CHANGES OF AEROBIC FITNESS AND CARDIOVASCULAR DISEASE RISK FACTORS
IN CHILDREN: A TWO-YEAR LONGITUDINAL STUDY

Bumsoo Ahn

A thesis submitted to the faculty of the University of North Carolina at Chapel Hill in partial fulfillment of the requirements for the degree of Master of Arts in the Department of Exercise and Sport Science (specialization: Exercise Physiology).

Chapel Hill
2011

Approved By:

Robert McMurray, Ph.D.

Claudio Battaglini, Ph.D.

Grayson Lipford, Ph.D.

ABSTRACT

Changes of Aerobic Fitness and Cardiovascular Disease Risk Factors in Children over Two
Years
(Under the direction of Robert McMurray, Ph.D.)

This study examined the relationship between two-year changes of aerobic fitness and cardiovascular disease (CVD) risk factors in children. Baseline and follow-up data were obtained from 120 children, the CHIC III Study (J.S. Harrell, PI). Aerobic fitness was estimated using a multi-stage submaximal test on the cycle ergometer. Six CVD risk factors were measured, and combined by using standardized z-score, CVD composite score. As a result, there was a trend of increasing CVD composite scores over two years ($p<0.001$). Despite the general trend, changes in CVD composite score were inversely related with changes in aerobic fitness ($r=-0.24$; $p=0.008$). BMI was the greatest contributor to the CVD composite change score (partial $R^2=0.38$; $p<0.001$). In conclusion, since aerobic fitness is significantly related with CVD composite score, and since moderate-to-vigorous physical activity can increase fitness while reducing fat mass, children should be encouraged to participate in moderate-to-vigorous physical activities.

ACKNOWLEDGEMENTS

TABLE OF CONTENTS

Chapter

CHAPTER I

INTRODUCTION

Studies have reported several factors that increase the risk of heart disease. According to the American Heart Association's Position Stand (Hunt et al., 2005), major cardiovascular disease (CVD) risk factors include unchangeable variables (age, sex and race), while there are other factors that can be modified by changing lifestyle, such as tobacco smoking, cholesterol, blood pressure, obesity, diabetes mellitus and physical activity (PA). Specifically, regular moderate-to-vigorous PA decreases CVD risk factors by lowering body fat, total cholesterol and blood pressure (Hewitt et al., 2008; Kasa-Vubu et al., 2005; Seibaek et al., 2003).

Childhood is a critical period for cardiovascular disease prevention because the pathophysiology starts in early stage of life. In support, Mattson et al. (2008) have shown that the presence of CVD risk factors during childhood is a predictor for type 2 diabetes mellitus and coronary heart disease during adulthood. In this regard, research on CVD risk factors in children may be a more meaningful approach than studies of adults.

One of the components of exercise is physical activity (PA). PA is defined as physical movement created by skeletal muscle contractions which result in energy expenditure (Ortega et al., 2007). Previous studies have shown a relationship between PA and CVD risk factors in adults (Thompson et al., 2003). In addition, habitual moderate-to-vigorous PA, aerobic exercise training, can increase aerobic fitness. Aerobic fitness is an individual attribute (Ortega, et al., 2007) and is typically quantified as maximal oxygen uptake (VO_2max) in units of mL O_2 per kilogram body mass per minute (mL/kg/min). For this

reason, aerobic fitness (an attribute) is measured more objectively and with greater reliability than physical activity (a behavior). Aerobic fitness is also related with CVD risk factors. In support, cross-sectional studies have shown that children with high aerobic fitness were associated with lower CVD risk factors (Eisenmann et al., 2007), and intervention studies have reported that improved fitness lowered CVD risk factors (Harrell et al., 1996). Most of the previous studies have focused on improved aerobic fitness. However, as children age their aerobic fitness tends to decrease (McMurray et al., 2003), and relatively fewer studies have focused on reduced aerobic fitness and CVD risk factors.

CVD risk factors naturally increase with aging (Berenson et al., 1995). In support, Daniels (2001) has shown a gradual increase in CVD risk factors of youth age from six to thirteen years. Since improved fitness reduces CVD risk factors, this natural progression of CVD risk factors may differ by fitness level. However, no studies have shown the effect of fitness level on the natural progression during childhood over two years. Therefore, the current study compared the natural progression of CVD risk factors between changed aerobic fitness (increased and decreased) and maintained-aerobic fitness.

When examining the effects of aerobic fitness on CVD risk factors, most previous studies (Eisenmann et al., 2005 a; J. Eisenmann et al., 2005 b; Ortega, et al., 2007) used maximal oxygen uptake (VO_2max) in units of mL O_2 per kilogram body mass per minute (mL/kg/min). However, the units of mL/kg/min can be of concern because total body mass includes fat mass as well as fat free mass (FFM). This inclusion of fat mass in the unit of VO_2max can confound the relationship between aerobic fitness and CVD risk factors (Freedman et al., 2007; Gregg et al., 2005; Thompson et al., 2007). This confusion occurs because fat mass increases oxygen uptake at a given workload, but does not contribute to

energy production. In support, Goran et al. (2000) reported that when aerobic fitness was expressed in units of mL/kg/min, aerobic fitness increased simply by losing weight. Therefore, when comparing physiological ability of muscle tissue to maximally consume oxygen, VO_2max should be presented relative to fat free mass (FFM), VO_2max in units of $mL/kg_{FFM}/min$.

Summary

Since cardiovascular complications occur in early stage of life, childhood is a critical period for cardiovascular disease prevention. CVD risk factors decrease with enhanced aerobic fitness, and thus previous intervention studies have shown training effects on CVD risk factors. However, CVD risk factors naturally increase with aging, and this natural progression of CVD risk factors may differ by fitness level. Therefore, the primary purpose of the current study was to examine the relationship between two-year changes of aerobic fitness and changes in CVD risk factors in children.

Operational Definitions

1. Cardiovascular Disease Risk Factors were defined as the causes which may contribute to the incidence of coronary artery disease, stroke and type 2 diabetes mellitus, and CVD risk factors include 6 factors: BMI, mean arterial pressure (MAP), insulin resistance (HOMA), total cholesterol (TC), HDL, and triglycerides (TG).

2. CVD risk composite score was obtained by the sum of z-scores from individual CVD risk factors: BMI + MAP + HOMA + TC - HDL + TG. This method was previously used and described by Andersen et al. (2003).

3. CVD risk change score was obtained by subtracting baseline CVD composite score from follow-up CVD composite score.

4. Fat free mass (FFM) was defined as nonfat components of human body. Skeletal muscle, bone and water are all examples of FFM.

5. Aerobic fitness was defined as maximum possible uptake of oxygen during exercise (Ortega et al., 2007).

6. Aerobic fitness Relative to Body Mass was defined as maximal oxygen uptake (VO_2max) in units of mL O_2 per kilogram body mass per minute (mL/kg/min).

7. Aerobic fitness Relative to Fat Free Mass (mL/kg_{FFM}/min) was defined as maximal oxygen uptake (VO_2max) in units of mL O2 per kilogram fat free mass per minute (mL/kg_{FFM}/min).

8. Decreased Aerobic fitness group was defined as 3 mL/kg_{FFM}/min decrease in aerobic fitness over 2 years.

9. Increased Aerobic fitness group was defined as 3 mL/kg_{FFM}/min increase in aerobic fitness over 2 years.

10. Maintained Aerobic fitness group was defined as change in aerobic fitness over 2 years that is less than 3 mL/kg_{FFM}/min.

Hypotheses

Hypothesis 1: There will be an inverse relationship between change in VO_2max (mL/kg_{FFM}/min) and changes in CVD composite score.

Hypothesis 2: The increased-fitness group will have a lower CVD risk composite change score than the maintained- and decreased-fitness groups.

Hypothesis 3: The decreased-fitness group will have a higher CVD risk composite change score than the maintained-fitness group.

Limitations

1. Diet is a complex and important factor that affect CVD risk factors, but was not controlled in this study.

2. The current study involved relatively small sample sizes in each group (3 groups) because total selected subjects were 120 youth.

3. Stress and smoking could affect CVD risk factors, but these factors were not measured.

Delimitations

1. Applications of the study are limited to 9-11 years old children.

2. Study participants were recruited only from North Carolina.

Significance

While adult studies are available on the relationship between aerobic fitness and CVD risk factors, relatively fewer studies focused on children. During childhood, however, natural progression of CVD risk factors occurs. In this regard, children studies are more important to prevent the development of cardiovascular disease. By removing fat mass in the unit of aerobic fitness ($mL/kg_{FFM}/min$), this study will examine the independent relationship of aerobic fitness on CVD risk factors. Enhanced understanding on childhood fitness and CVD risk factors can be critical to control cardiovascular disease in their later life.

CHAPTER II

REVIEW OF LITERATURE

Introduction

Cardiovascular disease (CVD) refers to the class of diseases related with heart and blood vessels, which is the leading cause of death in the United States. In 2006, cardiovascular diseases were responsible for 34.4% of all deaths with an estimated cost of 400 billion dollars (Lloyd-Jones et al., 2010). Epidemiological studies have shown that there are several CVD risk factors. These factors include unchangeable factors (age, sex and race) and modifiable factors (smoking, cholesterol, blood pressure, obesity, diabetes mellitus and physical activity). Initially physical activity was not considered as an important factor, but studies now have shown that physical activity (PA) may be the most influential factor because it decreases most other modifiable factors.

Previous studies have shown an inverse relationship between individual CVD risk factors and aerobic fitness (Blair et al., 1996; Farrell et al., 1998; Kannel et al., 1975; Lee et al., 1999; Slattery et al., 1988). These studies have focused on adults with fewer studies on children. However, pathophysiology of cardiovascular disease starts in early stages of life and persists over time (Dunn et al., 1997). Therefore, prevention of cardiovascular diseases should start during childhood. In this regard, studies on children may be a more meaningful approach than studies of adults with respect to preventing CVD risk factors.

This chapter will be divided into three parts. The first part will review individual CVD risk factors: obesity as measured by BMI, mean arterial pressure (MAP), homeostasis model

analysis (HOMA), total cholesterol (TC), high-density lipoproteins cholesterol (HDL) and triglycerides (TG). Specifically, the first part will 1) examine the relationship between individual CVD risk factors and cardiovascular disease incidence, 2) review natural progression of CVD risk factors and the importance of children study, and 3) examine the relationship between aerobic fitness and CVD risk factors in adults and children. The second part will discuss interactions of CVD risk factors to emphasize the utilization of CVD composite score. Aerobic fitness has been mainly expressed in units of mL O_2 intake per kg body mass per min. However, the unit of kg body mass can confound the relationship between aerobic fitness and CVD risk factors because fat mass increases oxygen uptake without contribution to energy production of skeletal muscles. The third part will emphasize why fat mass should be removed in the unit of aerobic fitness.

CVD Risk Factors

Obesity

   BMI is a value that is calculated by using a person's height and weight (BMI=kg/m^2). It is a surrogate measure for body fat and it is more widely used than percent body fat because height and weight values are more readily obtainable than percent body fat. Fat (or adipose tissue) is loose connective tissue composed of adipocytes. Adipose tissue is an active endocrine organ, and it releases adipokines, such as TNF-α and IL-6 (Kershaw and Flier 2004). The increased adipokines cause inflammation on the surface of blood vessel, which over time results in atherosclerosis and cardiovascular disease.

   Obesity is shown to be related with cardiovascular disease. Hubert et al. (1983) tracked the cardiovascular disease incidence of 2,252 men and 2,818 women (ages 28-62 years) of the Framingham cohort over 26 years . Since 8 years of follow-up, BMI became positively

associated (r=0.23) with cardiovascular disease incidence. Furthermore, Drenick et al. (1980) have examined a group of 200 morbidly obese men (average weight, 143.5 kg; age, 23 to 70 years) for a mean period of 7.5 years. In comparison with general population, the participants demonstrated 12-fold excess mortality in the 25-34 years age group and 6-fold excess mortality in the age group of 35-44 years age group, the major cause of death being cardiovascular disease.

Fatness tends to increase as children age and childhood obesity can persist over time from childhood to young adulthood. In a prospective study, Strak et al. (1981) have recorded BMI of 5,362 children at 6, 14 and 26 years. The prevalence of overweight increased from 1.7, 6.5 and 12.3% in male; and 2.9, 9.6 and 11.2% in female, respectively. Also, Freedman et al. (2001) examined 2,617 youth over a 17-year period. They have found significant correlations between childhood BMI levels and adulthood obesity (r=0.27). These findings suggest that obesity control during childhood is critical to delay or prevent adulthood obesity.

Aerobic fitness has been shown to help control obesity and cardiovascular disease. In an adult study, Slattery et al. (1988) have examined the relationship between physical fitness and fatness and cardiovascular disease mortality of the US railroad workers. A cohort of 3,043 white, middle-aged men were first examined in 1957-1960 and re-examined 20 years later. Results from this study suggest that men with lower physical fitness are at a greater risk of being obese and dying of coronary heart disease in an average follow-up of 20 years. In a children study, Eisenmann et al. (2005 b) have examined the relationship between adolescent fitness and adulthood CVD composite score. They examined 48 boys at their baseline (15.8 years) and follow-up (26.6 years), and found moderate relationship with adulthood BMI (r=-0.47) and CVD risk factors (r=-0.26).

<u>Blood pressure</u>

Blood pressure (BP) is the pressure exerted by circulating blood on the walls of blood vessels. BP is created by the pumping of the heart. It decreases as the circulating blood moves away from the heart through arteries. Thus, during each heartbeat BP varies between maximum (systolic) and a minimum (diastolic) blood pressure (SBP and DBP, respectively). According to the American Heart Association (Hunt et al., 2005), resting value of 140/90 mmHg or higher is considered as hypertension in adults; however, the criteria for children depend on age, sex and height and are yet to be defined.

High blood pressure causes greater shear force against artery walls. Over time, this pressure can damage the arteries which result in an inflammation process. The inflammation process can build up plaque (fatty material) which narrows the diameter of the vessel; hence atherosclerosis. Atherosclerosis can restrict blood flow to the heart which cause overload to the heart muscles. If untreated, this can develop cardiovascular diseases. In support, Franco et al. (2005) have examined hypertension and cardiovascular disease of 3,128 participants (aged 50) from the Framingham Heart Study. They found that normotensive participants survived 5.6 years longer than the hypertensive on average. Also, normotensive participants lived 7.2 years longer without cardiovascular disease compared with hypertensive subjects. These findings indicate the importance of blood pressure on life expectancy and cardiovascular disease incidence in adults.

Blood pressure (BP) tends to increase as children age, and the elevated BP persists over time (Tumer et al., 1999). In support, Hofman et al. (1985) have examined BP of 596 Dutch children (aged 5-19 years) for five consecutive years. They found significant correlations between age and systolic (r=0.4-0.6) and diastolic BP (r=0.2-0.5). Bao et al. (1995) have

tracked the elevated BP from childhood to adulthood. The 1,505 participants (659 male and 846 female) were 5 to 14 years old at baseline and 20 to 31 years old at follow-up (mean follow-up of 15 years). Persistence of BP was shown by correlations between childhood and adulthood levels (r=0.46 for systolic BP; r=0.38 for diastolic BP).

Aerobic fitness has been shown to be related with blood pressure. Blair et al. (1995) have evaluated the relationship between changes in physical fitness and blood pressure in adults. In this prospective study, two clinical examinations were conducted on 9,777 men (4.9 years of mean interval) to assess change or maintenance in physical fitness and blood pressure. Blood pressure was the highest in the men who were unfit at both examinations, and lowest in men who stayed fit at both examinations.

Although previous studies have shown the relationship between changes in physical fitness on blood pressure, these studies have focused only on adults (Blair et al., 1995; Blair et al., 1989), and there are relatively few studies on children. However, childhood studies are more important than adult studies because low aerobic fitness during childhood tends to be related with high blood pressure during adulthood. In an intervention study, McMurray et al. (2002) examined 1,140 children (aged 11 to 14 years; 630 girls and 510 boys), and found significant reductions in systolic (p=0.001) and diastolic blood pressure (p=0.001) after 8 weeks of 30-minute aerobic training (three times per week).

Insulin resistance

Insulin is a hormone which regulates carbohydrate and fat metabolism. It causes muscle, fat and liver tissues to take up glucose from blood. Insulin resistance decreases the amount of glucose taken up by a given insulin concentration. This pathological state is known as insulin resistance. One way of evaluating insulin resistance is homeostasis model assessment of

insulin resistance (HOMA). In children, HOMA has been shown to be strongly related (r=0.81) to the hyperglycemic euglycemic clamp (Guzzaloni et al., 2002). HOMA value 3.16 has been suggested as cut-off value for insulin resistance for children (Keskin et al., 2005).

Insulin resistance affects other CVD risk factors, subsequently causing atherosclerosis and cardiovascular disease over time (Lee et al., 1999). The physiological response to insulin resistance is an increased concentration of plasma insulin, which increases the release of free fatty acids (FFA) into circulation. Increased FFA to the liver stimulate the secretion of VLDL which causes excess triglycerides (Ginsberg 2000). Excess triglycerides can cause obesity and hypertension. Another effect of excess triglycerides is to decrease HDL and to increase LDL (Bruce et al., 1998). Taken together, insulin resistance is a potent risk factor for cardiovascular disease by affecting other CVD risk factors. In a longitudinal study, Magnussen et al. (2010) have examined 1,781 children at baseline (9-18 years) and follow-up (24-41 years). They found that children with high insulin resistance showed two to three times greater risk of having cardiovascular diseases as adults compared with children free of insulin resistance.

In pre-diabetic and diabetic children, insulin resistance tends to progress as they age. Nguyen et al. (2008) have examined normoglycemic (n=1,838), pre-diabetic (n=90) and type 2 diabetic (n=60) participants during childhood (4-11 years), adolescent (12-18 years), and adulthood (19-44 years). They found a significant trend of increasing insulin resistance. The same trend, however, was not found in normoglycemic children. These findings indicate the importance of controlling childhood insulin resistance to prevent further cardiovascular complications.

Aerobic fitness has been shown to be related with insulin resistance. Dwyer et al. (2009) have examined aerobic fitness of 647 children at baseline (7-15 years) and follow-up (17-25 years). The entire participants were divided into five groups depending on fitness change: 1) persistently unfit (lowest tertile in childhood and adulthood), 2) decreased fitness (those who dropped by one or two tertiles), 3) persistently moderate fitness (middle tertiles at both trials), 4) increased fitness (an increase of one or two tertiles), and 5) persistently fit (highest tertile at both trials). They found a tracking effect (p<0.001) from childhood fitness to adulthood insulin resistance. Also, persistently unfit and decreased-fitness groups were at a greater risk for insulin resistance than the other three groups.

Total Cholesterol

Cholesterol is a waxy substance produced by the liver. It is essential for normal body functions, such as signal transduction, cell membrane, and vitamin D and hormone productions. Since cholesterol is insoluble in blood, it transported in the circulatory system within lipoproteins; three which are very low-density lipoproteins (VLDL), low-density lipoproteins (LDL) and high-density lipoproteins (HDL). A sum of the three types of lipoproteins determines total cholesterol (TC). Specifically, LDL is elevated with high total cholesterol, and high levels of LDL particles promote cardiovascular disease. The American Heart Association suggested normal TC below 200 mg/dL with corresponding LDL below 100 mg/dL (Hunt et al., 2005).

Hypercholesterolemia is cholesterol above 240 mg/dL and is usually accompanied with high concentrations of LDL and low concentrations of HDL (Hunt et al., 2004). When excess LDL particles are present in the blood, they can circulate without receptors. These unbound LDL molecules are oxidized and taken up by macrophages, which causes inflammation. This

process occurs in the walls of blood vessels and results in atherosclerotic plaque formation. Conversely, HDL can transport cholesterol back to the liver for excretion or to other tissues, therefore decreasing plasma LDL cholesterol. For this reason, a combination of high LDL and low HDL (hypercholesterolemia) can increase cardiovascular disease. Lewington et al. (2007) have conducted a meta-analysis of 61 prospective observational studies. They demonstrated that total cholesterol levels have an exponential effect on cardiovascular disease, and associations were more pronounced in younger subjects.

Total cholesterol (TC) tends to increase as children age, and the elevated TC persists over time (Dunn et al., 1997). Lauer et al. (1990) have conducted a prospective study. 2,367 children were first examined at ages 8-18 years (baseline) and followed up to ages 20 to 30 years. The children were divided into 3 groups by their baseline cholesterol ranking: $<75^{th}$, 75th-95th, and $>95^{th}$ percentile. They found a gradual increase in TC as subjects' increasing age and a greater risk ratio for adulthood hypercholesterolemia with increasing childhood cholesterol levels. Regardless of the natural progression and tracking effect, guidelines for childhood cholesterol are still to be determined.

Aerobic fitness has been shown to be related with total cholesterol. In an intervention study, Dunn et al. (1997) examined initially sedentary 235 participants (116 men and 119 women). After 6-month aerobic training (20-60 minute per session; 3-5 days a week), they found 8 percent increase in aerobic fitness and 4 percent decrease in total cholesterol ($p<0.001$). In a cross-sectional study, Hoekstra et al. (2008) have examined 2,016 children (1,047 male and 998 female, aged 12 to 15 years), and found an inverse relationship ($r=-0.18$, $p<0.001$) between aerobic fitness and total cholesterol.

High-Density Lipoproteins

High-density lipoprotein (HDL) is one of the five major groups of lipoproteins. HDL can reverse atherosclerosis by removing cholesterol in the fatty steak. For that reason, it is also known as "good cholesterol." According to the American Heart Association (Hunt et al., 2005), HDL levels of above 60, 40-59, and below 40 mg/dL is considered as high, medium, and low HDL levels, respectively. However, HDL criteria for children are still to be developed.

HDL levels are related with cardiovascular disease in adults. Gordon et al. (1989) have reported the effect of HDL on cardiovascular disease by analyzing large cohort studies: the Framingham Heart Study (FSH) and Lipid Research Clinics Prevalence Mortality Follow-up Study (LRCF). In the FSH analysis, a 1 mg/dL increment in HDL was associated with a significant coronary heart disease risk reduction of 2% in men and 3% in women. In LRCF, the same analysis showed coronary heart disease risk decrement of 3.7 % in men and 4.7 % in women.

HDL tends to gradually decrease as children age, and the decreased HDL level persists over time. Freedman et al. (1985) have tracked HDL levels in children over an 8-year period; measurements were conducted at baseline, 3, 5, and 8 years of follow-up. They found a trend of decreasing HDL levels with advancing age. In addition, Beaglehole et al. (1980) have examined HDL levels of 1,639 children from ages 6 to 25 years, and found a significant inverse relationship between age and HDL. These findings suggest the importance of HDL levels during childhood.

Aerobic fitness has been shown to be related with HDL. In an intervention study, Dunn et al. (1997) examined initially sedentary 235 participants (116 men and 119 women), and

found 8 percent increase in fitness and 7 percent increase in HDL (p<0.001) after 6 months

aerobic training (3 to 5 times a week for 20-60 minute exercise per session). In a cross-

sectional study, Macek et al. (1989) have examined the relationship between VO_2max and

HDL in 93 adolescent participants (aged 16-18 years), and found a significant correlation

(r=0.420; p<0.001).

Triglycerides

Triglycerides play an important role in metabolism as energy sources and transporters of

dietary fat. Excess triglycerides in plasma are called hypertriglyceridemia and related to

cardiovascular disease. The National Cholesterol Education Program guidelines for

triglycerides are as follows (Hunt et al., 2005): normal (less than 150 mg/dL), borderline-

high (150 to 199 mg/dL), high (200 to 499 mg/dL), and very high (500 mg/dL or higher).

However, such criteria for children are still to be determined.

Hypertriglyceridemia is linked to atherosclerosis and, by extension, the risk of

cardiovascular disease. High concentration of triglycerides increases lipoprotein

concentration because fatty acids are bound to lipoprotein to circulate through blood vessels,

majority of produced lipoproteins being VLDL, precursor of LDL. The increased

concentration of LDL can cause atherosclerotic plaque formation and subsequently

cardiovascular disease.

Triglycerides (TG) tend to increase as children age, and the elevated level persists over

time (Dunn et al., 1997). In a cross-sectional study, Frerichs et al. (1976) have examined

serum lipid profiles of 3,446 children (ages 5-14 years) and found a significant relationship

(r=0.105) between triglycerides and age. In a longitudinal study, Widhalm et al. (1981)

annually measured blood profile of 109 children over 4 years (54 boys, 55 girls; aged 11 to 14 years), and found significant correlations (r=0.23 to 0.37).

Aerobic fitness has been shown to be inversely related with triglycerides. In a cross-sectional study, Eisenmann et al. (2005 a) have examined 416 boys and 345 girls (9-18 years old) from the Quebec Family Study. Participants were divided into four groups using a median split of age-adjusted physical working capacity (PWC) and BMI. They found that fitness levels had a reduction effect ($p < 0.001$) on triglycerides regardless of BMI. In addition, Macek et al. (1989) have examined the relationship between VO_2max and triglyceride levels in 93 adolescent participants (aged 16-18 years), and found an inverse relationship (r=-0.490; p<0.001) .

<u>Interactions among CVD risk factors</u>

Individual cardiovascular disease risk factors affect each other and increase cardiovascular disease incidence. Increased adipose tissues release adipokines, such as TNF-α, IL-6 and adiponectin (Kershaw & Flier 2004). These increased adipokines cause inflammation on the surface of arterial blood vessel, resulting in fatty streaks. Fatty streaks decrease the diameter of blood vessel which increases blood pressure because peripheral resistance is influenced by vessel diameter. Increased blood pressure has been shown to be positively related with insulin resistance (Kannel, 1985; Vasan et al., 2001). Insulin resistance is a cause of high blood pressure because insulin is a vasodilator and when resistant this effect is compromised. In response to insulin resistance, plasma insulin is elevated which increases the release of free fatty acids (FFAs) into circulation. Increased FFAs to the liver stimulate the secretion of VLDL which causes excess triglycerides (Ginsberg, 2000). Excess TGs can cause obesity and hypertension. Another effect of excess

triglycerides is to increase total cholesterol (decrease HDL and to increase LDL) through the actions of cholesteryl ester transfer protein (Bruce et al,. 1998). Combined effects of increase in TG, TC and LDL and decrease in HDL can cause cardiovascular disease. In addition to interactions among individual factors, using composite scores provides two other strong points. First, z-scores provide a continuous score which is more appropriate when investigating associations (McMurray & Andersen 2010). Second, composite score can compensate for the day-to-day fluctuation of individual factors (Andersen et al., 2008). By using CVD composite score, effect of aerobic fitness on overall CVD risk factors can be better evaluated.

Different units of VO$_2$max

When examining the effects of aerobic fitness on CVD risk factors, most previous studies (Blair et al., 1995; Eisenmann et al., 2005 a; Eisenmann et al., 2005 b; Ortega et al., 2007) used maximal oxygen uptake (VO$_2$max) in units of mL O$_2$ per kilogram body mass per minute (mL/kg/min). However, the units of mL/kg/min can be of concern because total body mass includes fat mass as well as fat free mass (FFM). This inclusion of fat mass in the unit of VO$_2$max can confound the relationship between aerobic fitness and CVD risk factors because fat mass is positively associated with them (Freedman et al., 2007; Goran et al., 2000; Gregg et al., 2005; Thompson et al., 2007). In support, Goran et al. (2000) reported that when aerobic fitness was expressed in units of mL/kg/min, increased aerobic fitness can occur simply by losing weight without exercise. The enhanced aerobic fitness, however, was not evident when fat mass was excluded (mL/kg$_{FFM}$/min). Therefore, when comparing physiological ability of skeletal muscle tissues to maximally consume oxygen, VO$_2$max should be presented relative to fat free mass (FFM), VO$_2$max in units of mL/kg$_{FFM}$/min.

According to the American Heart Association Position Stand (Hunt et al., 2005), aerobic fitness can help control cardiovascular disease (CVD) risk factors in adults. It has been also reported that CVD risk factors tend to gradually increase since childhood, and the elevated levels tend to persist over time (Tumer et al., 1999). However, previous studies on the relationship between aerobic fitness and CVD risk factors have mainly focused on adults, and relatively fewer studies were conducted on children. Finally, aerobic fitness has been mainly scaled in mL/kg/min in many previous studies where inclusion of fat mass can confound the relationship between aerobic fitness and CVD risk factors. Therefore, the current study will examine the relationship between change of aerobic fitness (mL/kg$_{FFM}$/min) and CVD risk composite score in children over two years.

CHAPTER III

METHODS

Participants

The current study was a retrospective secondary analysis based on participants in the

Cardiovascular Health in Children III (CHIC) study, Cohort 5 (J.S. Harrell, P.I.). The CHIC

study was a 5-year longitudinal study which measured CVD risk factors of children in North

Carolina from 1998 to 2003. Of the 1566 participants from the CHIC study, 120 children (62

girls and 58 boys) were purposefully selected from a larger study (CHIC III, Cohort 5, J.S.

Harrell, P.I.) to equally represent four possible weight changes: normal-weight to normal-

weight, normal-weight to overweight, overweight to overweight, and overweight to normal-

weight. The 120 children were classified into three groups by fitness change: increased-,

maintained- and decreased-fitness groups. Individual fitness groups' CVD risk factors were

compared. The measured CVD risk factors include age, sex, race, body fat (BMI), blood

pressure, HOMA, total cholesterol, high-density lipoprotein cholesterol (HDL),triglycerides

(TG) and aerobic fitness. Subjects' mean ages were 9.6 ±1.1 years at baseline and 11.5 ±1.0

years at follow-up. The sex and racial distribution was: 52% Female and 48% Male; and 53%

Black, 36% White, and 11% Others.

Procedures

The CHIC study included an informed consent and a written assent that were obtained

from the parents and participants, respectively. The approval from the Institutional Review

Board of the University was obtained before data collection. Baseline and 2-year follow-up

data were collected at the participants' school using teams of research assistants (RA) who were trained by the same investigator and met stringent criteria for reliability and precision before gathering data. In addition, to further ensure quality control physiologic variables of every tenth subject were measured by more than one RA.

Descriptive information such as age and gender were collected via self-report. Pubertal stage (1 to 5) was assessed using the Pubertal Development Scale (Petersen, Crockett, Richards, & Boxer, 1988). Height and body mass were measured to the nearest 0.1 cm and 0.1 kg using a stadiometer (Perspective Enterprises, Kalamazoo, MI) and electronic scale (Scaletronix, White Plains, NT). BMI was calculated by using kg/m^2. Percent body fat was estimated from sum of skinfolds taken at the subscapular and triceps sites (Slaughter et al., 1988). These measurements were taken in triplicate using Lange Calipers (Cambridge Scientific, Cambridge, MD). Seated blood pressure was measured in triplicate using a Hawksley Random Zero Sphygmomanometer (Hawksley & Sons Limited, Sussex, England). Participants rested for 5 minutes before the blood pressure measurements, and each of the three trials was separated by 1 minute. The average of the three values was used for analyses. Mean arterial pressure (MAP) were calculated by the following formula: diastolic BP + (systolic BP–diastolic BP)/3.

Aerobic fitness (VO_2max) was estimated using a multi-stage submaximal test on the cycle ergometer (McMurray et al., 1998). During this test, the workloads increased until the heart rate reached a range of 50-170 beats/min, and the heart rate/workload relationships were used to predict maximal capacity. This test showed strong correlations (r=0.807) with measured maximal values in children. From the exercise test, absolute oxygen uptake (L/min) was obtained. To convert the absolute oxygen uptake into oxygen uptake relative to FFM

(mL/kg$_{FFM}$/min), the absolute oxygen uptake was divided by kg FFM and multiplied by 1,000.

Blood samples were drawn early in the morning (between 7am and 9am) after a verified overnight fast. The blood samples were centrifuged, placed on dry ice (-80°C) and sent to the laboratories for analysis. Insulin levels were measured from the stored plasma using radioimmunoassay procedures (Linco, St. Charles, MO), while glucose levels were analyzed via automated hexokinase oxidase procedures. To calculate insulin resistance, homeostasis model assessment (HOMA) was computed by the following formula: fasting insulin (units per milliliter) $\times$ fasting glucose (milligrams per deciliter)/22.5. Blood samples were analyzed for high-density lipoproteain cholesterol (HDL), triglycerides (TG), and total cholesterol (TC) using a Hitachi 911 analyzer (Boehringer-Mannheim corporation) and coupled-enzymatic procedures.

Data analyses

Group analyses of aerobic fitness change

Total participants were divided into three groups based on change in aerobic fitness (mL/kg$_{FFM}$/min) over two years: decreased-, increased-, and maintained-fitness. Change score (follow up - baseline) of CVD composite z-score was used as dependent variable to conduct the between-group analyses.

CVD composite score

To examine the effects of aerobic fitness on overall CVD risk factors, the current study combined individual CVD risk scores based on a previous study (Andersen et al., 2003) as follows: CVD composite z-score = BMI + MAP + HOMA + TC - HDL + TG. Individual

CVD risk scores were derived from standardized z-scores. The standardized HDL score was multiplied by -1 because it is inversely related compared to the other risk factors.

Statistical analyses

Normality tests (descriptive statistics) were used to determine whether individual CVD risk factors (BMI, MAP, HOMA, TC, HDL and TG) were normally distributed. If skewness was greater than ±2, the assumptions of normality were not made. In such cases, log transformation was used to normalize the distribution. To describe the sample, subject characteristics were calculated and described by ANOVA.

Hypothesis #1 was that there will be an inverse relationship between changes in aerobic fitness (mL/kg$_{FFM}$/min) and CVD composite change score. This was evaluated by Pearson bivariate correlation using VO$_2$max (mL/kg$_{FFM}$/min) change score and CVD composite change scores (follow up - baseline).

Hypothesis #2 was that the increased-fitness group will show a lower CVD composite change score than maintained- and decreased-fitness groups. Research hypothesis #3 was that the decreased-fitness group will show a higher CVD risk composite change score than maintained -fitness group. Hypothesis #2 and #3 were evaluated by one-way (1×3) ANOVA (General Linear Model) comparing the CVD risk change scores between three groups of fitness: decreased-, maintained-, and increased-fitness group.

CHAPTER IV

RESULTS

Baseline characteristics of all participants are presented in Table 1. The participants were

categorized into three subgroups: improved-, maintained- or decreased-fitness group. The

three groups were similar in age, height, body mass, pubertal status, and CVD risk factors. At

baseline, the improved-fitness group had a lower fitness level ($mL/kg_{FFM}/min$) than

maintained-fitness group which was also lower than the decreased-fitness group ($p<0.001$).

Table 1. Baseline characteristics for 120 children (62 girls and 58 boys) by fitness group,

Mean ± SD

	Decreased-fitness	Maintained-fitness	Increased-fitness
Number of children (girls/boys)	47 (22/25)	28 (15/13)	45 (25/20)
Age (years)	9.8 ±1.2	9.9 ±0.8	9.8 ±1.0
Height (cm)	141.6 ±8.6	141.4 ±8.2	142.2 ±9.9
Body Mass (kg)	41.8 ±12.7	41.2 ±14.1	41.9 ±11.1
VO$_2$max ($mL/kg_{FFM}/min$) *	54.7 ±10.1	47.2 ±9.5	42.1 ±9.6
Body Mass Index (BMI)	20.5 ±4.4	20.3 ±5.0	20.5 ±4.0
Mean Arterial Pressure (mmHg)	75.6 ±8.6	72.5 ±10.0	74.9 ±11.4
HOMA	3.6 ±2.6	3.3 ±1.9	3.3 ±1.6
Total Cholesterol (mg/dl)	152.5 ±32.2	165.6 ±23.0	151.6 ±24.7
Triglycerides (mg/dl)	63.5 ±38.0	69.0 ±32.8	66.4 ±35.7
High-Density Lipoprotein (mg/dl)	48.5 ±13.4	51.7 ±16.0	52.5 ±12.6
CVD composite score	-0.57 ±3.08	-0.69 ±3.2	-0.95 ±3.3

* Significant difference between groups ($p = 0.05$); pubertal status was measured by tanner
stage (1-5 stages)
HOMA: homeostatic model assessment for insulin resistance

Over the two years, there was a general trend of increasing CVD composite score (p<0.001). Baseline composite score was -0.68 and increased to 0.28 at follow-up (p<0.001).

Table 2 shows change scores of individual CVD risk factors (z-score) presented by fitness. Between fitness groups, there were significant differences in change scores of VO_2max (mL/kg$_{FFM}$/min), BMI, total cholesterol, and CVD composite change score. However, the changes in body mass, HOMA, triglycerides, and high-density lipoprotein were not significantly different (p>0.05) between groups. Figure 1 shows that change of aerobic fitness was inversely related with CVD composite change score (r=-0.24; p<0.001).

Table 2. Change scores (z-scores) of individual CVD risk factors by fitness group

	Decreased-fitness	Maintained-fitness	Increased-fitness
VO_2max (mL/kg$_{FFM}$/min) #	-11.01 ±5.5	0.20 ±1.95	8.45 ±3.12
Body Mass	0.99 ±0.50	1.14 ±0.53	0.80 ±0.73
Body Mass Index (BMI) *	0.75 ±1.24	0.72 ±0.79	-0.18 ±1.57
Mean Arterial Pressure (MAP)	0.36 ±1.07	0.35 ±1.19	0.27 ±1.68
HOMA	-0.08 ±0.85	0.38 ±0.91	-0.02 ±0.68
Total Cholesterol	-0.01 ±1.08	0.21 ±1.04	-0.29 ±0.72
Triglycerides	0.23 ±0.89	0.34 ±0.58	0.20 ±1.36
High-Density Lipoprotein	-0.08 ±0.93	-0.40 ±0.69	-0.16 ±0.86
CVD composite change score *	1.37 ±2.78	1.64 ±1.72	0.27 ±3.20

* Significant difference in increased-fitness group compared with maintained- and increased-fitness groups (p=0.05)
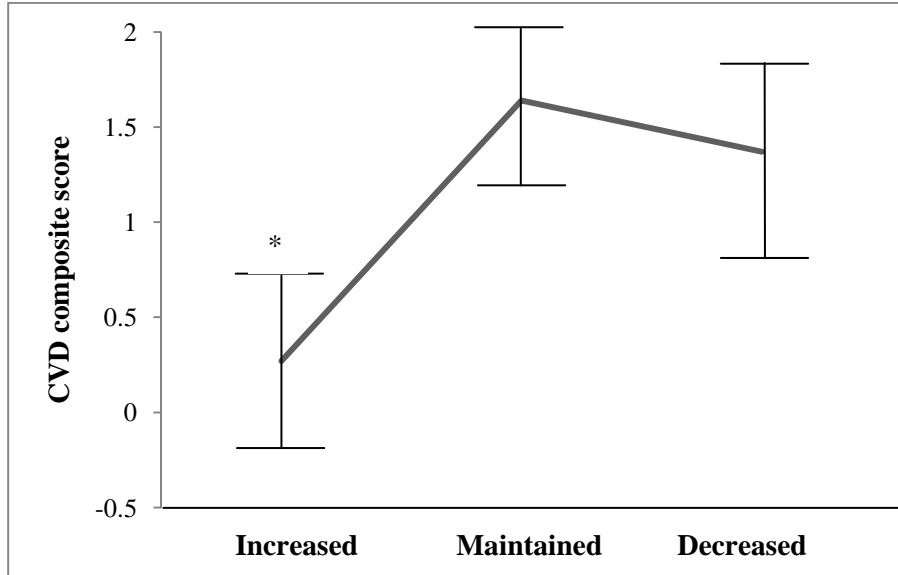# Significant difference between groups (p=0.05)

Figure 1. Scatter gram of individual changes in VO_2max ($mL/kg_{FFM}/min$) as related to CVD their changes in CVD composite score
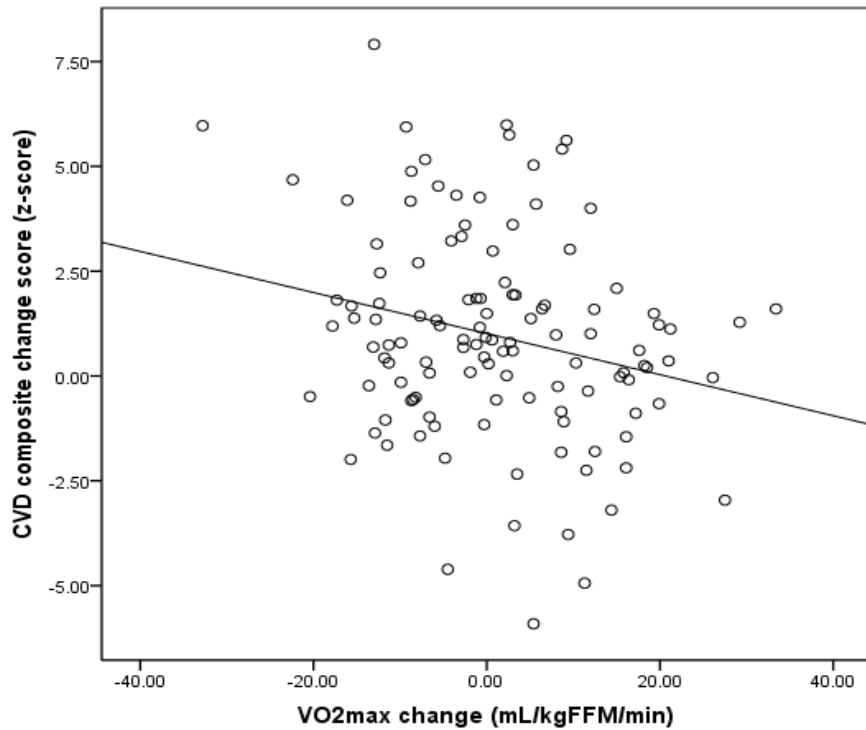
Based on the inverse relationship between aerobic fitness and CVD composite change score, CVD change scores within each fitness group were examined (Figure 2). The increased-fitness group showed a significantly lower change score than the maintained- ($p=0.008$) and decreased-fitness ($p=0.017$) groups. There was no significant difference between maintained- and decreased-fitness group (p=0.572).

Figure 2. CVD composite change score within each fitness group

* Significant difference between maintained- and decreased-fitness groups (*p*<0.05).

To further understand the relationship between CVD composite change score and individual components, stepwise regression was run. All of the individual risk factors were significantly related to the composite score (p<0.005). Partial R^2 of each factor was 0.38 for BMI, 0.16 for MAP, 0.18 for HOMA, 0.09 for TG, 0.02 for HDL and 0.07 for TC, respectively.

CHAPTER V

DISCUSSION

The primary purpose of this study was to examine the relationship between two-year changes of aerobic fitness and cardiovascular disease risk (CVD) composite change score in children. This study removed fat mass in the unit of aerobic fitness by using $mL/kg_{FFM}/min$, and found a significant inverse relationship between aerobic fitness and CVD composite change score. Furthermore, the change in composite score was least in the increased-fitness group. Interestingly, there was no significant difference in CVD composite change scores between the maintained-fitness group and the decreased-fitness group. This study also found a general trend of increasing CVD composite score over two years. Finally, among individual CVD risk factors BMI was the greatest determinant of the CVD composite change score.

The current study found an overall increase in CVD composite scores over two years. This trend has been shown by previous studies for specific risk factors. McTigue et al. (2002) have examined yearly BMI values over 12 years and found a gradual increase. Also, Webber et al. (1990) examined cholesterol levels at baseline and follow-up (12 years after). They found an increase in total cholesterol, LDL, VLDL with corresponding decrease in HDL. The observed natural progression of CVD composite score may be related to a decrease in physical activity during puberty. In support, McMurray et al. (2003) have examined 1,064 children and found gradual decreases in physical activity and corresponding reduction in aerobic fitness with advancing age. In a separate study, Carnethon et al. (2003) have

examined 2,748 children and found an inverse relationship between aerobic fitness and CVD risk factors.

Although CVD composite score generally increased, CVD composite change scores were inversely related to changes of aerobic fitness. Similar findings have been previously reported in studies that measured fitness adjusted for total body mass (mL/kg/min), but not when only adjusting for fat free mass (mL/kg$_{FFM}$/min). For example, Kovacs et al. (2009) have examined 51 overweight/obese children (23 boys, 28 girls) who participated in a 15-week aerobic training (three sessions per week with 60-minute duration). After the training program, the investigators found a significant increase in aerobic fitness (37.0 ±3.9 vs. 42.6 ±11.2 mL/kg/min) and corresponding decrease in CVD risk factors: waist circumference (85.9 ±12.4 vs. 80.9 ±10.2 cm), systolic blood pressure (113.3 ±11.2 vs. 106.7 ±11.6 mmHg), and low-density lipoproteins (2.4 ±0.6 vs. 1.9 ±0.6 mM/L). In a cross-sectional study, Anderson et al. (2006) have examined fitness quartiles and CVD composite scores of 2,845 randomly selected school children (aged 9-15 y). They found that lowest quartile of fitness showed 13.2 times greater risk ratio for CVD composite score than the highest quartile. These findings suggest the inverse relationship of aerobic fitness with CVD composite score either when adjusting fitness for total body mass or removing fat mass from the adjustment.

Aerobic fitness can be affected by other significant factors (Dunn et al., 1997). Some prospective studies have examined the relationship between initial aerobic fitness and CVD risk factors years later. However, significant changes in fitness can occur between the baseline and follow-up period which may affect the CVD risk factors, such as changes in body fat, physical activity levels, and even genetic influences. One of the significant factors

is physical activity; for example, increased physical activity level has been shown to be related with decreased CVD risk factors (McMurray et al., 2002; Eisenmann et al., 2005 b).

The overall results suggest that the natural progression was an increase in z-score of approximately one unit (-0.68 to 0.28). The natural progression was, however, greater than the expected average in the maintained- and increased-fitness groups. Table 2 shows that the maintained- and decreased-fitness groups showed approximately 50% greater change than the natural progression (one unit). This suggests that the maintenance of fitness was not adequate to even sustain the natural progression. Unexpectedly, CVD composite change scores were not significantly different between maintained- and decreased-fitness groups. Conjecture suggests that the reason for the non-significant difference could be that the duration of this study (2 years) was not long enough to result in an evident difference between the two groups. In this regard, the difference between the two groups may become evident if subsequent follow-up measurements are taken at a longer time point.

Among individual CVD risk factors, BMI was the greatest contributor to the change in CVD composite change score over two years. This may be related to the role of adipose tissues. Obesity increases adipokines, such as TNF-α and IL-6, which affect β-cells in pancreas to increase insulin resistance (Kershaw and Flier, 2004). Increased insulin resistance can affect blood pressure and blood lipid profiles (cholesterol, LDL, HDL and TG). Adipokines can also cause inflammation on the inner surface of blood vessel which decreases blood vessel diameter and increases blood pressure. The interaction of fat mass with other risk factors suggests its significant contribution to overall CVD risk factors. In support, Ondrak et al. (2007) have examined 1,824 children (8-16 y) and compared relative

importance of individual risk factors on total risk score. They found a strongest relationship between body fat and total risk score.

Although the change in BMI from the current study agrees with previous studies, the changes in mean arterial pressure (MAP), insulin resistance (HOMA), triglycerides (TG), total cholesterol (TC) and high density lipoproteins (HDL) were not significantly different between groups. These findings do not agree with previous studies (DuBose et al., 2007; Eisenmann et al., 2005 b). One possible reason for the discrepancy is the difference in the unit of aerobic fitness. While aforementioned previous studies used mL/kg/min, the current study used $mL/kg_{FFM}/min$ to eliminate the effect of fat mass. Containing fat mass in the unit of aerobic fitness includes a major contributor to the change in MAP, HOMA, TG and HDL in children. In support, Krekoukia et al. (2007) have reported a similar finding; aerobic fitness (mL/kg/min) was inversely related with HOMA (r=-0.24; p<0.5), but this relationship disappeared when adjusted for fat mass.

Limitations

This study has some limitations. First, two years of follow-up was a relatively short period of time because two years may have been too short to cause significant effects on other CVD risk factors. This could be why CVD composite change scores were not significantly different between maintained- and decreased-fitness groups. Second, physical activity (PA) was not measured and PA levels are related to both aerobic fitness and CVD risk factors. If PA data were obtained, the data could have been used to show the interplay of PA on the relationship between aerobic fitness and CVD risk factors. Third, while there are several different methods, HOMA was used to estimate insulin resistance. Although not the golden standard, HOMA was shown to be a valid and reliable method for quantifying insulin

resistance in children (Guzzaloni et al., 2002). Finally, small sample size limits generalization of findings.

Conclusion

Based on the results of the study, the following hypotheses were accepted or rejected. Research hypothesis #1. There will be an inverse relationship between changes in VO_2max ($mL/kg_{FFM}/min$) and CVD composite change score. This hypothesis was accepted because significant inverse relationship was found between VO_2max ($mL/kg_{FFM}/min$) and CVD composite change score.

Research hypothesis #2. Increased-fitness group will show a lower CVD composite change score than maintained- and decreased-fitness groups. This hypothesis was accepted because CVD composite change score in increased-fitness group was significantly lower than the other two groups.

Research hypothesis #3. Decreased-fitness group will show a higher CVD risk composite change score than maintained -fitness group. This hypothesis was rejected because there was non-significant difference in CVD composite change score between decreased-fitness group and maintained-fitness group.

Recommendations

The current study could have been more meaningful with the following modifications. First, future study can recruit participants to include more variations, which can help the generalization of the results. Specifically, natural progression of CVD composite score was first examined in this study. However, natural trend can be better determined in a larger sample size. Second, physical activity (PA) needs to be included in the future studies. Without the PA data, the interplay of PA on aerobic fitness and CVD composite score

31

remained a conjecture. Finally, longer duration study can be more meaningful to determine the effect of fitness on CVD composite score.

Despite the limitations of the study, there are meaningful insights that can be gained from the findings. This study showed that increased fitness can better control the natural progression of CVD composite score; even maintained-fitness level showed a greater natural progression that is similar to decreased-fitness group. Therefore, improving fitness is critical for cardiovascular prevention. One way to increase fitness level is to increase physical activity level, and another way is to lose weight because fatness was the strongest determinant of the changes of CVD composite score. Since moderate-to-vigorous physical activity can increase aerobic fitness while contributing on weight reduction, we should encourage and emphasize moderate-to-vigorous physical activities for children.

REFERENCES

Andersen, L., Wedderkopp, N., Hansen, H., Cooper, A., Froberg, K. (2003). Biological cardiovascular risk factors cluster in Danish children and adolescents: the European Youth Heart Study. *Preventive Medicine, 37*, 363-367.

Andersen, L., Harro, M., Sardinha, L. B., Froberg, K., Ekelund, U., Brage, S. (2006). Physical activity and clustered cardiovascular risk in children: a cross-sectional study (The European Youth Heart Study). *The Lancet, 368*, 299-304.

Andersen, L., Sardinha, L., Froberg, K., Riddonch, C., Page, A., Andersen S. (2008). Fitness, fatness and clustering of cardiovascular risk factors in children from Denmark, Estonia and Portugal: the European Youth Heart Study. *International Journal of Pediatric Obesity, 3*, 58-66.

Bao, W., Threefoot, S. A., Srinivasan, S. R., Berenson, G. S. (1995). Essential hypertension predicted by tracking of elevated blood pressure from childhood to adulthood: the Bogalusa Heart Study. *American Journal of Hypertension, 8*, 657-665.

Beaglehole, R., Trost, D., Tamir, I., Kwiterovich, P., Glueck, C., Insull, W. (1980). Plasma high-density lipoprotein cholesterol in children and young adults. The Lipid Research Clinics Program Prevalence Study. *Circulation, 62*, 83-92.

Berenson, G. S., Wattigney, W. A. Bao, W., Srinivasan R., Radhakrishnamurthy, B. (1995). Rationale to study the early natural history of heart disease: the Bogalusa Heart Study. *American Journal Medical Sciences, 310*, 22-28.

Blair, S., Kampert, J., Kohl III, H., Barlow, C., Macera, C., Paffenbarger Jr, R. (1996). Influences of cardiorespiratory fitness and other precursors on cardiovascular disease and all-cause mortality in men and women. *Journal of American Medical Association, 276*, 205-210.

Blair, S. N., Kohl III, H. W., Barlow, C. E., Paffenbarger Jr, R. S., Gibbons, L. W., Macera, C. A. (1995). Changes in physical fitness and all-cause mortality: a prospective study of healthy and unhealthy men. *Journal of American Medical Association, 273*, 1093-1098.

Blair, S. N., Kohl III, H. W., Paffenbarger Jr, R. S., Clark, D. G., Cooper, K. H., Gibbons, L. W. (1989). Physical fitness and all-cause mortality: a prospective study of healthy men and women. *Journal of American Medical Association, 262*, 2395-2401.

Bruce, C., Chouinard Jr, R. A., and Tall, A. R. (1998). Plasma lipid transfer proteins, high-density lipoproteins, and reverse cholesterol transport. *Annual review of nutrition, 18*, 297-330.

Carnethon, M., Gidding, S., Nehgme, R., Sidney, S., Jacobs, D., Liu, K. (2003). Cardiorespiratory fitness in young adulthood and the development of cardiovascular disease risk factors. *Journal of American Medical Association,* 290, 3092-3100.

Daniels, S. (2001). Cardiovascular disease risk factors and atherosclerosis in children and adolescents. *Current Atherosclerosis Reports, 3*, 479-485.

Drenick, E., Bale, G., Seltzer, F., Johnson, D. (1980). Excessive mortality and causes of death in mordidly obese men, 243, 443-445.

DuBose, K., Eisenmann, J., Donnelly, J. (2007). Aerobic fitness attenuates the metabolic syndrome score in normal-weight, at-risk-for-overweight, and overweight children. *Pediatrics, 120*, 1262-1268.

Dunn, A., Marcus, B., Kampert, J., Garcia, M., Kohl, H., Blair, S. (1997). Reduction in cardiovascular disease risk factors: 6-month results from Project Active. *Preventive Medicine, 26*, 883-892.

Dwyer, T., Magnussen, C. G., Schmidt, M. D., Ukoumunne, O. C., Ponsonby, A.-L., Raitakari, O. T. (2009). Decline in Physical Fitness From Childhood to Adulthood Associated With Increased Obesity and Insulin Resistance in Adults. *Diabetes Care, 32*, 683-687.

Eisenmann, J., DuBose, K., Donnelly, J. (2007). Fatness, Fitness, and Insulin Sensitivity Among 7-to 9-Year-Old Children. *Obesity, 15*, 2135-2144.

Eisenmann, J., Katzmarzyk, P., Perusse, L., Tremblay, A., Despres, J., Bouchard, C. (2005 a). Aerobic fitness, body mass index, and CVD risk factors among adolescents: the Quebec family study. *International Journal of Obesity, 29*, 1077-1083.

Eisenmann, J., Wickel, E., Welk, G., Blair, S. (2005 b). Relationship between adolescent fitness and fatness and cardiovascular disease risk factors in adulthood: the Aerobics Center Longitudinal Study (ACLS). *American Heart Journal, 149*, 46-53.

Farrell, S., Kampert, J., Kohl, H., Barlow, C., Macera, C., Paffenbarger JR, R. (1998). Influences of cardiorespiratory fitness levels and other predictors on cardiovascular disease mortality in men. *Medicine and Science in Sports and Exercise, 30*, 899-905.

Franco, O. H., Peeters, A., Bonneux, L., De Laet, C. (2005). Blood pressure in adulthood and life expectancy with cardiovascular disease in men and women: life course analysis. *Hypertension, 46*, 280-286.

Freedman, D., Kahn, H., Mei, Z., Grummer-Strawn, L., Dietz, W., Srinivasan, S. (2007). Relation of body mass index and waist-to-height ratio to cardiovascular disease risk factors in children and adolescents: the Bogalusa Heart Study. *American Journal of Clinical Nutrition, 86*, 33-40.

Freedman, D. S., Khan, L. K., Dietz, W. H., Srinivasan, S. R., Berenson, G. S. (2001). Relationship of childhood obesity to coronary heart disease risk factors in adulthood: the Bogalusa Heart Study. *Pediatrics, 108*, 712-718.

Freedman, D. S., Shear, C. L., Srinivasan, S. R., Webber, L. S., Berenson, G. S. (1985). Tracking of serum lipids and lipoproteins in children over an 8-year period: The Bogalusa heart study. *Preventive Medicine, 14*, 203-216.

Frerichs, R. R., Srinivasan, S. R., Webber, L. S., Berenson, G. (1976). Serum cholesterol and triglyceride levels in 3,446 children from a biracial community: the Bogalusa Heart Study. *Circulation, 54*, 302-309.

Ginsberg, H. N. (2000). Insulin resistance and cardiovascular disease. *Journal of Clinical Investigation, 106*, 453-458.

Goran, M., Fields, D. A., Hunter, G. R., Herd, S. L., Weinsier, R. L. (2000). Total body fat does not influence maximal aerobic capacity. *International Journal of Obesity Related Metabolic Disorder, 24*, 841-848.

Gordon, D. J., Probstfield, J. L., Garrison, R. J., Neaton, J. D., Castelli, W. P., Knoke, J. D. (1989). High-density lipoprotein cholesterol and cardiovascular disease. Four prospective American studies. *Circulation, 79*, 8-16.

Gregg, E., Cheng, Y., Cadwell, B., Imperatore, G., Williams, D., Flegal, K. (2005). Secular trends in cardiovascular disease risk factors according to body mass index in US adults. *Journal of American Medical Association, 293*, 1868-1875.

Guzzaloni, G., Grugni, G., Mazzilli, G., Moro, D., Morabito, F. (2002). Comparison between beta-cell function and insulin resistance indexes in prepubertal and pubertal obese children. *Metabolism, 51*, 1011-1016.

Harrell, J. S., McMurray, R. G., Bangdiwala, S. I., Frauman, A. C., Gansky, S. A., Bradley, C. B. (1996). Effects of a school-based intervention to reduce cardiovascular disease risk factors in elementary-school children: The Cardiovascular Health in Children (CHIC) Study. *The Journal of pediatrics, 128*, 797-805.

Hewitt, J., Whyte, G., Moreton, M., van Someren, K., Levine, T. (2008). The effects of a graduated aerobic exercise programme on cardiovascular disease risk factors in the NHS workplace: a randomised controlled trial. *Journal of Occupational Medicine and Toxicology, 3*, 7-15.

Hoekstra, T., Boreham, C. A., Murray, L. J., Twisk, J. (2008). Associations between aerobic and muscular fitness and cardiovascular disease risk: the northern Ireland young hearts study. *Journal of Physical Activity and Health, 5*, 815-822.

Hofman, A., Valkenburg, H. A., Maas, J., Groustra, F. N. (1985). The natural history of blood pressure in childhood. *International journal of epidemiology, 14*, 91-97.

Hubert, H. B., Feinleib, M., McNamara, P., Castelli, W. (1983). Obesity as an independent risk factor for cardiovascular disease: a 26-year follow-up of participants in the Framingham Heart Study. *Circulation, 67*, 968-975.

Hunt, S. A., Abraham, W. T., Chin, M. H., Feldman, A. M., Francis, G. S., Ganiats, T. G. (2005). ACC/AHA 2005 guideline update for the diagnosis and management of chronic heart failure in the adult: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines (Writing Committee to Update the 2001 Guidelines for the Evaluation and Management of Heart Failure): developed in collaboration with the American College of Chest Physicians and the International Society for Heart and Lung Transplantation: endorsed by the Heart Rhythm Society. *Circulation, 112*, 154-201.

Kannel, W., Wilson, P., Blair, S. (1985). Epidemiological assessment of the role of physical activity and fitness in development of cardiovascular disease. *The American Heart Journal, 109*, 876-885.

Kannel, W. B. (1975). Role of blood pressure in cardiovascular disease: The Framingham study. *Angiology, 26*, 1-7.

Kasa-Vubu, J., Lee, C., Rosenthal, A., Singer, K., Halter, J. (2005). Cardiovascular fitness and exercise as determinants of insulin resistance in postpubertal adolescent females. *Journal of Clinical Endocrinology and Metabolism, 90*, 849-856.

Kershaw, E. E., Flier, J. S. (2004). Adipose tissue as an endocrine organ. *Journal of Clinical Endocrinology and Metabolism, 89*, 2548-2555.

Keskin, M., Kurtoglu, S., Kendirci, M., Atabek, M. E., Yazici, C. (2005). Homeostasis model assessment is more reliable than the fasting glucose/insulin ratio and quantitative insulin sensitivity check index for assessing insulin resistance among obese children and adolescents. *Pediatrics, 115*, 500-507.

Kovacs, V., Fajcsak, Z., Martos, G. (2009). School-based exercise program improves fitness, body composition and cardiovascular risk profile in overweight/obese children. *Acta Physiologica Hungarica, 96*, 337-347.

Krekoukia, M., Nassis, G., Psarra, G., Skenderi, K., Chrousos, G., Sidossis, L. (2007). Elevated total and central adiposity and low physical activity are associated with insulin resistance in children. *Metabolism, 56*, 206–213.

Lauer, R., Clarke, W. (1990). Use of cholesterol measurements in childhood for prediction of adult hypercholesterolemia. *Journal of American Medical Association, 264*, 3034-3038.

Lee, C., Blair, S., Jackson, A. (1999). Cardiorespiratory fitness, body composition, and all-cause and cardiovascular disease mortality in men. *American Journal of Clinical Nutrition, 69*, 373-379.

Lewington, S., Whitlock, G., Clarke, R., Sherliker, P., Emberson, J., Halsey, J. (2007). Blood cholesterol and vascular mortality by age, sex, and blood pressure: a meta-analysis of individual data from 61 prospective studies with 55,000 vascular deaths. *Lancet, 370*, 1829-1839.

Lloyd-Jones, D., Adams, R., Brown, T., Carnethon, M., Dai, S., Simone, G., Ferguson, B., Ford, E., Furie, K., Gillespie, C., Go, A., Greenlund, K., Haase, N., Hailpern, S., Ho, P., Howard, V. Kissela, B., Kittner, S., Lackland, D., Lisabeth, L., Marelli, A., McDermott, M., Meigs, J., Mozaffarian, D., Mussolino, M., Nichol, G., Roger, V., Rosamond, W., Sacco, R., Sorlie, P., Stafford, R., Thom, R., Wasserthiel-Smoller, P., Wong, N., Wylie-Rosett, J. (2010). Excutive Summary: Hear Disease and Stroke Satistics-2010 Update. *American Heart Association, 121*, 948-954.

Macek, M., Bell, D., Rutenfranz, J., Vavra, J., Masopust, J., Neidhart, B. (1989). A comparison of coronary risk factors in groups of trained and untrained adolescents. *European journal of applied physiology and occupational physiology, 58*, 577-582.

Mattsson, N., T. Rönnemaa, M. Juonala, J. S. A. Viikari and O. T. Raitakari (2008). Childhood predictors of the metabolic syndrome in adulthood. The Cardiovascular Risk in Young Finns Study. *Annals of Medicine, 40*, 542-552.

Magnussen, C. G., Koskinen, J., Chen, W., Thomson, R., Schmidt, M. D., Srinivasan, S. R. (2010). Pediatric Metabolic Syndrome Predicts Adulthood Metabolic Syndrome, Subclinical Atherosclerosis, and Type 2 Diabetes Mellitus but Is No Better Than Body Mass Index Alone: The Bogalusa Heart Study and the Cardiovascular Risk in Young Finns Study. *Circulation, 122*, 1604-1611.

McMurray, R., Guion, W., Ainsworth, B., Harrell, J. (1998). Predicting aerobic power in children. A comparison of two methods. *The Journal of Sports Medicine and Physical Fitness, 38*, 227-233.

McMurray, R., Harrell, J., Bangdiwala. S., Bradley, C., Deng, S., Levine, A. (2002). A school-based intervention can reduce body fat and blood pressure in young adolescents. *Journal of Adolescent Health*, *31,* 125-132.

McMurray, R., Harrell, J., Bangdiwala, S., Hu, J. (2003). Tracking of physical activity and aerobic power from childhood through adolescence. *Medicine and Science in Sports and Exercise, 35*, 1914-1922.

McMurray, R., Andersen, L. (2010). The influence of exercise metabolic syndrome in youth: a review. *Americal Journal of Lifestyle Medicine, 4,* 176-186.

McTigue, K., Farrett, J., Popkin, B. (20002). The natural history of the development of obesity in a cohort of young U.S. adults between 1981 and 1998. *Annals of Internal Medicine, 136,* 923-925.

Nguyen, Q. M., Srinivasan, S. R., Xu, J.-H., Chen, W., Berenson, G. S. (2008). Changes in Risk Variables of Metabolic Syndrome Since Childhood in Pre-Diabetic and Type 2 Diabetic Subjects. *Diabetes Care, 31*, 2044-2049.

Ortega, F., Ruiz, J., Castillo, M., Sjöström, M. (2007). Physical fitness in childhood and adolescence: a powerful marker of health. *International Journal of Obesity, 32*, 1-11.

Ondrak, K., McMurray , R., Bangdiwala, S., Harrell, J. (2007). Influence of aerobic power and percent body fat on cardiovacular disease risk in youth. *Journal of Adolescent Health. 41,* 146-152.

Seibaek, M., Vestergaard, H., Burchardt, H., Sloth, C., Torp-Pedersen, C., Nielsen, S. L. (2003). Insulin resistance and maximal oxygen uptake. *Clinical Cardiology, 26,* 515-520.

Slattery, M., Jacobs JR, D. (1988). Physical fitness and cardiovascular disease mortality: the US Railroad Study. *American Journal of Epidemiology, 127,* 571-580.

Slaughter, M., Lohman, T., Boileau, R., Horswill, C., Stillman, R., VAN, L. (1988). Skinfold equations for estimation of body fatness in children and youth. *Human Biology, 60,* 709-723.

Strak, O., Atkins, E., Wolff, O., Douglas, J. (1981). Longitudinal study of obesity in the National Survey of Health and Development. *British Medical Journal, 283*, 13-17.

Thompson, D., Obarzanek, E., Franko, D., Barton, B., Morrison, J., Biro, F. (2007). Childhood overweight and cardiovascular disease risk factors: the National Heart, Lung, and Blood Institute Growth and Health Study. *The Journal of Pediatrics, 150,* 18-25.

Thompson, P., Buchner, D., Pina, I., Balady, G., Williams, M., Marcus, B. (2003). Exercise and physical activity in the prevention and treatment of atherosclerotic cardiovascular disease: a statement from the Council on Clinical Cardiology and the Council on Nutrition, Physical Activity, and Metabolism. *Arteriosclerosis, thrombosis, and vascular biology, 23,* 42-49.

Tumer, N., Yalcinkaya, F., Ince, E., Ekim, M., Kose, K., Cakar, N. (1999). Blood pressure nomograms for children and adolescents in Turkey. *Pediatric Nephrology, 13*, 438-443.

Vasan, R. S., Larson, M. G., Leip, E. P., Evans, J. C., O'Donnell, C. J., Kannel, W. B. (2001). Impact of high-normal blood pressure on the risk of cardiovascular disease. *New England Journal of Medicine, 345*, 1291-1297.

Webber, L., Srinivasan, S., Wattigney, W., Berenson, G. (1990). Tracking of serum lipids and lipoproteins from childhood to adulthood, the Bogalusa Heart Study. *American Journal of Epidemiology, 133*, 884-899.

Widham, K., Strobl, W., Westphal, G. (1981). Age dependency and tracking of serum lipids and lipoproteins in healthy children aged 11 to 14 years. *Atherosclerosis*, *38*, 189-196.