, authors were interested in exploring the role of the kallikreinkinin system (KKS) and the degradation of bradykinin in order to elicit an increased release of the vasodilator nitric oxide (NO) by determining the association between triathlon finishing times and certain NOS3 polymorphisms related closely to the KKS and nitric oxide mechanism. Since the production of NO is controlled within the NOS3 gene, it is important to assess the gene's polymorphisms and how these differences might influence performance in endurance events. The triathlon finishers' times were divided into tertiles, and it was found that the prevalence of the NOS3 GG genotype increased as the finishing times decreased [fastest times (35.0%) vs. middle times (40.4%) vs. slowest times (46.9%)] compared to the GA or AA genotype.[72] Therefore, it may be possible that those athletes with the slowest times and the GG genotype may have decreased NO production.

Although this study by Saunders et al.[72] focuses on performance within a sample of athletes, the findings help to establish a relationship between the cardiorespiratory system and the NOS3 genotype. The mechanisms proposed by the authors that the NOS3 gene plays a key role in endurance type activities can be applied to populations outside athletics as well, since the role of vasodilation and mitochondrial metabolism are essential to a wide variety of endurance

activities, either performance or health-related. This relationship can be used as building block for research questions aimed at a better understanding of aerobic fitness and how an individual's genetic background might dictate some aspects of fitness and the cardiorespiratory system.

Although there have been few studies assessing the relationship of NOS3, the studies have had a wide variety of variables and populations assessed. Hand and colleagues[73] assessed the NOS3 gene and the G894T and T-786c polymorphism in order to determine whether or not relationships in exercise hemodynamics, including heart rate, blood pressure, cardiac output, stroke volume, arteriovenous oxygen difference (a-vO2 diff), and total peripheral resistance existed among different polymorphisms. Subjects included 63 postmenopausal women who were divided into three groups: sedentary, physically active and endurance trained. No significant NOS3 G894T allele differences for habitual physical activity, blood pressure, cardiac output or a-vO2 differences were noted. There were differences in both submaximal and maximal heart rate. Specifically, non-carriers of the G894T genotype had higher submaximal exercise heart rates than the carriers (120 ± 2 vs. 112 ± 2 bpm, $P = 0.007$). Non-carriers also had higher maximal heart rates during exercise testing (165 ± 2 vs. 158 ± 2 bpm, $P = 0.04$).[73] G984T heterozygote genotypes tended to have higher stroke volume compared to the non-carriers during both submaximal and maximal exercise (78 ± 2 ml/beat vs. 72 ± 2 ml/beat, $P = 0.03$, and 70 ± 2 ml/beat vs. 64 ± 2 ml/beat, $P = 0.08$, respectively).[73]

One of the main strengths of the study by Hand et al.[73] was the inclusion of a variety of different activity levels. By including sedentary, physically active and trained individuals, the authors were able to get a basic understanding of exercise hemodynamics and how the NOS3 G894T genotype is distributed among the wide range of exercise responses. These findings are an important contribution to the literature due to the applicable nature of their outcome variables

in many different populations. A better grasp of how individuals with different genotypes will respond to graded exercise testing in terms of heart rate and stroke volume responses, both which are important to an individual's aerobic fitness, may help with future testing and exercise prescription.

Interleukin – 6 Gene and Aerobic Fitness

The role of the Interleukin – 6 (IL6) gene lies primarily in the role of inflammation and the maturation of Beta cells, and is released mainly at the site of both acute and chronic inflammation. This cytokine is also discharged into the bloodstream following a muscle contraction, and acts in decreasing insulin resistance and also increasing lipid breakdown within the individual. Previous studies have linked individuals with high IL6 markers and C-reactive proteins (CRP) with the development of Type II Diabetes, decreased muscle mass, decreased muscular strength, as well as risk of low fitness levels.[74-75] Low fitness levels may be the result of negative atherosclerotic changes in the artery elicited by high circulating inflammatory markers, specifically IL6 and CRP. Inversely, high fitness levels could possibly offset the damages caused by circulating inflammatory markers. These potential implications related to high levels of circulating IL6 and CRP, deem it necessary to assess the effect of this gene on aerobic fitness, and also its interaction with physical activity on fitness.

Fitness and physical activity have both been linked with the IL6 gene,[76] showing an inverse relationship between individuals with a lower fitness typically have inflammatory and markers such as IL6 as well as CRP. A study by Isasi et al.[76] assessed physical fitness and CRP levels of 205 subjects, ages 6-24 years. In the study, fitness was measured using a treadmill test, and the study determined that fitness and CRP levels were inversely related, meaning that as an individual's fitness decreased, their CRP levels increased.

Ortlepp et al.[77] assessed a group of 1,929 healthy young male smokers, and a secondary objective of the study was to determine the relationship of the IL6 gene to physical fitness. Physical fitness was measured using a cycle ergometer, and genotyping was determined using blood samples. Results of the study demonstrated that those who smoked and had the C/C genotype had lower aerobic fitness than those individuals who smoked with the G/G genotype.[77] However, within non-smokers, there were no differences in aerobic fitness.

Although these studies do not directly assess the IL6 gene's involvement in aerobic fitness, the gene's function in the production of inflammatory markers, including CRP levels, warrants the assessment of how the gene may relate to aerobic fitness.

Insulin-Like Growth Factor - I Gene and Aerobic Fitness

The Insulin-like Growth Factor – 1 (IGF-1) gene regulates cell growth and differentiation in a variety of different ways, including the growth of cartilage, bone, muscle and other tissues. More specifically within the skeletal muscle, IGF-1 has been associated with increased muscle protein content, reduced muscle degradation, and hypertrophy of skeletal muscles. In addition, due to its relationship with insulin sensitivity, IGF-I is also heavily involved with glucose uptake into the muscles.[78-81]

Possibly the most significant study on IGF-1 and its relation to fitness was done by Lopez-Alarocon et al.[80] The group assessed the IGF-I_{189} polymorphism(189/189 and 189/NC) and those who were non-carriers of the polymorphism (NC/NC), and its association with lean mass, exercise economy, and exercise performance, all of which play a role in aerobic fitness.[80] The research involved 114 sedentary women, all of whom were genotyped for the IGF-I_{189} polymorphism, and then assessed their endurance performance and submaximal oxygen consumption as markers for fitness. The results demonstrated significantly longer treadmill

times with the IGF-1_{189} genotype (189/189 and 189/NC) compared to the NC/NC genotype. In addition, as a submaximal workload, there was a negative association between oxygen uptake and the carriers of the polymorphism.[80] A negative association indicates lower energy expenditure at submaximal workloads, and a higher exercise economy. This may translate into improved fitness in individuals with the heterozygote IGF-1_{189}.

These results support the potential influence of the IGF-1 gene on fitness in adults, and it is possible that a similar relationship exists in children. Due to the IGF-1 gene's function in skeletal muscle and growth, as well as insulin sensitivity, it is also possible that in the selected population of this study, there may be a wide variation of fitness values associated with the IGF-1 polymorphism due to the potential for increased insulin resistance in children of a higher weight status.

Apolipoprotein-E Gene and Aerobic Fitness

Apolipoprotein-E gene (APO-E) is responsible for making the protein, Apolipoprotein, which fuses with lipids in order to form the lipoproteins necessary for the carrying of cholesterol throughout the bloodstream. More specifically, APO-E functions in the production of very low density lipoproteins, which are used in the transport of excess cholesterol for hepatic breakdown, and aiding in triglyceride clearance.[82] The APO-E gene is considered a candidate gene for this study due to its relationship to lipid transport and breakdown, as well as for the role that physical activity plays on the regulation of cholesterol regulation. Most research concerning the APO-E gene has been done in terms of the body's response to exercise training. The rationalization for exercise training programs is because APO-E has been shown to be responsible for lipid transport, and literature has demonstrated that physical activity may aide in maintaining healthy lipid profiles.

A study by Leon et al.[83] explored the APO-E gene through a variety of questions, one being how APO-E influences an individual's aerobic fitness both at baseline and following a 20-week training program. The results demonstrated that there were no differences in maximal aerobic fitness at baseline or 20 weeks across the different APO-E genotypes. Although there were no differences shown, it does establish the need for more detailed research on the APO-E gene because of the strong associations shown between cardiovascular fitness profiles and physical activity.

A similar study done by Thompson et al.[84] assessed a group of 120 males and females who were equally distributed for the APO-E genotypes E2/3, E3/3, and E3/4 genotypes. Results showed that there were no differences between the E2/3, E3/3, and E3/4 genotypes at baseline (2.49 ± 0.86 L·min^{-1} vs. 2.62 ± 0.75 L·min^{-1} vs. 2.57 ± 0.76 L·min^{-1}; $P = 0.753$, respectively).[84] However, there was a significant increase in VO_{2max} following the 24 week aerobic exercise program among the three genotypes (0.30 ± 0.39 L·min^{-1} vs. 0.09 ± 0.25 L·min^{-1} vs. 0.34 ± 0.32 L·min^{-1}; $P = 0.001$, respectively).[84] The exercise training program applied by Thompson and colleagues[84] did demonstrate differences after a 24-week exercise training program that included exercise sessions where individuals exercised at 60 – 85% of their maximal oxygen uptake and the findings suggest that individuals with various APO-E genotypes may respond differently to exercise. This relationship between APO-E, exercise and improved aerobic fitness is encouraging because it suggests that genetic profiling may aid in the implementation of an exercise prescription that is going to be most effective for the individual.

The obesity epidemic implies that there is an increase in unhealthy lipid profiles of adults and children. Previous literature has shown a significant relationship between coronary artery plaque and dyslipidemia in youth,[85] and additional information is need to establish how

improvements in aerobic fitness may help modify these risk factors. Since aerobic fitness may vary among APO- E genotypes, it is important that we assess this gene in more detail in order to better understand physiological responses to exercise and physical activity. Although there is little research on the associations between APO-E genotype and aerobic fitness, it is necessary that we explore this relationship in children in order to delay the onset of cardiovascular disease through the use of proper exercise and physical activity prescription.

Gene-Physical Activity Interactions and Aerobic Fitness

As stated earlier, a gene-environment interaction (GxE) study is one way of assessing the interaction between habitual physical activity, the identified candidate gene, and the outcome variable. By definition, a GxE may influence the outcome variable in two different ways. The first mode by which a GxE may influence is to assess the effects of varying environments, such as physical activity levels, and how that environment may influence individuals with the same genotype. The second mode is to assess how the same environment, such as a physical activity, affects individuals with different genotypes.[86] Although research in this field is limited, there have been studies done highlighting the influence of genes and an individual's environment on key factors pertaining to fitness. Studies have linked certain genes to cardiovascular disease risk factors including aerobic fitness (ACE), obesity (ADRβ2), blood lipids (APO-E), and blood pressure (ACE) in adults. It is unknown whether these genes act in a similar manner in children compared with adults or if the mechanism of an environmental factor (inactivity or activity) alter the gene expressions from childhood into adulthood.

Unfortunately, the vast majority of studies assessing the gene-physical activity interaction do not use aerobic fitness as the outcome variable. Instead, the studies' outcome variables are

critical cardiovascular phenotypes that contribute to overall fitness. This section will focus primarily on those studies and their potential relationship to fitness in children.

A study by Hagberg et al.[87] assessed the effect of the ACE II, ID, and DD genotypes on VO_{2max} among a group of postmenopausal women with varying physical activity levels. The study determined that there was no significant interaction between the different genotypes and physical activity levels on VO_{2max}, indicating that the influence of genotype on fitness was relatively similar across all physical activity levels (II, 33.4 ± 7.6 vs. ID, 30.1 ± 8.5 vs. DD, 27.1 ± 5.8 $ml \cdot kg^{-1} \cdot min^{-1}$). It has been established that the ACE gene is related to fitness; however to what extent the gene dictates a fitness level is unknown. Gene interaction studies such as this are crucial to the understanding of how genetics contributes to fitness across a variety of physical activity levels.

Additional GxE studies on physical activity and fitness focused on fitness level, but also include hemodynamic responses in order to potentially tease out if certain hemodynamic factors of fitness have an increased genetic contribution. Roltsch et al.[88] not only assessed fitness (VO_{2max}), but also variables of submaximal and maximal exercise including: cardiac output, stroke volume, heart rate, blood pressure, total peripheral resistance and arteriovenous oxygen difference. Results found that, similar to Hagberg et al.[87] that there were no significant associations of the ACE II, ID or DD genotypes on hemodynamic responses or VO_{2max}. In addition, there was no significant interaction effect of genotype and physical activity level on VO_{2max}, indicating that genotype effect was consistent across sedentary and endurance-trained young women.[88]

A third study assessing the GxE question includes the ADRβ2 gene. McCole et al[89] assessed the ADRβ2 and ADRβ3 gene in 62 postmenopausal women in order to help better

understand the influence of genotype and habitual physical activity on fitness levels. Physical activity was self-reported, DNA samples were obtained via venous blood samples, and aerobic fitness was assessed using a VO_2 submaximal and maximal treadmill test. Results of the study showed that there were significant interactions between ADRβ2 genotype and submaximal a-vO2 differences in the adult women, however there was no significant interaction effect between the Gln/Gln, Gln/Glu, and Glu/Glu genotypes and physical activity levels on VO_{2max} (Gln/Gln, 29.1± 1.6 vs. Gln/Glu 32.4 ± 1.4 vs. Glu/Glu 24.8 ± 2.5 $ml \cdot kg^{-1} \cdot min^{-}1$).[89] Despite the lack of a significant interaction of genotype and physical activity, it was still determined that there were statistically different VO_{2max} differences between genotype groups. This finding is important because it reiterates the potential genetic differences in fitness, and provides evidence that a GxE may still exist despite the study's findings.

Unfortunately, the limited research on the gene-physical activity interaction has shown inconclusive results in adults. Previous GxE studies have included the ACE and ADRβ2 gene, but due to the associations found between the remaining candidate genes of this study (NOS3, IGF-1, IL6 and APO-E) and fitness, all six will be assessed in terms of a GxE.

Summary

Although there have been a number of studies done assessing genetic variation, the majority of the research on aerobic fitness, physical activity and each of the genes discussed in the earlier sections conducted in the field of genetics have been done in adults. It is important to note that it is likely that the mechanisms that occur in adults likely occur in a similar manner in children, but also that the impact of physical activity on aerobic fitness in children is not as well established. However, due to the limited increasing health risks related to low physical

activity and fitness in children, it is critical that we explore these potential relationships within the pediatric population.

CHAPTER 3: MANUSCRIPT

Abstract

Purpose: To determine the association of certain aerobic fitness and physical activity genotypes and the gene-physical activity interaction effect on aerobic fitness in pre-pubertal, African American, obese children. **Methods:** Subjects were 30 pre-pubertal, African American, obese children (9.5 ± 1.7 years) who were free of clinical disease. Height and weight were measured according to standard procedures. Body fat was assessed using dual energy x-ray absorptiometry, and DNA samples were collected using buccal swabs. Aerobic fitness was assessed using a cycle ergometer and the McMaster cycle protocol. ANOVAs were used to determine associations and interaction effects of the ACE, ADRB2, NOS3, IL6, IGF-1, and APO-E genes, physical activity and aerobic fitness. **Results:** Age, height, weight, body mass index, and waist circumference were significantly lower in girls compared to boys. Subjects averaged approximately 51 minutes of moderate-to-vigorous intensity activity per day, and girls were significantly more active than boys. There were no significant associations between the candidate genes and aerobic fitness level. $P > 0.05$). There were trends towards significance for the IL6 rs2069845 gene for absolute and relative VO_{2peak} measures ($P = 0.078$, and $P = 0.094$, respectively). There was also a trend toward significance for the ADRβ2 rs1042717 gene for leanVO_{2peak} ($P = 0.092$). **Conclusions:** In children, further research is needed that includes diverse populations and large sample sizes in order to more accurately assess the association and interaction effects of the candidate genes, physical activity and fitness.

Introduction

Low aerobic fitness has been identified as an independent risk factor for cardiovascular disease and increased mortality in both adults and children.[1-2] Specifically, associations between

aerobic fitness, referred to as fitness for the remainder of the paper, blood pressure, lipid values, and obesity have all been shown in the pediatric population, and may play a significant role in the development of chronic disease later in adulthood.[3] Aerobic fitness is an important aspect of health, and various factors play a key role in the development and maintenance of fitness levels including weight status, physical activity levels, and genetics.

Weight status may play one of the most important roles in relation to fitness. Approximately 35.5% of children, ages 6-11 years, in the United States fall at or above the 85th percentile for Body Mass Index (BMI) using Centers for Disease Control and Prevention (CDC) age- and sex- growth charts.[4] Furthermore, in the African American population, 37.6% of 6-11 year old children are above the 85th percentile.[4] Not only is high weight status related to an increased number of cardiovascular disease risk factors, but the presence of obesity has also been found to be inversely related to fitness levels.[5] Research has shown that physical inactivity may also negatively impact fitness levels. Therefore, it is important to better understand variables contributing to fitness within the pediatric population. [6]

High levels of physical activity are associated with reducing the risk of all-cause mortality, diabetes, and cardiovascular disease in the adult population, and it is possible that a similar relationship exists in children.[7-8] Previous research has shown that physical activity is positively associated with aerobic fitness in children; however, these studies have only shown moderate/weak correlations.[9-10] Research has also shown that race and maturation may also influence the fitness level of a child.[29, 90-91]

Genetic make-up is another contributing factor to fitness levels. Genetic studies have only been conducted in adults and have found that an individual's genotype explains a portion of the variability (25-66%) in fitness.[11-16] Unfortunately, few studies[87-89] have assessed the

interaction between habitual physical activity and genotype and the role these two variables may play on fitness.

Aerobic fitness is a multi-factorial phenotype, meaning environmental interactions (i.e. lifestyle, diet) play a role in the development of fitness.[17] Candidate genes have been identified that relate to both aerobic fitness and physical activity. Angiotensin Converting Enzyme (ACE) is the gene associated with physical activity and fitness in adults, and the remaining genes β2 Adrenergic Receptor (ADRβ2), Endothelial Nitric Oxide Synthase 3 (NOS3), Interleukin 6 (IL6), Insulin-like Growth Factor 1 (IGF-1), and Apolipoprotein-E (APO-E) are all related to fitness in adults. [18] These candidate genes have been established in the adult literature, and it is possible that these same relationships exist in children. In order to control for the effect of race, maturity, and body composition on fitness levels and genetic variation, only African American, pre-pubertal, obese children will be included in the study.

Therefore, the purpose of this study was to determine the association of genotype and fitness in the specified population. A secondary purpose of the study was to assess the interaction effect of genotype and physical activity level on aerobic fitness.

Methods

Study Participants

Subjects were 30 African American children (boys aged 6-12 years old and girls aged 6-9 years old) who were obese, pre-pubertal, and had no clinical diagnosis of any other physical disease except obese weight status.

Recruitment and Testing Sites

This was a multi-site study that included clinics in Memphis, TN and Knoxville, TN. The Memphis site was overseen by pediatric endocrinologists at a diabetes and obesity lifestyles

clinic. The Knoxville site included pediatricians at a weight management clinic. These sites specialized in the care of overweight and obese children, and assessed pubertal status as standard of care.

Primary recruitment of the subjects was performed by a study investigator during, or shortly after, the child's initial visit to one of the clinics. During the initial visit to the pediatric endocrinologist or pediatrician, BMI was calculated, pubertal status was assessed, and inclusion/exclusion criteria were determined by the physician, and once the child was screened and deemed eligible for the study, the parent and child were contacted.

Assessments were conducted at either the Memphis site [University of Tennessee Clinical and Translational Institute, General Clinical Research Unit (CTSI/CRU)] or the Knoxville site [University of Tennessee, Applied Physiology Laboratory (APL)].

Subject Screening

The screening was conducted at the clinics. Height and weight were measured, and used to calculate a BMI percentile based on CDC age and sex-specific growth charts.[92] The percentile was then used to determine eligibility for the study and all children at or above the 95th percentile were considered eligible. The race of the child was reported by parents/guardian at the child's initial clinic visit.

Pubertal status was determined by stages using of Tanner's Criteria.[93] Medical practioners assessed developmental stages by examining the breasts (girls), genitals (boys) and pubic hair (girls and boys). This is part of the standard evaluation at the selected clinics. Children who were classified as Stage 1 or 2 for all variables were considered pre-pubertal, and therefore eligible for inclusion in the study.

Exclusion criteria for the study included diagnosis of disease as well as contraindications for maximal exercise testing in children. These criteria included: children being treated with medication for hypertension and/or diabetes, heart disease (including inflammatory cardiac disease), pulmonary disease, acute renal disease, acute hepatitis, drug overdose affecting the cardiorespiratory response to exercise, severe aortic or pulmonary stenosis, serious ventricular dysrhythmia, seizure disorder, hemorrhagic disease or asthma with the use of medication within the previous two weeks or a hospital/emergency room visit within the past year.[94]

Initial Visit

Upon arriving at the CTSI/CRU or APL, informed written consent was obtained from the parent or guardian of the child. Informed, written assent was obtained from the child. All subjects were asked to fast for at least four hours prior to the appointment and instructed to wear comfortable, loose fitting clothes and closed toe shoes.

Anthropometrics

Height and weight were assessed using standard procedures[95], and anthropometrics were then used to calculate (BMI). CDC BMI-for-age and sex-specific growth charts were then used to determine BMI percentile.[92] Waist circumference measurements (cm) were also obtained using a Gulick tape measure at the end of normal expiration halfway between the last rib and the iliac crest.[95]

Body Composition

Body composition was assessed by certified X-ray technicians via dual energy X-Ray absorptiometry (DXA) using a Hologic Discovery-A Fan Beam Bone Densitometer (S/N 80044) at the CTSI/CRU or GE Medical Systems Lunar IDXA (40782) at the APL. Previously measured height and weight were used for analysis. Subjects' hands and feet were secured in

place in order to avoid unwanted movements during the body scan. The scan took approximately 10 minutes to complete and was analyzed using pediatric software. Lean mass (kg), fat free mass (kg) and percent fat were used in analysis.

Aerobic Fitness Test

Aerobic fitness was assessed using a cycle ergometer. Prior to testing, resting systolic and diastolic blood pressure and heart rate were measured using an automatic blood pressure cuff (OMRON, Vernon Hills, IL), and the testing protocol was described to the subject in detail. The McMaster Cycle Protocol (Table 1) required the subject to pedal at 50 rpm and the initial workload was determined based on the child's height (cm). The test was a continuous protocol that increased in intensity every two minutes until volitional exhaustion.

Subjects were fitted with a face mask to continuously measure oxygen consumption (VO_2) and carbon dioxide production (VCO_2) throughout the test using a metabolic system [CardioCoachCO_2 Model 9000, Korr Medical Technologies (Salt Lake City, UT) or the Oxycon Mobile portable metabolic system (CareFusion, Yorba Linda, CA)]. Peak oxygen

Table 1: McMaster Protocol

Rate (RPM)	Height (cm)	Initial Load Watts (W)	Increment Watts (W)	Stage Duration Minutes (min)
50	<120	12.5	12.5	2
50	120-140	12.5	12.5	2
50	140-160	25	25	2
50	>160	25	50.0 (males) 25.0 (females)	2

uptake (VO_{2peak}) was determined as the highest value obtained during the last stage of the test. Calibration of the metabolic equipment was completed prior to each test. Telemetry was used to continuously monitor heart rate during the test (Polar, Lake Success, NY). During the last 15 seconds of each exercise stage, the subject was asked to point out their rating of perceived exertion (RPE) on the OMNI Scale[96] (Appendix C). The RPE scale ranges from 0 – 10 and includes pictures in order to better assess how hard the subjects feel they are working during the stage. Subjects' HR_{peak}, RER_{peak}, RPE, test time were measured, and peak MET level was calculated from the values obtained during the cycle test. The test was stopped when subjects could no longer maintain the pedal cadence, or requested to stop. Subjects were then asked to cool down for at least five minutes at a self-selected pace. The subjects' VO_{2peak} was determined as the highest value attained during the last 30 seconds of the final stage.

Physical Activity Assessment

The Actigraph GT3X (Pensacola, FL) was used to assess each subject's habitual physical activity. The monitor is tri-axial and was set to 15 second epochs in order to capture the subject's activity as accurately as possible. The cutpoints used have been validated in this population by Evenson et al.[43] Cutpoints for each intensity were set at: sedentary (0-25 counts), light (26-573 counts), moderate (574-1002 counts), vigorous ($\geq$ 1003 counts). It is recommended at least three days of activity are monitored to ensure a correct representation of the subject's habitual activity.[42] The Actigraph was placed on an elastic belt around the waist and worn over the right hip for seven days. After monitoring was complete, the subject placed the Actigraph in a pre-paid, stamped and addressed box and sent it back to the study investigator. Actilife software was used to download the data. Data was then designated into four difference

intensity categories (sedentary, light, moderate, and vigorous) using pre-determined activity count cut points and validated regression equations.[43] The number of minutes and percentage of time spent at each intensity was then calculated.

DNA Sample

Each subject's DNA was obtained by the primary investigator using buccal brushes. In order to maximize the yield of DNA from the brush, two samples were obtained. Before swabbing, each subject was instructed to rinse his/her mouth thoroughly twice with water for 30 seconds each time. DNA collection was done by firmly rolling the brush on the inside of each cheek for 20 seconds on each side. The brush was then placed in a sterile area where it was set to dry for at least 15 minutes, and then placed into a -20^{0}C freezer for storage until analysis took place.

DNA Extraction and Genotyping

DNA extraction from the buccal swabs is done based on protocols from Epicentre Biotechnologies (Madison, WI).[97] Genotypes were determined using the Applied Biosystems Instruments by LifeTech (Carlsbad, CA) Prism 7000, Taqman single nucleotide polymorphism microarrays, and SDS software.

Single nucleotide polymorphism (SNP) identification markers were identified for the following genes: Angiotensin Converting Enzyme (ACE), β_2 Adrenergic Receptor (ADRB2), Nitric Oxide Synthase 3 (NOS3), Insulin-like growth factor 1 (IGF1), Interleukin 6 (IL6), and Apolipoprotein E (APO-E). More specifically, the SNPs of the following genes were explored: ACE rs4353, rs4293, and rs4311 (chromosome 17), ADRβ2rs1042717 (chromosome 5), NOS3 rs1007311 and rs891512 (chromosome 7), IGF-1 rs2288378 and rs11111272 (chromosome 12),

IL6 rs2069845 and rs1554606 (chromosome 7) and APOE rs7412 (chromosome19). Each of these polymorphisms are present in different frequencies in a gene, and have all been identified as valid single nucleotide polymorphism for the target sample population.

Statistical Analysis

Means and standard deviations were calculated for all data. Allele frequencies were calculated from genetic data. ANOVAs were used to assess the main effect of genotype, the main effect of moderate-to-vigorous physical activity, and the interaction of the two variables on fitness. An alpha level of 0.05 was used to determine significance, and an alpha value of 0.10 was set to determine a significant trend.

Results

Subject Characteristics

A total of 30 pre-pubertal, African American, obese subjects participated in the study. Descriptive characteristics of these subjects are given in Table 2. All anthropometric data were significantly less in girls than in boys, except BMI percentile, which was not significantly different between the two groups.

Table 2: Mean Anthropometric Data of Girls, Boys, and Total Group (mean±SD)

Variable	Girls (n = 15)	Boys (n = 15)	Total (n = 30)
Age	8.7 ± 0.8	10.4 ± 2.0*	9.5 ± 1.7
Height (cm)	135.7 ± 6.9	148.5 ± 15.5*	141.9 ± 13.4
Weight (kg)	54.0 ± 8.1	75.8 ± 23.6*	64.5 ± 20.3
BMI (kg/m2)	29.3 ± 3.6	33.5 ± 6.8*	31.3 ± 5.7
BMI %ile	99.2 ± 0.5	99.2 ± 0.4	99.2 ± 0.4
Waist Circ. (cm)	81.4 ± 7.5	93.1 ± 17.1*	87.1 ± 14.1

* denotes significant differences between girls and boys ($P < 0.05$)

Genotypes

The allele frequencies of the sample compared to the general population for each individual candidate gene are reported in Table 3. The genotype frequencies for each SNP are included in Table 4.

Physical Activity Assessment

All subjects were asked to wear a physical activity monitor (Actigraph GT3X) for seven consecutive days following the lab visit. The mean minutes of wear time per day was 699 minutes. The mean number of minutes spent in each activity intensity is included in Table 5. There was a significant difference in minutes of moderate activity accumulated daily in girls compared to boys. The percentage of time spent in various activity intensities is shown in Table 6.

Table 3: Allele Frequency for All Genes in Sample Population and General African American Population[98]

Gene		Allele	Sample (%)	General (%)	Gene		Allele	Sample (%)	General (%)
ACE	**rs4353**	G	45	41	**IL6**	**rs2069845**	G	29	33
		A	55	59			A	77	68
ACE	**rs4293**	A	15	27	**IL6**	**rs1554606**	T	29	33
		G	85	73			G	71	67
ACE	**rs4311**	T	32	30	**IGF-1**	**rs2288378**	T	26	17
		C	68	70			C	74	83
ADRB2	**rs1042717**	A	34	39	**IGF-1**	**rs11111272**	G	44	36
		G	66	61			C	56	64
NOS3	**rs1007311**	A	42	38	**APOE**	**rs7412**	T	11	13
		G	58	62			C	89	87
NOS3	**rs891512**	A	2	5					
		G	98	95					

Table 4: Genotype Distribution and Frequency of Total Sample

Gene	SNP	Genotype	Total	Frequency	Gene	SNP	Genotype	Total	Frequency
ACE	**rs4353**	GG	6	20	**IL6**	**rs2069845**	GG/GA	18	60
		GA	15	50			AA	12	40
		AA	9	30					
ACE	**rs4293**	GA/AA	8	26.7	**IL6**	**rs1554606**	TT/TG	16	53.3
		GG	22	73.3			GG	14	46.7
ACE	**rs4311**	TT/TC	18	60	**IGF-1**	**rs2288378**	TT/TC	14	46.7
		CC	12	40			CC	16	53.3
ADR B2	**rs1042717**	AA/GA	16	53.3	**IGF-1**	**rs11111272**	GG	5	16.7
		GG	14	46.7			GC	16	53.3
NOS3	**rs1007311**	AA	6	20			CC	9	30
		GA	13	43.3	**APOE**	**rs7412**	TT/TC	6	20
		GG	11	36.7			CC	24	80
NOS3	**rs891512**	GA/AA	1	3.3					
		GG	29	96.7					

Table 5: Mean Daily Time Spent in Various Activities for Girls, Boys, and Total Group (mean±SD)

Time Spent in Activity	Girls (n = 15)	Boys (n = 15)	Total (n = 30)
Sedentary	384.9 ± 65.8	439.9 ± 121.1	412.4 ± 99.7
Light	237.3 ± 46.6	224.5 ± 53.5	230.1 ± 49.7
Moderate	56.0 ± 27.5	36.1 ± 20.1	46.1 ± 25.7
Vigorous	5.8 ± 3.3	4.2 ± 5.3	5.0 ± 4.4
MVPA	61.7 ± 30.1	40.3 ± 24.0	51.0 ± 28.9
Wear Time	683.9 ± 117.2	713.6 ± 126.9	698.8 ± 121.0

* denotes significant differences between boys and girls (*P < 0.05)*

Table 6: Mean Daily Time Spent in Various Activities for Girls, Boys, and Total Group

Time Spent in Activity	Girls (n = 15)	Boys (n = 15)	Total (n = 30)
Sedentary	56%	62%	59%
Light	35%	32%	33%
Moderate	8%	5%	7%
Vigorous	1%	1%	1%
MVPA	9%	6%	8%

Peak Oxygen Uptake

Physiological data during rest and for oxygen uptake are included in Table 7. There were no significant differences in resting variables between girls and boys, except in diastolic blood pressure. For VO_{2peak}, there was a significant difference in absolute VO_{2peak} ($L \cdot min^{-1}$), indicating that girls had lower oxygen uptake than boys. Girls also had a lower respiratory exchange ratio (RER_{peak}) compared to boys. VO_{2peak} measures grouped by genotype are included in Table 8. There were no significant differences in VO_{2peak}, HR_{peak}, RER_{peak}, RPE, test time, and peak MET level across genotypes.

Table 7: Mean Physiological Data for Girls, Boys, and Total Group (mean±SD)

Variable	Girls (n = 15)	Boys (n = 15)	Total (n = 30)
% Fat	43.2 ± 4.1	40.9 ± 4.7	42.1 ± 4.5
Resting Heart Rate (bpm)	75 ± 10.5	79 ± 9	77 ± 10
Resting Systolic BP (mm/Hg)	106 ± 12	113 ± 11	109 ± 12
Resting Diastolic BP (mm/Hg)	58 ± 8	65 ± 9*	61 ± 9
Peak Heart Rate (bpm)	174 ± 16	179 ± 14	176 ± 15
RER	1.01 ± 0.06	1.07 ± 0.09*	1.04 ± 0.08
RPE	8 ± 2	8 ± 2	8 ± 2
Test Length (min)	9.0 ± 2.7	8.8 ± 1.8	8.7 ± 2.2
Peak MET Value	6.3 ± 2.4	6.7 ± 1.3	6.5 ± 1.9
VO_{2peak} (L/min)	1.06 ± 0.03	1.61 ± 0.06*	1.31 ± 0.08
VO_{2peak} (ml/kg/min)	19.6 ± 4.8	22.7 ± 7.2	21.1 ± 6.0
VO_{2peak} ($ml/kg_{FFM}/min$)	35.9 ± 8.9	38.7 ± 11.6	37.2 ± 10.2

* denotes significant differences between girls and boys ($P < 0.05$)

Table 8: Mean VO2peak Measures (mean ± SE) by Genotype (mean±SD)

Gene	SNP	Genotype	n	VO_{2peak} ($L \cdot min^{-1}$)	VO_{2peak} ($mL \cdot kg^{-1} \cdot min^{-1}$)	VO_{2peak} ($mL \cdot kgFFM^{-1} \cdot min^{-1}$)	Gene	SNP	Genotype	n	VO_{2peak} ($L \cdot min^{-1}$)	VO_{2peak} ($mL \cdot kg^{-1} \cdot min^{-1}$)	VO_{2peak} ($mL \cdot kgFFM^{-1} \cdot min^{-1}$)
ACE	**rs4353**	AA	9	1300.4 ± 397.2	20.2 ± 2.1	37.0 ± 3.5	**IL6**	**rs2069845**	GG/GA	18	1185.9 ± 147.8	19.7 ± 1.4	36.4 ± 2.4
		GA	15	1361.8 ± 646.3	20.9 ± 1.6	36.7 ± 2.7			AA	12	1542.1 ± 151.0	23.5 ± 1.7	39.0 ± 2.9
		GG	6	1286.8 ± 535.0	23.6 ± 2.5	39.9 ± 4.2							
ACE	**rs4293**	GA	8	1330.1 ± 195.7	20.0 ± 2.2	35.4 ± 3.6	**IL6**	**rs1554606**	TT/TG	16	1231.9 ± 135.8	20.7 ± 1.5	37.5 ± 2.6
		GG	22	1327.8 ± 118.0	21.7 ± 1.3	38.2 ± 2.2			GG	14	1438.6 ± 145.1	21.9 ± 1.7	37.3 ± 2.7
		AA	--										
ACE	**rs4311**	TT/TC	18	1393.1 ± 129.0	20.9 ± 1.5	37.3 ± 2.4	**IGF-1**	**rs2288378**	TT/TC	14	1312.2 ± 147.8	22.3 ± 1.6	39.7 ± 2.7
		CC	12	1231.4 ± 158.0	21.7 ± 1.8	37.6 ± 3.0			CC	16	1342.6 ± 138.3	20.3 ± 1.5	35.4 ± 2.5
ADRβ2	**rs1042717**	AA/GA	16	1305.8 ± 138.2	19.8 ± 1.5	34.5 ± 2.4	**IGF-1**	**rs11111272**	CC	9	1541.4 ± 178.3	19.8 ± 2.1	36.6 ± 3.4
		GG	14	1354.3 ± 147.8	22.9 ± 1.6	40.7 ± 2.6			GC	16	1300.2 ± 133.7	22.1 ± 1.6	38.7 ± 2.6
									GG	5	1035.2 ± 239.2	21.0 ± 2.8	34.8 ± 4.6
NOS3	**rs1007311**	AA	16	1329.3 ± 229.5	23.5 ± 2.5	41.0 ± 4.2	**APOE**	**rs7412**	TT/TC	6	1315.0 ± 225.9	18.3 ± 2.5	32.6 ± 4.1
		GA	13	1365.8 ± 155.9	20.5 ± 1.7	36.2 ± 2.8			CC	24	1331.8 ± 113.0	22.0 ± 1.2	38.6 ± 2.0
		GG	11	1283.7 ± 169.5	20.9 ± 1.9	36.9 ± 3.1							
NOS3	**rs891512**	AA/GA	1	1218.0 ± 553.0	17.1 ± 6.2	31.9 ± 10.2							
		GG	29	1332.2 ± 102.7	21.4 ± 1.1	37.6 ± 1.9							

Results of the one-way ANOVAs indicated there were no significant associations between genotype and VO_{2peak} ($ml \cdot kg_{FFM}^{-1} \cdot min^{-1}$) for any of the candidate genes (Table 8). There were trends towards significance for the IL6 rs2069845 gene for absolute and relative VO_{2peak} measures (P = 0.078, and P = 0.094, respectively). There was also a trend toward significance for the ADRβ2 rs1042717 gene for leanVO_{2peak} ($P = 0.092$). These trends were determined as an alpha value between 0.05 and 0.10. ANOVA source tables are shown in Appendix F.

Discussion

The purpose of this study was to determine the association of genotype and aerobic fitness, and the interaction of genotype and physical activity on aerobic fitness. The current study revealed no association of genotype and no gene-activity interaction effect on fitness.

The study is unique to the literature because of the use of objectively monitored physical activity and measured peak aerobic fitness levels. Previous literature has identified potential associations of the candidate genes ACE, ADRB2, NOS3, IL6, IGF-1 and APO-E with fitness levels in adults; however the results of the current study are equivocal. Furthermore, few studies have focused on children, and even less has been done assessing the gene-activity interaction effect of fitness levels in children.

It was hypothesized that there would be a significant association of the genotype on VO_{2peak}. The results demonstrate no association of any candidate gene with VO_{2peak}, and this finding has been demonstrated previously in adult literature and in the limited research in children. The second hypothesis was that there would be a significant interaction effect between the genotype and activity level on VO_{2peak}. Again, this finding was insignificant, and consistent with the small body of literature published assessing the gene-activity interaction on fitness in adults and children.

However, the results of the study did show interesting findings in the genotype frequencies. Although the allele frequencies are comparable to the general African American population, some of the SNPs have an underrepresentation of genotypes in the sample. Specifically, in the ACE gene, SNP rs4293 the AA genotype is absent, and in the NOS3 SNP rs891512, the GG genotype was absent. Although this has not been previously shown in these SNPs, it is possible that there is a clustering effect of specific genotypes in the pre-pubertal, African American, obese population. This may indicate a predisposition to lower fitness and increased weight status based on the genotype, however, due to the limited number subjects, this is difficult to conclude.

Regardless, the lack of association and interaction effects found, in previous studies and the current study, emphasize the importance behavioral determinants of fitness, including physical activity and lifestyle. As mentioned previously, body composition, racial differences, maturation, and activity may all impact the fitness level of a child. The current study identifies the demographic, physiological, and physical activity level profile on the subjects that may aide in future understanding of an individual's environment and its contribution to fitness.

Obese children are significantly less physically active compared to their normal weight counterparts.[99] Although these data had no normal weight comparison group, it is evident from the data included in Table 5 that subjects were not meeting the recommended 60 minutes of moderate to vigorous physical activity guideline for children. A study by Trost et al.[99] assessed non-obese and obese children and found that on average, the obese children spent approximately 63 $min \cdot day^{-1}$ in moderate intensity activity, and 7 $min \cdot day^{-1}$ in vigorous intensity activity. The total sample of the current study spent approximately 51 $min \cdot day,^{-1}$ however the time spent in vigorous intensity activity is roughly the same as reported in Trost's study (Table 5). This low

accumulation of time in vigorous intensity activity may partially explain the results of the peak exercise test. As shown in Table 7, at peak exercise, the children barely reached a MET value that could be deemed a vigorous intensity ($\geq$ 6 METs). Therefore, this low accumulation of vigorous intensity activity may be as a result of the children's inability to physiologically sustain a MET value greater than 6.0 METs. Since our subjects' MET_{peak} during the exercise test was 6.6 ± 1.9 METs, it would be expected that very few children are accumulating much vigorous activity, and that a majority of their time is spent in sedentary, light or moderate activity.

The literature has also shown that boys are more active than girls, and results of this study demonstrate the opposite. There are a number of potential reasons for the significantly higher accumulated minutes of moderate and moderate-to-vigorous activity in girls. The girls included in this study were significantly younger than the boys (8.6 ± 1.0 years vs. 10.5 ± 1.9 years) and they were also lighter in weight and had a lower percent fat compared to the boys. The younger age of the girls may impact the activity levels because they are engaging in more active play outside, instead of participating in sedentary activities, and being lighter may allow for the girls to be more active by not limiting what types of activities or intensities they want to participate in during the day.

There are many strengths of this study. The homogenous nature of the sample population allowed for a number of different factors, including pubertal status, weight status and race to be controlled for in the study. The use of objective monitoring of physical activity is an important strength of the study because the accelerometer data allows for a valid and accurate representation of the subject's habitual physical activity, including intensity and duration of each subject's physical activity. In addition, the subjects averaged approximately 699 minutes of wear time a day, which allowed for an accurate assessment of daily activity. The use of a

maximal, graded exercise test allowed for peak exercise values to be obtained for an accurate assessment of fitness.

A major limitation of this study is the small sample size. The 30 subjects included in the analysis may not allow for an accurate representation of the activity levels or genetic associations on fitness of children of a similar age. Previous association and interaction studies have suggested that samples include in excess for 200 subjects in order to ascertain the statistical power in order to draw an accurate conclusion. Unfortunately, for this study, it was necessary for the physicians at the clinics to recruit the participants and as a result, the appropriate sample size was not able to be obtained. Future studies should include a site near the campus that allows for study investigators to take on the primary role of study recruiter during the study in order to avoid issues with subject recruitment.

CHAPTER 4: CONCLUSIONS

The main objectives of this study were to assess the association of genotype with aerobic fitness, and secondly to assess the interaction effect of genotype and physical activity on aerobic fitness in children. This study found no significant association of genotype and fitness level, and no interaction effect of candidate gene and activity level on aerobic fitness in pre-pubertal, African-American, obese children. Future studies should continue to use objective measures of physical activity and measured aerobic fitness on a larger, more diverse sample in order to further assess associations of genotype, activity levels, and fitness in children.

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APPENDICES

APPENDIX A:

Informed Parental Consent and Assent Form for Knoxville, TN

Parental or Legal Guardian Permission for Child to Act as a Subject in a Research Study

The association of certain genotypes and obesity-related disorders in pre-pubertal Black youth

Co-Principal Investigators: Dawn P. Coe, Ph.D., Assistant Professor
Department of Exercise, Sport, and Leisure Studies
338 HPER
1914 Andy Holt Avenue
Knoxville, TN 37996
(865)974-0294

Bruce Alpert, M.D., Professor
Plough Foundation Chair of Excellence
Department of Pediatrics (Cardiology)
215 Le Bonheur Physicians Office Building
777 Washington Avenue
Memphis, TN 38105
(901) 287-6380

Co-Investigator: Cheryl J. Kojima, Ph.D., Associate Professor
Naima Moustaid-Moussa, Ph.D., Professor
Dixie L. Thompson, Ph.D, Professor
Jennifer I. Flynn, B.S.

1. INTRODUCTION:

In this permission form, the word "you" means you and/or your child.

You/your child are being given the opportunity to participate in this study because you have not yet experienced puberty (when the sex organs mature), are African American and considered to be obese (very overweight). You are being given an opportunity to donate a sample, using a cheek swab, to this research study for genetic analysis. Your participation in this research study is voluntary. Please read this permission form carefully and take your time making your decision. As the study staff discusses this permission form with you, please ask him/her to explain any words or information that you do not clearly understand. We encourage you to talk with your family and friends before you decide to take part in this research study. The nature of the study, risks, inconveniences, discomforts, and other important information about the study are listed below.

Please tell the study doctor or study staff if you are taking part in another research study.

The purpose of this study is to find out whether your genes increase your chance for gaining weight, and having problems that may be caused by weight gain (high blood sugar, high blood pressure, and heart problems). Genes are like blueprints in each of our cells that determine traits that we inherit, like eye color and hair color. Genes may also influence what diseases we get and how we respond to treatment. DNA is the substance that makes up our genes.
This is a single center study and approximately 100 subjects will be participating in this study.

The study will take place at the University of Tennessee Applied Physiology Laboratory (UTAPL) located at 1914 Andy Holt, Room 309 HPER, Knoxville, TN 37996.

Your participation in this study will last approximately seven days. One and a half hours (1 ½ hours – one visit only) will be spent at the UTAPL and then you will be asked to wear an activity monitor (explained later in this form) for seven days.

2. PROCEDURES TO BE FOLLOWED:

If you choose to participate in this research, samples will be taken from your mouth. These samples will be used for the extraction of DNA. You will also be asked to have three (3) assessments completed as part of the study. Body composition (body fat and lean muscle mass) will be assessed using Dual Energy X-Ray Absorptiometry (DEXA) and waist measurement, fitness level (how much exercise you can handle at one time) will be assessed during a cycle exercise test using a stationary cycle, and activity will be measured using a physical activity monitor. These assessments will all take place at the UTAPL and will be performed for research purposes only.

Genetic Sample
You will be asked to donate a tissue sample from your mouth for genetic analysis. You will rinse out your mouth with water and a nurse/research assistant will collect tissue by rubbing a small brush on the inside of you cheek, approximately 20 times on each side. The DNA specimen, the data associated with the specimen, data generated from analysis of the specimen will be owned by the sponsor of this research study, the University of Tennessee. Your DNA sample will be retained until the study is completed (approximately 12 months). The University of Tennessee will have ownership of the genetic sample. No profit making activities will result from the research use of the sample. Results of the genetic analyses are not relevant to your health and therefore will not be shared with you. Once the genetic sample has been analyzed and the protocol is completed, your sample will no longer be needed and will be destroyed. We will hold on to your data until full analyses are complete, for approximately one year, and you will not be re-contacted to take part in the study in the future.

DEXA
The DEXA scan is able to determine the amount of fat and lean mass (bone, organs, and muscle) that makes up your body. For the DEXA assessment, you will be asked to lie still on a padded table while the machine scans your body. A whole body scan takes between 10 – 20 minutes. This x-ray scan takes pictures of your body and from these pictures we can tell how much fat and lean mass (bone, organs, muscle) make up your body.

Waist Measurement
A tape measure will be used to measure the distance around your waist.

Cycle Test
The purpose of this test is to determine your fitness level. This will be done by performing an exercise test on a cycle that gradually becomes more difficult. Prior to the test, height, weight and blood pressure will be measured. The pedal rate, how fast the pedals are moving, will remain the same throughout the test and every two minutes resistance, how hard it is to pedal, will be added to make the test a little more difficult. The test will end when you request to stop or when you

are no longer able to pedal at a certain speed. You may request to stop the test at any time. During the test, heart rate will be monitored using strap around your chest and you will wear a mask covering your mouth and nose to assess how much oxygen your body is using to make energy. Although your mouth and nose are covered with the mask you will still be able to talk during the test. The more oxygen you can use the higher fitness level you have. You will also be asked how hard you feel you are working during the test using a 0-10 rating of perceived exertion (RPE) scale. If you develop any pain or have any abnormal response to the exercise test, the test will immediately be stopped. If you wish to stop the test at any time, you can either ask for the test to stop or just stop pedaling.

Physical Activity Monitoring
You will be asked to wear a monitor to assess physical activity levels. This device is a small box, the size of a small pager that will be worn on a belt around the waist. The device will be worn for one week and you will wear the monitor at all times except during water activities (swimming, bathing, showering) and when you are sleeping. At the end of the week, you will mail the monitor to the study center using a self-addressed stamped box (provided by the researchers).

The DNA specimen, the data associated with the specimen, data generated from analysis of the specimen will be owned by the sponsor of this research study, the University of Tennessee. Your DNA sample will be retained until the study is completed (approximately 12 months). The University of Tennessee will have ownership of the genetic sample. No profit making activities will result from the research use of the sample. Results of the genetic analyses are not relevant to your health and therefore will not be shared with you. Once the genetic sample has been analyzed and the protocol is completed, your sample will no longer be needed and will be destroyed. We will hold on to your data until full analyses are complete, for approximately one year, and you will not be re-contacted to take part in the study in the future.

The following information will be collected from your medical record: date of birth, maturity status, a list of your current medications, and the results from the blood sample drawn in the clinic. This information will be collected from the medical chart because these procedures will have already been done during a standard care clinic visit, and will not need to be repeated. All of the other procedures in the study, including the collection of the genetic sample, the DEXA scan, cycle test, waist measurement, and activity monitoring, are being performed for research purposes only.

3. RISKS ASSOCIATED WITH PARTICIPATION:

Every effort will be made to minimize any discomforts or risks.

There are potential risks associated with an exercise test that may include lightheadedness, chest discomfort, leg cramps, falling, muscle sprain/strain, and abnormal blood pressure. The risk of these problems is rare (1-5% chance). Additionally, there are some very rare (<1%) risks that include: irregular heart rates/rhythms, heart attack, stroke or death. Personnel trained in pediatric CPR, will be available to take care of these situations and emergency equipment will be readily available at all times.

There are only minor risks associated with the DEXA scan. It may feel uncomfortable, as you have to lie on an open table for 12-20 minutes as the DEXA scans your body. The amount of

radiation to which you will be exposed is 1/20th of what you would receive from a chest x-ray or about the same amount of radiation received during an airline trip from New York to Los Angeles.

There is minimal risk associated with the heart rate and physical activity monitors. The belts, worn around the chest and waist that contain the device may rub on the skin, causing irritation.

There is a potential risk of unintended disclosure of confidential information to parties outside the research context that might affect your ability to get insurance or a job. However, these risks are quite remote since appropriate confidentiality measures will be taken to protect any information about your health that is revealed by your DNA sample.

4. BENEFITS ASSOCIATED WITH PARTICIPATION:

There are no direct benefits to you for participating in this research study. Your participation in this research study may provide additional information regarding the possible causes of obesity and obesity-related disorders in children.

5. ALTERNATIVES TO PARTICIPATION:

An alternative to participating in this study is to not participate in the study. You will receive the same medical care whether or not you participate in this research study.

6. CONFIDENTIALITY:

All of your paper research records will be stored in locked file cabinets and will be accessible only to research personnel.

All of your electronic research records will be computer password protected and accessible only to research personnel.

Your DNAsample will be maintained at the University of Tennessee, Knoxville campus during the study and will be labeled with a code.

Your research records and your DNA sample will be transmitted to the University of Tennessee, Knoxville campus and will be labeled with a code. A master key that links your name with the code on your research record and specimen will be maintained at the local investigative site (UTAPL).

Under federal privacy regulations, you have the right to determine who has access to your personal health information (called "protected health information" or PHI). PHI collected in this study may include your medical history, the results of physical exams, lab tests, x-ray exams, and other diagnostic and treatment procedures, as well as basic demographic information. By signing this permission form, you are authorizing the researchers at the University of Tennessee to have access to your PHI collected in this studyand to receive your PHI from your physician and facilities where you have received health care. The Institutional Review Board (IRB) at the University of Tennessee may review your PHI as part of its responsibility to protect the rights and welfare of research subjects. Your PHI will not be used or disclosed to any other person or

entity, except as required by law, or for authorized oversight of this research study by other regulatory agencies, or for other research for which the use and disclosure of your PHI has been approved by the IRB. Your PHI will be used only for the research purposes described in the Introduction of this permission form. Your PHI will be used until the study is completed.

You may cancel this authorization in writing or by phone at any time by contacting the principal investigator listed on the first page of the permission form. If you cancel the authorization, continued use of your PHI is permitted if it was obtained before the cancellation and its use is necessary in completing the research. However, PHI collected after your cancellation may not be used in the study. If you refuse to provide this authorization, you will not be able to participate in the research study. If you cancel the authorization, then you will be withdrawn from the study. Finally, the federal regulations allow you to obtain access to your PHI collected or used in this study.

Information about your participation in this study will be placed in your medical record; as such, this information could be made available to your employer or insurer.

You will not be identified in any presentations or publications based on the results of the research study.

7. COMPENSATION AND TREATMENT FOR INJURY:

You are not waiving any legal rights or releasing the University of Tennessee, or its agents, from liability for negligence. In the event of physical injury resulting from research procedures, the University of Tennessee has not budgeted funds for compensation either for lost wages or for medical treatment. Therefore, the University of Tennessee does not provide for treatment or reimbursement for such injuries. No compensation will be available for any ancillary expenses incurred as a result of research related physical injuries, additional hospital bills, lost wages, travel expenses, etc.

If you suffer a research-related injury, your study nurse will provide acute medical treatment and will provide you with a subsequent referral to appropriate health care facilities.

You and/or your insurance carrier will be billed for the costs associated with the medical treatment of a research-related injury.

Additionally, no compensation will be available to subjects for any non-physical injuries that may be incurred as a result of research participation, such as exposure to criminal or civil liability, or damage to their reputation, financial standing, or employability

8. QUESTIONS:

If you have any questions about this study, you may contact Dawn P. Coe, Ph.D.at 865-974-0294 You may contact the ETCH IRB Chairman, at 865-541-8477 if you have any questions about your rights as a participant in this study or your rights as a research subject.

9. PAYMENT FOR PARTICIPATION:

You (the child) will receive a $25.00 gift card to Toys R Us and you (the parent/legally authorized representative) will receive a $10 gas card for volunteering your time to be a part of this study when the physical activity monitor is returned to the researchers.

10. COSTS OF PARTICIPATION:

There is no cost to you to participate in this study.

11. VOLUNTARY PARTICIPATION:

Your participation in this research study is completely voluntary. You may choose not to participate or to drop out of the study without any penalty or loss of benefits to which you are otherwise entitled.

If you give your permission to Dr. Coe to use your DNA sample for the research specified above, and then you decide later, once the specimen has been collected, that you do not want the specimen to be used, you may contact Dr. Coe and indicate your wishes. If you withdraw from this research study, your specimen (DNA) and related data will be destroyed; however, any analysis that was completed before your child chooses to withdraw from the research study will be retained if it is necessary for completion of the research.

12. PERMISSION OF SUBJECT:

You have read or have had read to you a description of the research study as outlined above. The investigator or his/her representative has explained the research study to you and has answered all the questions you have at this time. You knowingly and freely choose to allow your child to participate in the research study. A copy of this permission form will be given to you for your records.

_

_____ _____ _____

Signature of Research Subject **Date** **Time**

_____

Printed Name of Research Subject

_____ _____

Signature of Person Obtaining Permission **Date** **Time**

_____

Printed Name of Person Obtaining Permission

In my judgment, the subject or the legally authorized representative has voluntarily and knowingly given permission and possesses the legal capacity to give permission to participate in this research study.

_____ _____ _____

Signature of Investigator **Date** **Time**

_____ _____ _____

Signature of Legally Authorized Representative **Date** **Time**

_____

Relationship of Legally Authorized Representative

The association of certain genotypes and obesity-related disorders in pre-pubertal Black youth

☐ The assent discussion was initiated on ______________(date) at __________ (time).

The information was presented in age-appropriate terms.

The minor: ____(Subject's Name)

☐ Agreed to take part in the study on _________________(date) at _______________ (time).
☐ Declined to take part in the study. The minor declined for the following reason(s):

● ●

☐ An assent discussion <u>was not</u> initiated with the minor for the following reason(s):

- ☐ Minor is under 8 years of age
- ☐ Minor is physically incapacitated
- ☐ Minor is cognitively or emotionally unable to participate in an assent discussion
- ☐ Minor refused to take part in the discussion
- ☐ Other____

RESEARCHER/DESIGNEE STATEMENT: I hereby certify that I have discussed the research project with the research participant and/or his/her parent(s) or legal guardian(s). I have explained all the information contained in the permission document, including any risks that may be reasonably expected to occur. I further certify that the research participant was encouraged to ask questions and that all questions were answered.

____
Researcher/Designee Printed Name

______________________________ ________________ _______________
Researcher/Designee Signature Date Time (AM/PM)

____
Minor Subject Printed Name
OPTIONAL

________________________________ __________________ ________________
Minor Subject Signature (8-13 years) Date Time (AM/PM)
OPTIONAL

APPENDIX B:

Parental Informed Consent and Subject Assent Form for Memphis, TN

The association of certain genotypes and obesity-related disorders in pre-pubertal Black youth

Principal Investigator: Dawn P. Coe, Ph.D., Assistant Professor
Department of Exercise, Sport, and Leisure Studies
338 HPER
1914 Andy Holt Avenue
Knoxville, TN 37996
(865)974-0294

Bruce Alpert, M.D., Professor
Plough Foundation Chair of Excellence
Department of Pediatrics (Cardiology)
215 Le Bonheur Physicians Office Building
777 Washington Avenue
Memphis, TN 38105
(901) 287-6380

Co-Investigator: Cheryl J. Kojima, Ph.D., Associate Professor
Naima Moustaid-Moussa, Ph.D., Professor
Dixie L. Thompson, Ph.D, Professor
Pedro Velasquez, M.D.
Nicole Barnes, M.D.
Claudia P. Neira, DNP, FNP
Jennifer I. Flynn, B.S.

1. INTRODUCTION:

In this consent form, the word "you" means you and/or your child.

You/your child are being given the opportunity to participate in this study because you have not yet experienced puberty (when the sex organs mature), are African American and considered to be obese (very overweight). You are being given an opportunity to donate a sample, using a cheek swab, to this research study for genetic analysis. Your participation in this research study is voluntary. Please read this consent form carefully and take your time making your decision. As the study staff discusses this consent form with you, please ask him/her to explain any words or information that you do not clearly understand. We encourage you to talk with your family and friends before you decide to take part in this research study. The nature of the study, risks, inconveniences, discomforts, and other important information about the study are listed below.

Please tell the study doctor or study staff if you are taking part in another research study.

The purpose of this study is to find out whether your genes increase your chance for gaining weight, and having problems that may be caused by weight gain (high blood sugar, high blood pressure, and heart problems). Genes are like blueprints in each of our cells that determine traits that we inherit, like eye color and hair color. Genes may also influence what diseases we get and how we respond to treatment. DNA is the substance that makes up our genes.
This is a single center study and approximately 100 subjects will be participating in this study.

The study will take place at the Clinical and Translational Science Institute Clinical Research Unit (CTSI/CRU) (1265 Union Ave, 8th Floor East Wing, Memphis, TN 38104).

Your participation in this study will last approximately seven days. One and a half hours (1 ½ hours – one visit only) will be spent at the CTSI/CRU and then you will be asked to wear an activity monitor (explained later in this form) for seven days.

2. PROCEDURES TO BE FOLLOWED:

If you choose to participate in this research, samples will be taken from your mouth. These samples will be used for the extraction of DNA. You will also be asked to have three (3) assessments completed as part of the study. Body composition (body fat and lean muscle mass) will be assessed using Dual Energy X-Ray Absorptiometry (DEXA) and waist measurement, fitness level (how much exercise you can handle at one time) will be assessed during a cycle exercise test using a stationary cycle, and activity will be measured using a physical activity monitor. These assessments will all take place at the CTSI/CRU and will be performed for research purposes only.

Genetic Sample
You will be asked to donate a tissue sample from your mouth for genetic analysis. You will rinse out your mouth with water and a nurse/research assistant will collect tissue by rubbing a small brush on the inside of you cheek, approximately 20 times on each side. The DNA specimen, the data associated with the specimen, data generated from analysis of the specimen will be owned by the sponsor of this research study, the University of Tennessee. Your DNA sample will be retained until the study is completed (approximately 12 months). The University of Tennessee will have ownership of the genetic sample. No profit making activities will result from the research use of the sample. Results of the genetic analyses are not relevant to your health and therefore will not be shared with you. Once the genetic sample has been analyzed and the protocol is completed, your sample will no longer be needed and will be destroyed. We will hold on to your data until full analyses are complete, for approximately one year, and you will not be re-contacted to take part in the study in the future.

DEXA
The DEXA scan is able to determine the amount of fat and lean mass (bone, organs, muscle) that makes up your body. For the DEXA assessment, you will be asked to lie still on a padded table while the machine scans your body. A whole body scan takes between 10 – 20 minutes. This x-ray scan takes pictures of your body and from these pictures we can tell how much fat and lean mass (bone, organs, muscle) make up your body.

Waist Measurement
A tape measure will be used to measure the distance around your waist.

Cycle Test
The purpose of this test is to determine your fitness level. This will be done by performing an exercise test on a cycle that gradually becomes more difficult. Prior to the test, height, weight and blood pressure will be measured. The pedal rate, how fast the pedals are moving, will remain the

same throughout the test and every two minutes resistance, how hard it is to pedal, will be added to make the test a little more difficult. The test will end when you request to stop or when you are no longer able to pedal at a certain speed. You may request to stop the test at any time. During the test, heart rate will be monitored using strap around your chest and you will wear a mask covering your mouth and nose to assess how much oxygen your body is using to make energy. Although your mouth and nose are covered with the mask you will still be able to talk during the test. The more oxygen you can use the higher fitness level you have. You will also be asked how hard you feel you are working during the test using a 0-10 rating of perceived exertion (RPE) scale. If you develop any pain or have any abnormal response to the exercise test, the test will immediately be stopped. If you wish to stop the test at any time, you can either ask for the test to stop or just stop pedaling.

Physical Activity Monitoring
You will be asked to wear a monitor to assess physical activity levels. This device is a small box, the size of a small pager that will be worn on a belt around the waist. The device will be worn for one week and you will wear the monitor at all times except during water activities (swimming, bathing, showering) and when you are sleeping. At the end of the week, you will mail the monitor to the study center using a self-addressed stamped padded envelope (provided by the researchers).

The DNA specimen, the data associated with the specimen, data generated from analysis of the specimen will be owned by the sponsor of this research study, the University of Tennessee. Your DNA sample will be retained until the study is completed (approximately 12 months). The University of Tennessee will have ownership of the genetic sample. No profit making activities will result from the research use of the sample. Results of the genetic analyses are not relevant to your health and therefore will not be shared with you. Once the genetic sample has been analyzed and the protocol is completed, your sample will no longer be needed and will be destroyed. We will hold on to your data until full analyses are complete, for approximately one year, and you will not be re-contacted to take part in the study in the future.

The following information will be collected from your medical record: date of birth, maturity status, a list of your current medications, and the results from the blood sample drawn in the clinic. This information will be collected from the medical chart because these procedures will have already been done during a standard care clinic visit, and will not need to be repeated. All of the other procedures in the study, including the collection of the genetic sample, the DEXA scan, cycle test, waist measurement, and activity monitoring, are being performed for research purposes only.

3. RISKS ASSOCIATED WITH PARTICIPATION:

Every effort will be made to minimize any discomforts or risks.

There are potential risks associated with an exercise test that may include lightheadedness, chest discomfort, leg cramps, falling, muscle sprain/strain, and abnormal blood pressure. The risk of these problems is rare (1-5% chance). Additionally, there are some very rare (<1%) risks that include: irregular heart rates/rhythms, heart attack, stroke or death. Personnel trained in pediatric CPR, will be available to take care of these situations and emergency equipment will be readily available at all times.

There are only minor risks associated with the DEXA scan. It may feel uncomfortable, as you have to lie on an open table for 12-20 minutes as the DEXA scans your body. The total amount of radiation you will receive in this study is about the same as you would receive from exposure to about one day of natural sources of radiation (about one mrem).

There is minimal risk associated with the heart rate and physical activity monitors. The belts, worn around the chest and waist that contain the device may rub on the skin, causing irritation.

There is a potential risk of unintended disclosure of confidential information to parties outside the research context that might affect your ability to get insurance or a job. However, these risks are quite remote since appropriate confidentiality measures will be taken to protect any information about your health that is revealed by your DNA sample.

4. BENEFITS ASSOCIATED WITH PARTICIPATION:

There are no direct benefits to you for participating in this research study. Your participation in this research study may provide additional information regarding the possible causes of obesity and obesity-related disorders in children.

5. ALTERNATIVES TO PARTICIPATION:

An alternative to participating in this study is to not participate in the study. You will receive the same medical care whether or not you participate in this research study.

6. CONFIDENTIALITY:

All of your paper research records will be stored in locked file cabinets and will be accessible only to research personnel.
All of your electronic research records will be computer password protected and accessible only to research personnel.

Your DNAsample will be maintained at the University of Tennessee, Knoxville campus during the study and will be labeled with a code.

Your research records and your DNA sample will be transmitted to the University of Tennessee, Knoxville campus and will be labeled with a code. A master key that links your name with the code on your research record and specimen will be maintained at the local investigative site (CTSI/CRU in Memphis, TN).

Under federal privacy regulations, you have the right to determine who has access to your personal health information (called "protected health information" or PHI). PHI collected in this study may include your medical history, the results of physical exams, lab tests, x-ray exams, and other diagnostic and treatment procedures, as well as basic demographic information. By signing this consent form, you are authorizing the researchers at the University of Tennessee to have access to your PHI collected in this study and to receive your PHI from your physician and facilities where you have received health care. The Institutional Review Board (IRB) at the University of Tennessee Health Science Center may review your PHI as part of its responsibility to protect the rights and welfare of research subjects. Your PHI will not be used or disclosed to

any other person or entity, except as required by law, or for authorized oversight of this research study by other regulatory agencies, or for other research for which the use and disclosure of your PHI has been approved by the IRB. Your PHI will be used only for the research purposes described in the Introduction of this consent form. Your PHI will be used until the study is completed.

You may cancel this authorization in writing or by phone at any time by contacting the principal investigator listed on the first page of the consent form. If you cancel the authorization, continued use of your PHI is permitted if it was obtained before the cancellation and its use is necessary in completing the research. However, PHI collected after your cancellation may not be used in the study. If you refuse to provide this authorization, you will not be able to participate in the research study. If you cancel the authorization, then you will be withdrawn from the study. Finally, the federal regulations allow you to obtain access to your PHI collected or used in this study.

Information about your participation in this study will be placed in your medical record; as such, this information could be made available to your employer or insurer.

You will not be identified in any presentations or publications based on the results of the research study.

7. COMPENSATION AND TREATMENT FOR INJURY:

You are not waiving any legal rights or releasing the University of Tennessee or the LifeDOC Clinic, or the agents of either, from liability for negligence. In the event of physical injury resulting from research procedures, neither the University of Tennessee nor the LifeDOC Clinic has funds budgeted for compensation either for lost wages or for medical treatment. Therefore, neither the University of Tennessee nor the LifeDOC Clinic provides for treatment or reimbursement for such injuries. No compensation will be available for any ancillary expenses incurred as a result of research related physical injuries, additional hospital bills, lost wages, travel expenses, etc.

If you suffer a research-related injury, your study nurse will provide acute medical treatment and will provide you with a subsequent referral to appropriate health care facilities.

You and/or your insurance carrier will be billed for the costs associated with the medical treatment of a research-related injury.

Additionally, no compensation will be available to subjects for any non-physical injuries that may be incurred as a result of research participation, such as exposure to criminal or civil liability, or damage to their reputation, financial standing, or employability

8. QUESTIONS:

If you have any questions about this study, you may contact Dawn P. Coe, Ph.D.at 865-974-0294 In the event of a research-related injury, contact Dr. Bruce Alpert at 901-287-6781. This telephone number is an office number.

You may contact Dr. Terrence F. Ackerman, Ph.D., UTHSC IRB Chairman, at 901-448-4824 if you have any questions about your rights as a participant in this study or your rights as a research subject.

9. PAYMENT FOR PARTICIPATION:

You (the child) will receive a $25.00gift card to Toys R Us and you (the parent/legally authorized representative) will receive a $10 gas card for volunteering your time to be a part of this study when the physical activity monitor is returned to the researchers.

10. COSTS OF PARTICIPATION:

There is no cost to you to participate in this study.

11. VOLUNTARY PARTICIPATION:

Your participation in this research study is completely voluntary. You may choose not to participate or to drop out of the study without any penalty or loss of benefits to which you are otherwise entitled.

If you give your permission to Dr. Coe to use your DNA sample for the research specified above, and then you decide later, once the specimen has been collected, that you do not want the specimen to be used, you may contact Dr. Coe and indicate your wishes. If you withdraw from this research study, your specimen (DNA) and related data will be destroyed; however, any analysis that was completed before your child chooses to withdraw from the research study will be retained if it is necessary for completion of the research.

12. CONSENT OF SUBJECT:

You have read or have had read to you a description of the research study as outlined above. The investigator or his/her representative has explained the research study to you and has answered all the questions you have at this time. You knowingly and freely choose to allow your child to participate in the research study. A copy of this consent form will be given to you for your records.

_

_____ _____ _____

Signature of Research Subject **Date** **Time**

_____

Printed Name of Research Subject

_____ _____ _____

Signature of Person Obtaining Consent **Date** **Time**

_____

Printed Name of Person Obtaining Consent

In my judgment, the subject or the legally authorized representative has voluntarily and knowingly given informed consent and possesses the legal capacity to give informed consent to participate in this research study.

_____ _____ _____

Signature of Investigator **Date** **Time**

_____ _____ _____

Signature of Legally Authorized Representative **Date** **Time**

_____

Relationship of Legally Authorized Representative

The association of certain genotypes and obesity-related disorders in pre-pubertal Black youth

☐ The assent discussion was initiated on _____(date) at _____ (time).

The information was presented in age-appropriate terms.

The minor: ____(Subject's Name)

☐ Agreed to take part in the study on _________________(date) at _______________ (time).
☐ Declined to take part in the study. The minor declined for the following reason(s):

● ●

☐ An assent discussion <u>was not</u> initiated with the minor for the following reason(s):

- ☐ Minor is under 8 years of age
- ☐ Minor is physically incapacitated
- ☐ Minor is cognitively or emotionally unable to participate in an assent discussion
- ☐ Minor refused to take part in the discussion
- ☐ Other____

RESEARCHER/DESIGNEE STATEMENT: I hereby certify that I have discussed the research project with the research participant and/or his/her parent(s) or legal guardian(s). I have explained all the information contained in the informed consent document, including any risks that may be reasonably expected to occur. I further certify that the research participant was encouraged to ask questions and that all questions were answered.

____
Researcher/Designee Printed Name

______________________________ ________________ _______________
Researcher/Designee Signature Date Time (AM/PM)

____
Minor Subject Printed Name

________________________________ __________________ ________________
Minor Subject Signature (8-13 years) Date Time (AM/PM)

APPENDIX C:

RPE Scale

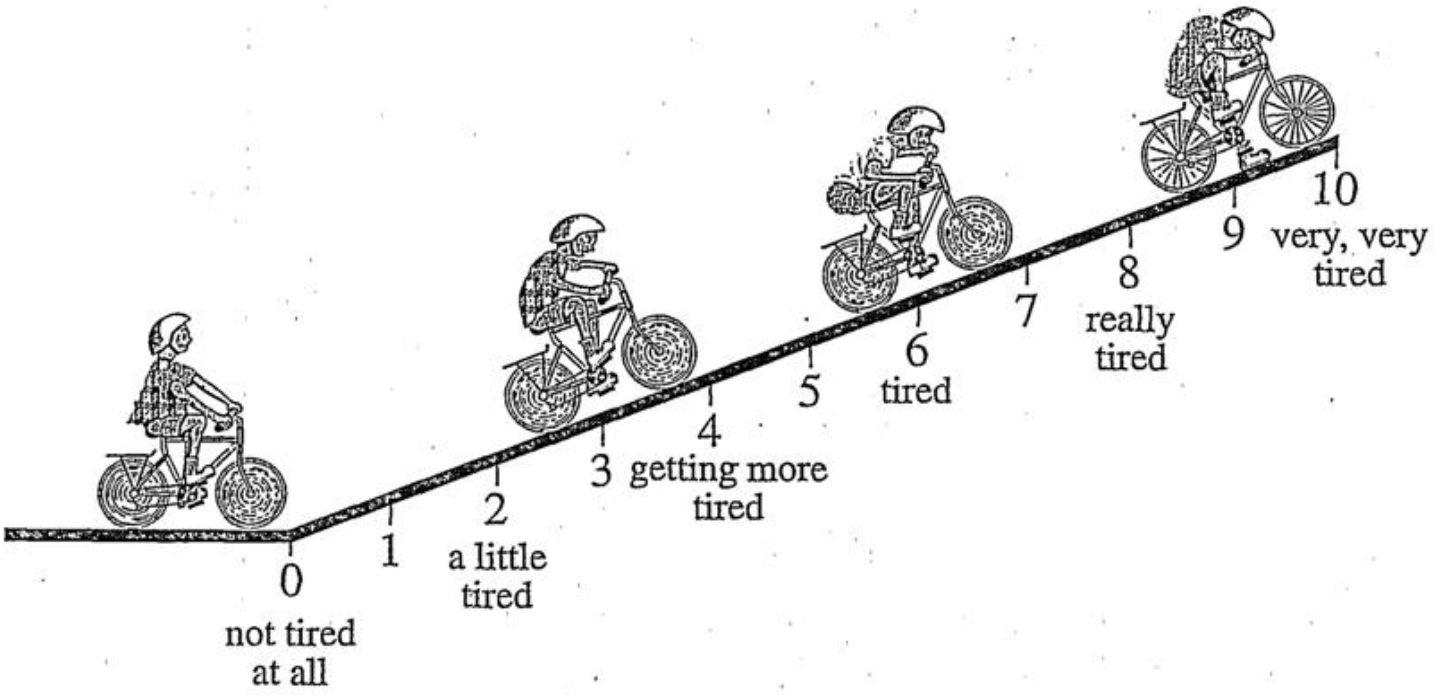

Figure 1—Children's OMNI Scale of Perceived Exertion

Robertson, R. et al. Children's OMNI Scale of Perceived Exertion: mixed gender and race validation. Med Sci Sports Exerc. 2000;32:452-458.

APPENDIX D:

SNP Assay Information

Gene Name: ACE rs4353
Angiotensin I Converting Enzyme (peptidyl-dipeptidase A) 1
Assay ID: C____589818_10
Location: Chr. 17 – 61570422
Context Sequence (VIC/FAM):
CAGTTGTTTCCTTCAGGGAGCCTCC[A/G]TCTTGGGAGATAAAGCATGTGTAC
A
Polymorphism: A/G, Transition Substitution

1) **Gene Name:** ACE rs4293
Angiotensin I Converting Enzyme (peptidyl-dipeptidase A) 1;similar to TRIMCyp
Assay ID: C___1247701_10
Location: Chr. 17 - 61555666
Context Sequence (VIC/FAM):
GGGGAGTTACTTTCTGTTAAAGGAA[A/G]CATTCTGGAGTAGGAAGCCAAATT
C
Polymorphism: A/G, Transition Substitution

2) **Gene Name:** ACE rs4311
Angiotensin I Converting Enzyme (peptidyl-dipeptidase A) 1
Assay ID: C___1247707_1_
Location: Chr. 17 - 61560763
Context Sequence (VIC/FAM):
GGTGGAAATGCCTTTTCTACAAAAG[C/T]TAAATCCATCTGTTTGCAACCTCTA
Polymorphism: C/T, Transition Substitution

3) **Gene Name:** ADRβ2 rs1042717
Adrenergic, beta-2-, receptor, surface
Assay ID: C___2084766_10
Location: Chr. 5 - 148206646
Context Sequence (VIC/FAM):
CCTGTGCTGATCTGGTCATGGGCCT[A/G]GCAGTGGTGCCCTTTGGGGCCGCC
C
Polymorphism: A/G, Transition Substitution

4) **Gene Name:** NOS3 rs1007311
Nitric Oxide Synthase 3 (endothelial cell)
Assay ID: C__11631000_1_
Location: Chr. 7 - 150696008
Context Sequence (VIC/FAM):
AGGAGGGCATGAGGCTCAGCCCCAG[A/G]ACCCCCTCTGGCCCACTCCCCAC
AG
Polymorphism: A/G, Transition Substitution

5) **Gene Name:** NOS3 rs891512
Nitric Oxide Synthase 3 (endothelial cell);ATG9 autophagy related 9 homolog B (S. cerevisiae)
Assay ID: C___7599646_1_
Location: Chr. 7 - 150708089
Context Sequence (VIC/FAM):
TTCATCCGGGGGTAAGTGAGATGGA[A/G]GACTTGGTGGGGAGCTGCCCAGG
GT
Polymorphism: A/G, Transition Substitution

6) **Gene Name:** IL6 rs2069845
Interleukin 6 (interferon, beta 2);hypothetical LOC541472
Assay ID: C___1839699_10
Location: Chr. 7 - 22770149
Context Sequence (VIC/FAM):
GTTTCCCAGTCCTCTTTACACCACC[A/G]GATCAGTGGTCTTTCAACAGATCCT
Polymorphism: A/G, Transition Substitution

7) **Gene Name:** IL6 rs1554606
Interleukin 6 (interferon, beta 2);hypothetical LOC541472
Assay ID: C___9394731_10
Location: Chr. 7 – 22768707
Context Sequence (VIC/FAM):
TTAGTTCATCCTGGGAAAGGTACTC[G/T]CAGGGCCTTTTCCCTCTCTGGCTGC
Polymorphism: G/T, Transversion Substitution

8) **Gene Name:** IGF-1 rs2288378
Insulin-like Growth Factor 1 (somatomedin C)
Assay ID: C__16184374_10
Location: Chr. 12 - 102830008
Context Sequence (VIC/FAM):
TTTTATTTGCACAGTCTGTGTCCTT[T/C]TGAATTCCAGCTAGCACCTGAAGAC
Polymorphism: T/C, Transition Substitution

9) **Gene Name:** IGF-1 rs11111272
Insulin-like Growth Factor 1 (somatomedin C)
Assay ID: C___2801102_10
Location: Chr. 12 - 102827441
Context Sequence (VIC/FAM):
GCAGACACTGTCATCTCATCCTTCT[C/G]TGTTTAGAAGCACTACACTGGGCAT
Polymorphism: C/G, Transversion Substitution

10) **Gene Name:** APOE rs7412
Apolipoprotein C-I; hypothetical LOC100129500;apolipoprotein E;translocase of outer mitochondrial membrane 40 homolog (yeast)
Assay ID: C____904973_10
Location: Chr. 19 - 45412079
Context Sequence (VIC/FAM):
CCGCGATGCCGATGACCTGCAGAAG[C/T]GCCTGGCAGTGTACCAGGCCGGG
GC
Polymorphism: C/T, Transition Substitution

APPENDIX E:

DNA and Plate Preparation Protocol

DNA Extraction:

1) If frozen, swab was first thawed for approximately ten minutes
2) Place swab in DNA extraction solution (MasterAmp Buccal Swab DNA Extraction Kit) and rotate in solution a minimum of five times, with the brush being spun against the walls of the tube to ensure DNA yield.
3) Tubes must then be sealed and swabs disposed of in biohazard bin.

DNA Purification:

1) Vortex solution for 10 seconds.
2) Incubate solution at 60°C at 1000 rpm for 30 minutes.
3) Vortex solution for 15 seconds.
4) Incubate solution at 98°C at 1000-1400 rpm for eight minutes.
5) Vortex solution for 15 seconds
6) Incubate solution at 98°C at 1000-1400 rpm for an additional eight minutes.
7) Vortex solution for 15 seconds
8) Chill tubes on ice briefly to allow tube to cool
9) Centrifuge solution at 4°C for five minutes
10) Transfer supernate from original tube to clean tube using 350 µL pipet, being careful not to include any of the cellular debris at the bottom of the tube in the process

DNA Template Dilution:

1) Thaw DNA for approximately 10 minutes
2) 90 µL added to the first 32 wells of the plate
3) 10 µL of designated, purified DNA added to each well
4) Plate covered with film in order to avoid evaporation of the template

Plate Preparation:

1) Master Mix created, containing 660 µL of Taqman GXTpress Master Mix, 33 µL of designated probe solution, and 297 µL of water.
2) Mastermix added to the PCR plate in duplicate (62 wells).
3) DNA template added to the Master Mix in duplicate (2 wells for every DNA sample).
4) Film placed over top of plate to avoid spilling and cross contamination.
5) Plate centrifuged, and placed in ABI 7000.

APPENDIX F:

ANOVA Source Tables

TableA1: ANOVA Source Table for the Association of the ACE rs4353 SNP with VO_{2peak}($L \cdot min^{-1}$)

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	34133.744[a]	2	17066.872	.054	.948
Intercept	45276431.501	1	45276431.501	143.126	.000
ACE rs4353	34133.744	2	17066.872	.054	.948
Error	8541151.456	27	316338.943		
Total	61514682.000	30			
Corrected Total	8575285.200	29			

a. R Squared = .004 (Adjusted R Squared = -.070)

TableA2: ANOVA Source Table for the Association of the ACE rs4293 SNP with VO_{2peak}($L \cdot min^{-1}$)

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	32.461[a]	1	32.461	.000	.992
Intercept	41444599.261	1	41444599.261	135.325	.000
ACE rs4293	32.461	1	32.461	.000	.992
Error	8575252.739	28	306259.026		
Total	61514682.000	30			
Corrected Total	8575285.200	29			

a. R Squared = .000 (Adjusted R Squared = -.036)

TableA3: ANOVA Source Table for the Association of the ACE rs4311 SNP with VO_{2peak}($L \cdot min^{-1}$)

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	188115.339[a]	1	188115.339	.628	.435
Intercept	49592552.006	1	49592552.006	165.561	.000
ACE rs4311	188115.339	1	188115.339	.628	.435
Error	8387169.861	28	299541.781		
Total	61514682.000	30			
Corrected Total	8575285.200	29			

a. R Squared = .022 (Adjusted R Squared = -.013)

TableA4: ANOVA Source Table for the Association of the ADRβ2 rs1042717 SNP with VO_{2peak}(L·min^{-1})

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	17589.343[a]	1	17589.343	.058	.812
Intercept	52832565.343	1	52832565.343	172.863	.000
ADRβ2 rs1042717	17589.343	1	17589.343	.058	.812
Error	8557695.857	28	305631.995		
Total	61514682.000	30			
Corrected Total	8575285.200	29			

a. R Squared = .002 (Adjusted R Squared = -.034)

TableA5: ANOVA Source Table for the Association of the APOE rs7412 with VO_{2peak}(L·min^{-1})

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	1346.700[a]	1	1346.700	.004	.948
Intercept	33625370.700	1	33625370.700	109.811	.000
APOE rs7412	1346.700	1	1346.700	.004	.948
Error	8573938.500	28	306212.089		
Total	61514682.000	30			
Corrected Total	8575285.200	29			

a. R Squared = .000 (Adjusted R Squared = -.036)

TableA6: ANOVA Source Table for the Association of the IGF-1 rs2288378 with VO_{2peak}(L·min^{-1})

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	6876.905[a]	1	6876.905	.022	.882
Intercept	52623870.372	1	52623870.372	171.965	.000
IGF-1 rs2288378	6876.905	1	6876.905	.022	.882
Error	8568408.295	28	306014.582		
Total	61514682.000	30			
Corrected Total	8575285.200	29			

a. R Squared = .001 (Adjusted R Squared = -.035)

TableA7: ANOVA Source Table for the Association of the IGF-1 rs11111272 with VO_{2peak}($L \cdot min^{-1}$)

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	851057.740[a]	2	425528.870	1.487	.244
Intercept	40228530.358	1	40228530.358	140.619	.000
IGF-1 rs11111272	851057.740	2	425528.870	1.487	.244
Error	7724227.460	27	286082.499		
Total	61514682.000	30			
Corrected Total	8575285.200	29			

a. R Squared = .099 (Adjusted R Squared = .033)

TableA8: ANOVA Source Table for the Association of the IL6 rs2069845 with VO_{2peak}($L \cdot min^{-1}$)

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	913211.339[a]	1	913211.339	3.337	.078
Intercept	53583376.006	1	53583376.006	195.813	.000
IL6 rs2069845	913211.339	1	913211.339	3.337	.078
Error	7662073.861	28	273645.495		
Total	61514682.000	30			
Corrected Total	8575285.200	29			

a. R Squared = .106 (Adjusted R Squared = .075)

TableA9: ANOVA Source Table for the Association of the IL6 rs1554606 with VO_{2peak}($L \cdot min^{-1}$)

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	319029.048[a]	1	319029.048	1.082	.307
Intercept	53252262.515	1	53252262.515	180.598	.000
IL6 rs1554606	319029.048	1	319029.048	1.082	.307
Error	8256256.152	28	294866.291		
Total	61514682.000	30			
Corrected Total	8575285.200	29			

a. R Squared = .037 (Adjusted R Squared = .003)

TableA10: ANOVA Source Table for the Association of the NOS3 rs1007311 with VO_{2peak}($L \cdot min^{-1}$)

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	40111.377[a]	2	20055.689	.063	.939
Intercept	47327778.867	1	47327778.867	149.716	.000
NOS3 rs1007311	40111.377	2	20055.689	.063	.939
Error	8535173.823	27	316117.549		
Total	61514682.000	30			
Corrected Total	8575285.200	29			

a. R Squared = .005 (Adjusted R Squared = -.069)

TableA11: ANOVA Source Table for the Association of the NOS3 rs891512 with VO_{2peak}($L \cdot min^{-1}$)

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	12608.441[a]	1	12608.441	.041	.841
Intercept	6286770.041	1	6286770.041	20.558	.000
NOS3 rs891512	12608.441	1	12608.441	.041	.841
Error	8562676.759	28	305809.884		
Total	61514682.000	30			
Corrected Total	8575285.200	29			

a. R Squared = .001 (Adjusted R Squared = -.034)

TableA12: ANOVA Source Table for the Association of the ACE rs4353 with VO_{2peak}($mL \cdot kg^{-1} \cdot min^{-1}$)

Dependent Variable:relativeVO2peak

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	45.152[a]	2	22.576	.588	.563
Intercept	12163.602	1	12163.602	316.560	.000
ACE rs4353	45.152	2	22.576	.588	.563
Error	1037.457	27	38.424		
Total	14612.490	30			
Corrected Total	1082.610	29			

a. R Squared = .042 (Adjusted R Squared = -.029)

TableA13: ANOVA Source Table for the Association of the ACE rs4293 with VO_{2peak}($mL \cdot kg^{-1} \cdot min^{-1}$)

Dependent Variable:relativeVO2peak

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	16.016[a]	1	16.016	.420	.522
Intercept	10202.600	1	10202.600	267.837	.000
ACE rs4293	16.016	1	16.016	.420	.522
Error	1066.594	28	38.093		
Total	14612.490	30			
Corrected Total	1082.610	29			

a. R Squared = .015 (Adjusted R Squared = -.020)

TableA14: ANOVA Source Table for the Association of the ACE rs4311 with VO_{2peak}($mL \cdot kg^{-1} \cdot min^{-1}$)

Dependent Variable:relativeVO2peak

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	4.769[a]	1	4.769	.124	.727
Intercept	13088.433	1	13088.433	340.010	.000
ACE rs4311	4.769	1	4.769	.124	.727
Error	1077.840	28	38.494		
Total	14612.490	30			
Corrected Total	1082.610	29			

a. R Squared = .004 (Adjusted R Squared = -.031)

TableA15: ANOVA Source Table for the Association of the ADRβ2 rs1042717 with VO_{2peak}($mL \cdot kg^{-1} \cdot min^{-1}$)

Dependent Variable:relativeVO2peak

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	68.931[a]	1	68.931	1.904	.179
Intercept	13598.531	1	13598.531	375.621	.000
ADRβ2 rs1042717	68.931	1	68.931	1.904	.179
Error	1013.679	28	36.203		
Total	14612.490	30			
Corrected Total	1082.610	29			

a. R Squared = .064 (Adjusted R Squared = .030)

TableA16: ANOVA Source Table for the Association of the APOE rs7412 with VO_{2peak}($mL \cdot kg^{-1} \cdot min^{-1}$)

Dependent Variable:relativeVO2peak

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	63.948[a]	1	63.948	1.758	.196
Intercept	7789.185	1	7789.185	214.102	.000
APOE rs7412	63.948	1	63.948	1.758	.196
Error	1018.662	28	36.381		
Total	14612.490	30			
Corrected Total	1082.610	29			

a. R Squared = .059 (Adjusted R Squared = .025)

TableA17: ANOVA Source Table for the Association of the IGF-1 rs2288378 with VO_{2peak}($mL \cdot kg^{-1} \cdot min^{-1}$)

Dependent Variable:relativeVO2peak

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	29.283[a]	1	29.283	.778	.385
Intercept	13553.616	1	13553.616	360.288	.000
IGF-1 rs2288378	29.283	1	29.283	.778	.385
Error	1053.327	28	37.619		
Total	14612.490	30			
Corrected Total	1082.610	29			

a. R Squared = .027 (Adjusted R Squared = -.008)

TableA18: ANOVA Source Table for the Association of the IGF-1 rs11111272 with VO_{2peak}($mL \cdot kg^{-1} \cdot min^{-1}$)

Dependent Variable:relativeVO2peak

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	31.798[a]	2	15.899	.409	.669
Intercept	10601.951	1	10601.951	272.411	.000
IGF-1 rs11111272	31.798	2	15.899	.409	.669
Error	1050.812	27	38.919		
Total	14612.490	30			
Corrected Total	1082.610	29			

a. R Squared = .029 (Adjusted R Squared = -.043)

TableA19: ANOVA Source Table for the Association of the IL6 rs2069845 with VO_{2peak}($mL \cdot kg^{-1} \cdot min^{-1}$)

Dependent Variable:relativeVO2peak

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	104.729[a]	1	104.729	2.999	.094
Intercept	13459.401	1	13459.401	385.388	.000
IL6 rs2069845	104.729	1	104.729	2.999	.094
Error	977.880	28	34.924		
Total	14612.490	30			
Corrected Total	1082.610	29			

a. R Squared = .097 (Adjusted R Squared = .064)

TableA20: ANOVA Source Table for the Association of the IL6 rs1554606 with VO_{2peak}($mL \cdot kg^{-1} \cdot min^{-1}$)

Dependent Variable:relativeVO2peak

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	11.058[a]	1	11.058	.289	.595
Intercept	13521.255	1	13521.255	353.315	.000
IL6 rs1554606	11.058	1	11.058	.289	.595
Error	1071.552	28	38.270		
Total	14612.490	30			
Corrected Total	1082.610	29			

a. R Squared = .010 (Adjusted R Squared = -.025)

TableA21: ANOVA Source Table for the Association of the NOS3 rs1007311 with VO_{2peak}($mL \cdot kg^{-1} \cdot min^{-1}$)

Dependent Variable:relativeVO2peak

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	37.402[a]	2	18.701	.483	.622
Intercept	12570.438	1	12570.438	324.722	.000
NOS3 rs1007311	37.402	2	18.701	.483	.622
Error	1045.208	27	38.711		
Total	14612.490	30			
Corrected Total	1082.610	29			

a. R Squared = .035 (Adjusted R Squared = -.037)

TableA22: ANOVA Source Table for the Association of the NOS3 rs891512 with VO_{2peak}($mL \cdot kg^{-1} \cdot min^{-1}$)

Dependent Variable:relativeVO2peak

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	17.702[a]	1	17.702	.465	.501
Intercept	1431.302	1	1431.302	37.634	.000
NOS3 rs891512	17.702	1	17.702	.465	.501
Error	1064.908	28	38.032		
Total	14612.490	30			
Corrected Total	1082.610	29			

a. R Squared = .016 (Adjusted R Squared = -.019)

TableA23: ANOVA Source Table for the Association of the ACE rs4353 with VO_{2peak}($mL \cdot kg_{FFM}^{-1} \cdot min^{-1}$)

Dependent Variable:leanVO2peak

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	45.940[a]	2	22.970	.214	.809
Intercept	37458.014	1	37458.014	348.785	.000
ACE rs4353	45.940	2	22.970	.214	.809
Error	2899.688	27	107.396		
Total	44974.592	30			
Corrected Total	2945.628	29			

a. R Squared = .016 (Adjusted R Squared = -.057)

TableA24: ANOVA Source Table for the Association of the ACE rs4293 with VO_{2peak}($mL \cdot kg_{FFM}^{-1} \cdot min^{-1}$)

Dependent Variable:leanVO2peak

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	47.137[a]	1	47.137	.455	.505
Intercept	31724.382	1	31724.382	306.464	.000
ACE rs4293	47.137	1	47.137	.455	.505
Error	2898.491	28	103.518		
Total	44974.592	30			
Corrected Total	2945.628	29			

a. R Squared = .016 (Adjusted R Squared = -.019)

TableA25: ANOVA Source Table for the Association of the ACE rs4311 with VO_{2peak}($mL \cdot kg_{FFM}^{-1} \cdot min^{-1}$)

Dependent Variable:leanVO2peak

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.848[a]	1	.848	.008	.929
Intercept	40421.820	1	40421.820	384.345	.000
ACE rs4311	.848	1	.848	.008	.929
Error	2944.780	28	105.171		
Total	44974.592	30			
Corrected Total	2945.628	29			

a. R Squared = .000 (Adjusted R Squared = -.035)

TableA26: ANOVA Source Table for the Association of the ADRβ2 rs1042717 with VO_{2peak}($mL \cdot kg_{FFM}^{-1} \cdot min^{-1}$)

Dependent Variable:leanVO2peak

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	288.187[a]	1	288.187	3.036	.092
Intercept	42306.452	1	42306.452	445.760	.000
ADRβ2 rs1042717	288.187	1	288.187	3.036	.092
Error	2657.441	28	94.909		
Total	44974.592	30			
Corrected Total	2945.628	29			

a. R Squared = .098 (Adjusted R Squared = .066)

TableA27: ANOVA Source Table for the Association of the APOE rs7412 with VO_{2peak}($mL \cdot kg_{FFM}^{-1} \cdot min^{-1}$)

Dependent Variable:leanVO2peak

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	173.611[a]	1	173.611	1.754	.196
Intercept	24367.851	1	24367.851	246.138	.000
APOE rs7412	173.611	1	173.611	1.754	.196
Error	2772.018	28	99.001		
Total	44974.592	30			
Corrected Total	2945.628	29			

a. R Squared = .059 (Adjusted R Squared = .025)

TableA28: ANOVA Source Table for the Association of the IGF-1 rs2288378 with VO_{2peak}($mL \cdot kg_{FFM}^{-1} \cdot min^{-1}$)

Dependent Variable:leanVO2peak

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	140.863[a]	1	140.863	1.406	.246
Intercept	42166.496	1	42166.496	420.949	.000
IGF-1 rs2288378	140.863	1	140.863	1.406	.246
Error	2804.765	28	100.170		
Total	44974.592	30			
Corrected Total	2945.628	29			

a. R Squared = .048 (Adjusted R Squared = .014)

TableA29: ANOVA Source Table for the Association of the IGF-1 rs11111272 with VO_{2peak}($mL \cdot kg_{FFM}^{-1} \cdot min^{-1}$)

Dependent Variable:leanVO2peak

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	68.795[a]	2	34.397	.323	.727
Intercept	32447.435	1	32447.435	304.530	.000
IGF-1 rs11111272	68.795	2	34.397	.323	.727
Error	2876.834	27	106.549		
Total	44974.592	30			
Corrected Total	2945.628	29			

a. R Squared = .023 (Adjusted R Squared = -.049)

TableA30: ANOVA Source Table for the Association of the IL6 rs2069845 with VO_{2peak}($mL \cdot kg_{FFM}^{-1} \cdot min^{-1}$)

Dependent Variable:leanVO2peak

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	52.324[a]	1	52.324	.506	.483
Intercept	40931.089	1	40931.089	396.111	.000
IL6 rs2069845	52.324	1	52.324	.506	.483
Error	2893.305	28	103.332		
Total	44974.592	30			
Corrected Total	2945.628	29			

a. R Squared = .018 (Adjusted R Squared = -.017)

TableA31: ANOVA Source Table for the Association of the IL6 rs1554606 with VO_{2peak}($mL \cdot kg_{FFM}^{-1} \cdot min^{-1}$)

Dependent Variable:leanVO2peak

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.394[a]	1	.394	.004	.952
Intercept	41825.045	1	41825.045	397.626	.000
IL6 rs1554606	.394	1	.394	.004	.952
Error	2945.234	28	105.187		
Total	44974.592	30			
Corrected Total	2945.628	29			

a. R Squared = .000 (Adjusted R Squared = -.036)

TableA32: ANOVA Source Table for the Association of the NOS3 rs1007311 with VO_{2peak}($mL \cdot kg_{FFM}^{-1} \cdot min^{-1}$)

Dependent Variable:leanVO2peak

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	96.809[a]	2	48.405	.459	.637
Intercept	38931.215	1	38931.215	368.975	.000
NOS3 rs1007311	96.809	2	48.405	.459	.637
Error	2848.819	27	105.512		
Total	44974.592	30			
Corrected Total	2945.628	29			

a. R Squared = .033 (Adjusted R Squared = -.039)

TableA33: ANOVA Source Table for the Association of the NOS3 rs891512 with VO_{2peak}($mL \cdot kg_{FFM}^{-1} \cdot min^{-1}$)

Dependent Variable:leanVO2peak

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	31.542[a]	1	31.542	.303	.586
Intercept	4672.938	1	4672.938	44.900	.000
NOS3 rs891512	31.542	1	31.542	.303	.586
Error	2914.086	28	104.074		
Total	44974.592	30			
Corrected Total	2945.628	29			

a. R Squared = .011 (Adjusted R Squared = -.025)

VITA

Jennifer Irene Flynn was born on July 31, 1987 in Tawas, Michigan, and raised in Harrisville, Michigan. She graduated from Alcona Community High School in June 2005. Her college career began at Saginaw Valley State University in Saginaw, Michigan in August 2005, and she received a Bachelor of Science degree in Exercise Science in August 2009. She started at the University of Tennessee, Knoxville in August 2009. In August 2011, she graduated with a Master of Science degree in Exercise Physiology from the Department of Kinesiology, Recreation, and Sport Studies. She then began her doctoral studies, in August 2011, in Kinesiology at University of Tennessee, Knoxville with a specialization in Exercise Physiology.