EFFECTS OF A 16 WEEK PHYSICAL ACTIVITY INTERVENTION ON SERUM C - REACTIVE PROTEIN CONCENTRATIONS IN 8-11 YEAR OLD AFRICAN AMERICAN AND CAUCASIAN CHILDREN.

by

Joshua Reid Vick

December, 2013

Director of Thesis: Robert C. Hickner, Ph.D.

Major Department: Kinesiology

C-reactive protein (CRP) is a non-specific marker of systemic inflammation that has been associated with heart disease, obesity, and metabolic disorders in adults and children. Previous physical activity interventions have yielded inconsistent results regarding the effects of exercise training on CRP concentrations in children. **Purpose:** The purpose of this study was to determine the effects of a 16-week aerobic activity intervention on serum CRP concentrations in 8-11 year old African American and Caucasian children, and to evaluate the extent to which body composition influenced this outcome. **Methods:** Serum CRP was analyzed from blood samples collected before and after 16 weeks of an aerobic physical activity intervention in 60 healthy pre-pubescent children (Tanner stage < 2) who were not taking medication other than for attention deficit hyperactivity disorder or seasonal allergies, and whose baseline and follow-up CRP concentrations were $\leq$ 10 mg/L. Participants were placed into the physical activity intervention (n = 39) or control (n = 21) groups. **Results:** Analyses revealed that CRP concentrations remained unchanged, and BMI %tiles did not decrease, with increases in absolute peak oxygen consumption in the exercise and control groups. Baseline CRP was associated with baseline age ($r = 0.356$; $p = 0.006$), BMI percentile ($r = 0.397$; $p = 0.002$), percent body fat ($r = 0.603$; $p = 0.000$), absolute peak aerobic oxygen consumption ($r = 0.314$; $p = 0.016$) and relative peak aerobic oxygen consumption ($r = -0.455$; $p = 0.000$) yet not with sex, race, or baseline waist-to-

hip ratio. No significant differences existed for CRP change across racial or BMI categories. **Conclusion:** Physical activity intervention does not seem to lower CRP concentration in the absence of BMI percentile or percent body fat reductions in African American and Caucasian children.

EFFECTS OF A 16 WEEK PHYSICAL ACTIVITY INTERVENTION ON SERUM C - REACTIVE PROTEIN CONCENTRATIONS IN 8-11 YEAR OLD AFRICAN AMERICAN AND CAUCASIAN CHILDREN.

A Thesis

Presented To the Faculty of the Department of Kinesiology

East Carolina University

In Partial Fulfillment of the Requirements for the Degree

Master of Science in Exercise and Sport Science

Exercise Physiology

by

Joshua Reid Vick

December, 2013

EFFECTS OF A 16 WEEK PHYSICAL ACTIVITY INTERVENTION ON SERUM C-REACTIVE PROTEIN CONCENTRATIONS IN 8-11YEAR OLD AFRICAN AMERICAN AND CAUCASIAN CHILDREN

by

Joshua Reid Vick

APPROVED BY:

DIRECTOR OF THESIS: _____
Robert Hickner, Ph.D.

COMMITTEE MEMBER: _____
Michael McCammon, M.A.

COMMITTEE MEMBER: _____
Katrina DuBose, Ph.D.

COMMITTEE MEMBER: _____
Kimberly Myers, Ph.D.

CHAIR OF THE DEPARTMENT
OF KINESIOLOGY: _____
Stacey Altman, J.D.

DEAN OF THE COLLEGE OF HEALTH AND HUMAN PERFORMANCE

_____
Glen G. Gilbert, Ph.D.

DEAN OF THE
GRADUATE SCHOOL: _____
Paul J. Gemperline, PhD

Acknowledgement:

I would like to acknowledge the Lord Jesus Christ for dying in my place, that I may inherit life through Him. I also acknowledge him for giving me strength to complete this thesis. May all glory be given to Him.

I would like to thank Dr. Robert Hickner for allowing me to work with him on this project, having an open door office policy, and encouraging a collaborative work environment. I couldn't have asked for a better mentor.

I would like to thank Mr. Mike McCammon, Dr. Kimberly Myers, Dr. Katrina Dubose, Dr. Matthew Mahar, Dr. Terry Jones, Morgan Pearce, Joe Peirce, Dr. Andrada Ivanescu, and Chuck Tanner for their help with this project. I would also like to acknowledge the students and personnel involved in the activity intervention and data collection from which this study was derived.

I would like to acknowledge my beautiful wife for her love, prayer, encouragement, and support throughout this process. She is truly my helpmate; what a gift. I would also like to acknowledge my parents, wonderful family, and brothers and sisters in Christ who prayed for me during this process.

TABLE OF CONTENTS

List of Tables

List of Figures

Chapter I: Introduction

C-reactive protein (CRP) is a 5 ringed acute-phase reactant that is produced in the liver, and functions in non-specific host defense. Because CRP is capable of binding to numerous membrane receptors throughout the body, and is overexpressed in times of infection or tissue injury, it has been recognized as a non-specific marker of systemic inflammation (Volankis, 2001). Concentrations of C-reactive protein may vary by age (Ford, et al., 2001) and normative values for men, women, and children have been cited as 4.1 mg/L, 5.5 mg/L, and 0.3 mg/L, respectively (Wong, Pio, Valencia, & Thakal, 2001; Ford, et al., 2001). However, though these values represent average CRP concentration among children and adults, they by no means indicate acceptable levels for optimal health. CRP concentrations as low as 2.22-4.99 mg/L have been associated with heart disease in adults (Wong, Pio, Valencia, & Thakal, 2001).

Increased C-reactive protein levels have been associated with heart disease (Rikder, Cushman, Stampfer, Tracy, & Hennekens, 1997), type II diabetes (Ford, Body Mass Index, Diabetes, and C-Reactive Protein Among U.S. Adults., 1999), metabolic syndrome (Laaksonen, et al., 2004), increased BMI (Wee, et al., 2008; Siervo, et al., 2012), and low cardiorespiratory fitness (Isasi, et al., 2003) in adults and children. Several studies have shown associations between risk factors for disease present in childhood and disease risk in adulthood (Berenson, 2002; Sun, et al., 2008; Morrison, Friedman, Wang, & Glueck, 2008) which may imply that increased inflammation during childhood could lead to disease in adulthood.

Furthermore, improved symptoms of some of the adverse health conditions associated with increased CRP concentration appear to be coupled with lowering CRP values

(Yokoe, et al., 2003; Steiropoulos, et al., 2007; Li, et al., 2008). Therefore, treatments designed to lower CRP concentrations may be worth investigating.

Physical activity interventions in adults and children have been shown to lower CRP levels (Balducci, et al., 2010; Martins, Neves, Coelho-Silva, Veríssimo, & Teixeira, 2010; Ounis, et al., 2010; Roberts, Chen, & Barnard, 2007). However, some evidence suggests that only specific types of exercise, such as mixed aerobic and resistance training, may be useful in accomplishing these results (Balducci, et al., 2010), and that improved CRP values with physical activity are more pronounced in African Americans than Caucasians (Heffernan, et al., 2009). Furthermore, some studies indicate that the improvement in CRP values with physical activity may be mediated through changes in body composition, not necessarily physical activity alone (Ounis, et al., 2010; Nemet, Oren, Pantanowitz, & Eliakim, 2013; Garanty-Bogacka, et al., 2011), while others have found no improvement in CRP values with physical activity (Nassis, et al., 2005; Kelly, Steinberger, Olson, & Dengel, 2007).

Limited research has focused primarily on examining the influence of race on CRP concentrations, however, there is evidence to suggest that African American race may affect both initial and post exercise intervention CRP concentrations (Heffernan, et al., 2009). Taken together, these findings suggest that further investigation regarding the influence of specific types of physical activity on CRP concentrations in children of varying race, habitus, and inflammatory characteristics may be useful.

Purpose of the Study

The purpose of this study was to determine the effects of a 16-week aerobic physical activity intervention on serum CRP concentrations in 8-11 year old African American and Caucasian children, and to evaluate the extent to which body composition influenced this outcome.

Hypothesis

Based on previous findings from studies involving similar physical activity interventions that have led to reductions in CRP concentrations in children, it was hypothesized that CRP concentrations for children in the physical activity intervention would be reduced compared to those in the control group. Similarly, because CRP concentrations have been reported to be elevated among African Americans compared to Caucasians, and because reductions in CRP have been shown to be greater in African American than Caucasian adults, it was hypothesized that CRP concentrations would be increased at baseline, and decreased more as a result of the physical activity intervention, in African American than Caucasian children.

Delimitations

1. Data for participants with missing CRP data, or with CRP values $\geq$ 10mg/L were not analyzed.
2. Pre-pubescent children between the ages of 8-11 years and Tanner Stage < 2 were selected to avoid very young children and to avoid pubertal confounders.

Limitations

1. The sample sizes for some participant groups were smaller than others included in the study, as well as those in the literature.
2. ADHD and seasonal allergy medication use was present during the study. It is unknown whether the medications used influenced CRP concentrations.
3. Participation in physical activity outside the scope of the study for children in the exercise or control group had the potential to affect the results.

Operational Definitions

C-reactive protein: an acute phase protein and non-specific measure of systemic inflammation

Body composition: a measure of body fatness

Obesity: having a BMI $\geq$ 30 kg/m2 in adults, or > 95th %tile for children of the same age and sex

Overweight: having a BMI of 25-29.9 kg/m^2 in adults, or $\geq$ 85th %tile for children of the same age and sex

Aerobic Fitness/Aerobic Capacity: a measure of the body's maximum ability to use oxygen to perform activity

Absolute Fitness: aerobic fitness expressed in L/min

Relative Fitness: aerobic fitness expressed in terms of one's body weight as mL/kg/min

Steady State Heart Rate: last two measured heart rates within 5 beats per minute during any given stage of an exercise testing protocol

Acronyms and Abbreviations

ADHA: Attention Deficit Hyperactivity Disorder

BMI: Body Mass Index

bpm: beats per minute

CDC: Center for Disease Control

CRP: C-reactive protein

DM: diabetes mellitus

HOMA-IR: homeostatic model assessment of insulin resistance

IDEFICS: Identification and prevention of Dietary- and lifestyle-induced health Effects in Children and Infants Study

IGT: impaired glucose tolerance

NC: normal control

NCEPATP III: The Third Report of the National Cholesterol Program Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Program)

NHANES III: Third National Health and Nutrition Examination Survey

PALs: Physical Activity Leaders

VO2 max: Maximal Volume of Oxygen Consumption

WHR: Waist-to-Hip Ratio

Chapter II: Review of Literature

C-reactive protein (CRP) has been associated with several disease states in adults and children which, if developed early in life, could lead to compromised health status later in life. Literature regarding the effects of physical activity on CRP concentration in children has yielded mixed results. The following literature review will examine associations between (a) CRP and disease, and (b) CRP and physical activity in adults and children.

CRP and Disease

Heart Disease. Among the most alarming disease states associated with CRP concentrations is atherosclerosis. Ridker and colleagues reported a positive association between baseline CRP values and myocardial infarction risk during 8 years of follow up in 1,086 physicians enrolled in the Physician's Health Study (Rikder, Cushman, Stampfer, Tracy, & Hennekens, 1997). Miller and colleagues found associations between CRP concentrations and the presence of 1 or more risk factors for heart disease in a cohort of 15,341 men and women from the NHANES III study (Miller, Zhan, & Havas, 2005).

Participants of the Physician's Health study were without history of stroke, myocardial infarction, transient ischemic attacks, or cancer at baseline (Rikder, Cushman, Stampfer, Tracy, & Hennekens, 1997), and those of the NHANES III study were selected on the basis of risk factor information (Miller, Zhan, & Havas, 2005). Regardless of smoking status, BMI, diabetes, high blood pressure, or family history of premature coronary artery disease, physicians whose baseline CRP concentrations were at least 2.11 mg/L were 2.9 times as likely (95% CI: 1.8-4.6) to experience myocardial infarction compared to those with baseline CRP values of no more than 0.55 mg/L (Rikder, Cushman, Stampfer, Tracy, & Hennekens, 1997). Similarly, Miller et al

found that, with the presence of at least 1 borderline or abnormal measurement for heart disease risk factors of total cholesterol, fasting blood glucose, blood pressure, BMI, HDL, triglycerides, or smoking status risk, , the risk for high CRP concentrations was nearly 3 fold higher than for those without any risk factors for heart disease. Further, they identified excess weight, being female, high blood pressure, smoking, diabetes, and low HDL-C values as risk factors contributing most to increased CRP values after adjustments for age and sex (Miller, Zhan, & Havas, 2005). From these studies it was concluded that increased CRP values may be used to predict myocardial infarction (Rikder, Cushman, Stampfer, Tracy, & Hennekens, 1997) and may also be attributable to risk factors for coronary heart disease (Miller, Zhan, & Havas, 2005). Though neither study was able to determine whether heart disease and associated risk factors are a cause or result of increased CRP concentrations, the associations seem to indicate a relationship between systemic inflammation and adverse cardiovascular health.

In another study by Wong et al, CRP concentrations were found to be associated with 10 year cardiovascular risk among 9,684 men and women without cardiovascular disease at baseline. Ten year risk, calculated from algorithms of the Framingham Heart Study risk factor information was analyzed with categories of increasing CRP across racial stratifications of non-Hispanic black, non-Hispanic white, and Mexican American. While CRP concentrations were found to be higher in African American men and women than Caucasian men and women, increased CRP concentrations were more common in women than men. CRP concentrations were 4.7 ± 7.5 mg/L and 6.1 ± 8.6 mg/L in African American men and women, respectively, compared to 3.7 ± 5.2 mg/L and 4.6 ± 6.2 mg/L in Caucasian men and women, respectively. CRP concentrations ≥ 1 mg/L were associated with risk factors of smoking, increased age, increased BMI, and high blood pressure in men, as well as with increased BMI and diabetes

prevalence in women. Furthermore, ten year cardiovascular disease risk progressively increased from 13.4% to 21.1% in men, and from 2.4% to 4.2% in women across increasing categories of CRP concentration. Researchers concluded that CRP concentration differed by race and gender, and seemed to be related to coronary heart disease (Wong, Pio, Valencia, & Thakal, 2001).

Associations of elevated CRP levels with heart disease risk have also been found in children (Wijnstok, et al., 2010; Cook, et al., 2000). Significant associations between CRP and cardiovascular disease (CVD) risk were noted among 276 12-15 year olds (Wijnstok, et al., 2010), and 699 10-11 year old British children (Cook, et al., 2000), though some of the associations were attributed to adiposity. Wijnstok et al studied clustered cardiovascular risk from mean arterial pressure, LDL/HDL ratio, aerobic fitness, skinfolds, and triglyceride information in obese, overweight, and normal weight children matched for age, sex, and smoking status that were sampled from the Young Hearts 2000 Study (Wijnstok, et al., 2010). British children of the Ten Towns Children Study were selected from different towns with low and high indices of cardiovascular disease mortality in adults (Cook, et al., 2000).

Both studies identified associations between cardiovascular disease risk factors and CRP. Cook et al found that CRP was 47% higher in girls than boys, increased with age, and was associated with ponderal index (weight/height^3). After adjustments for age, sex, town, and ethnicity, CRP concentrations were 270% higher in the highest quintile of current ponderal index than in the lowest. Of all cardiovascular disease risk factors identified by the researchers in the British children, only HDL concentration ($r = -0.13$), resting heart rate ($r = 0.12$), and fibrinogen levels ($r = 0.33$) were found to be significantly associated with CRP concentrations after adjustments for age, sex, town, ethnicity, and ponderal index, while adiposity was related to all cardiovascular risk factors analyzed, independent of CRP concentrations (Cook, et al., 2000).

Similarly, Wijnstok et al. reported positive associations between CRP concentrations and cardiovascular disease risk in children ($r = 0.39$) that continued after multiple regression analysis included all significant predictors for CVD risk. However, associations between lifestyle factors, such as diet composition or physical activity, and CVD risk, diminished after multiple regression analyses. Furthermore, CRP was associated with four of the five CVD risk factors identified in the study, including LDL/HDL ratio ($r = -0.21$), triglycerides ($r = 0.21$), aerobic fitness ($r =0.30$), and skinfold thickness ($r = 0.38$) (Wijnstok, et al., 2010). Though both groups of researchers acknowledged the relationship between CRP and risk factors for heart disease, similar conclusions regarding how strongly the two were associated was not drawn. Wijnstok et al. concluded that inflammatory markers such as CRP were strong indications of cardiovascular disease risk (Wijnstok, et al., 2010), while Cook et al. concluded that adiposity was more related to CRP concentration than the other parameters studied, and that the positive association between adiposity and cardiovascular risk was independent of CRP (Cook, et al., 2000). However, from the associations between CRP, HDL, and fibrinogen, after adjusting for adiposity in the Cook et al. study, it could also be implied that CRP does influence heart disease risk independent of adiposity. Furthermore, given the negative association between resting heart rate and aerobic fitness, the positive association between resting heart rate and CRP values in these children, independent of adiposity, may be an indication that unstudied physical fitness levels corresponded to lower CRP values in individuals of varying body fat content. The measure of ponderal index could have; however, been a better measure of adiposity in this population than those used by Wijnstok et al, which may explain the difference in findings. Nonetheless, there seems to be evidence to suggest CRP is positively associated with heart disease risk in adults and children.

Body Composition. While the relationship between heart disease and obesity has been well established, a positive relationship between CRP and obesity has also been noticed among populations of adults (Yudkin, Stehouwer, Emeis, & Coppack, 1999; Ford, 1999) and children (Ford, et al., 2001; Nappo, et al., 2013; Alvarez, Higgins, Oster, Darnell, & Gower, 2009). Not only have CRP concentrations in children been associated with current measures of body composition, but have also been reported to be an indication of body composition later in life. Ford et al. noticed a significant association between BMI and CRP values among a cohort of 5,305 8-18 year old children from the NHANES III study (Ford, et al., 2001) while Nappo and colleagues found associations between baseline CRP and baseline BMI, baseline cardiovascular risk factors, and BMI at 2-year follow up in a group of over 6,500 2-9 year old children of the Identification and Prevention of Dietary- and Lifestyle-Induced Health Effects in Children and Infants study (IDEFICS) (Nappo, et al., 2013).

Both studies found increasing adiposity with categories of increasing CRP concentrations. CRP was classified as either $\leq$ 2.1 mg/L or > 2.1 mg/L ("elevated") by Ford et al. (Ford, et al., 2001), and was sectioned into three sex specific categories ranging from < 0.2 mg/L to > 1.8 mg/L in the study by Nappo et al. (Nappo, et al., 2013). After statistical adjustment for age, sex, ethnicity, poverty income ratio, white blood cell count, and history of chronic bronchitis, Ford et al. found that odds ratios for having elevated CRP within groups of BMI categories $<15^{th}$, 85^{th}- $< 95^{th}$, and $\geq 95^{th}$ percentiles were 1.71 (95% CI: 0.96-3.01), 2.20 (95% CI: 1.3-3.75), and 4.92 (95% CI: 3.39-7.15), respectively (Ford, et al., 2001). Also note-worthy was that CRP was not associated with sex or ethnicity. Similarly, Nappo et al. reported that average baseline BMI z-score increased from -0.059 to 0.611 and average baseline waist circumference increased from 53.3 cm to 57.5 cm, across increasing categories of baseline CRP

concentration in boys, while baseline BMI z-score was increased from -0.288 to 0.688 and baseline waist circumference increased from 52.1 cm to 57.2 cm, on average, across increasing categories of baseline CRP concentration in girls. Furthermore, though BMI z-score increased across all categories of baseline CRP concentration in the 4,110 children participating in 2-year follow-up, higher increases were seen among children with increasing baseline CRP categories, regardless of baseline BMI z-score. BMI z-scores increased by an average of 0.107, 0.196 and 0.279 in boys and 0.071, 0.188, 0.235 in girls across categories I, II, and III of baseline CRP values, respectively and the odds ratios for becoming overweight/obese for children in baseline CRP categories I, II, and III were computed as 1.0, 2.0 (95% CI: 1.49-2.9), and 2.0 (95% CI: 1.1-3.5) in boys, and 1.0, 2.0 (95% CI: 1.2-3.2), and 2.6 (95% CI: 1.4-4.7) in girls, respectively (Nappo, et al., 2013). Findings from both studies seem to indicate an increased risk for overweight/obese status in boys and girls with increasing CRP values.

Similarly, in a group of 59 healthy 7-12 year old children, CRP concentrations were found to be higher in obese participants before and after meal consumption than in lean counterparts. Children were in Tanner stage 1-3, and were excluded from the study if they were taking medications that could potentially affect body composition or physical activity participation. Obese children displayed significantly higher concentrations of CRP during fasting, and after meal consumption, than did lean children, with fasting CRP concentration of 0.17 ± 0.21 mg/L and 1.10 ± 1.11 mg/L observed in lean and obese children, respectively. Though no markers of inflammation were significantly associated with lean body mass, CRP was significantly associated with fasting and postprandial measures of BMI ($r = 0.51$ and -0.36, respectively), BMI percentile ($r = 0.52$ and -0.47, respectively), total body fat ($r = 0.67$ and -0.45), and percent body fat ($r = 0.71$ and 0.47, respectively). (Alvarez, Higgins, Oster, Darnell, &

Gower, 2009). Given that CRP has been associated with increased BMI measures in children, the relationship between CRP concentrations and other disease states related to obesity is worth investigating.

Metabolic Syndrome and Type II Diabetes. Associations have also been shown between CRP concentrations, metabolic syndrome, and type II diabetes (Santos, Lopes, Guimara˜es, & Barros, 2005; Laaksonen, et al., 2004; Yuan, et al., 2006), which may not come as a surprise given the similarity in the risk factors for these conditions with those of heart disease and obesity.

In a group of 957 Portugal residents, Santos and colleagues found associations between CRP and risk factors for metabolic syndrome. Participants were 18-92 years of age, most of whom were between 40-60 years old. CRP values >10 mg/L were excluded from the analysis. After statistical adjustment for age, sex, smoking status, and alcohol consumption, values for each of the 5 risk factors for metabolic syndrome were associated with higher mean CRP concentrations than were normal values for these risk factors. A significant positive trend ($p < 0.001$) was also noticed between the number of metabolic syndrome risk factors present and CRP values among the population, with CRP values progressing from 0.97 mg/L (95% CI: 0.84-1.12) to 3.18 mg/L (95% CI: 1.42-7.10) across risk number categories of 0-5. Furthermore, the combination of increased waist circumference and high blood pressure showed a 2 fold increase in CRP values compared to the reference group, and the addition of 1 of the 3 other NCEPATPIII risk factors for metabolic syndrome to these two did not correspond to significant increases in CRP concentrations. The researchers concluded that central obesity and high blood pressure seemed to be the 2 characteristics of metabolic syndrome most associated with inflammation (Santos, Lopes, Guimara˜es, & Barros, 2005)

A longitudinal study of 762 Finnish men without diabetes or metabolic syndrome at baseline showed associations between increased serum CRP concentrations at baseline and the risk of developing either condition at 11 year follow-up (Laaksonen, et al., 2004). Participants were 42, 58, 54, or 60 year old at baseline and followed for 11 years. CRP was classified as 0.1-1.99 mg/L, 1.0-2.99 mg/L, and 3.0-9.99 mg/L and participants with CRP concentrations of 10 mg/L or more were excluded from the analysis. Participants with high baseline CRP concentrations were 2.91 (95% CI: 1.65-5.10) and 3.60 (95% CI: 1.98-6.57) times more likely to develop metabolic syndrome according to NCEP and WHO criteria, respectively, than those with low baseline CRP concentrations. However, after adjusting for BMI, waist girth, WHR, blood pressure, insulin, glucose and triglyceride levels, these findings were no longer significant. Similarly, after statistical adjustment for age, participants with high baseline CRP concentrations were 3.6 (95% CI: 1.84-7.41) times more likely to develop diabetes later on than those with low baseline CRP concentrations. Furthermore, after additional adjustments for cardiovascular disease, socioeconomic status, lifestyle factors, family history of diabetes, BMI and WHR, blood pressure, insulin, glucose and triglyceride levels, a significant 2.3 (95% CI: 1.04-5.07) fold increased risk of developing diabetes was seen among participants with high baseline CRP concentrations compared to those with low baseline CRP concentrations. Interestingly, no significant associations were noticed between BMI, CRP concentrations, and the development of either metabolic syndrome or diabetes, perhaps suggesting that BMI may not mediate the association between CRP and the two conditions. The researchers concluded that CRP concentrations were more associated with diabetes than metabolic syndrome at follow up, though inflammation seemed to increase the risk of both conditions (Laaksonen, et al., 2004).

Associations have also been seen between CRP concentration and insulin resistance in a group of 90 middle aged participants with and without impaired glucose tolerance (IGT) and type II diabetes (DM) (Yuan, et al., 2006). After adjustments for BMI and WHR, serum CRP concentration was significantly increased in IGT and DM groups compared with the normal control (NC) group. Mean concentrations of CRP were 0.40 mg/L (0.21–1.67), 3.56 mg/L, (1.73–9.47), and 2.46 mg/L (1.04-6.7) in NC, IGT, and DM groups, respectively. Increased CRP concentrations in the IGT and DM groups were significantly positively correlated with BMI ($r = 0.528$), WHR ($r = 0.359$), fasting plasma glucose levels ($r = 0.281$), and fasting insulin concentration ($r = 0.456$), while increased CRP concentrations were negatively associated with insulin sensitivity index ($r = -0.271$). The researchers concluded that CRP is strongly associated with components of metabolic syndrome in patients with impaired glucose tolerance or type II diabetes, though many of the associations may be mediated through body composition. Furthermore, the researchers acknowledged that whether chronic systemic inflammation leads to increased insulin sensitivity, or increased insulin sensitivity leads to increased systemic inflammation is not well understood (Yuan, et al., 2006). These findings seem to indicate that increased CRP concentrations have been associated with characteristics of disease, though whether or not the CRP values are directly related with the cause of these conditions, their risk factors, or are a result of them, remains unclear.

Physical Activity and CRP

Physical Activity and CRP in Adults. There seems to be an association between CRP concentrations and physical activity interventions in the literature, though some findings indicate specific exercise modalities may be necessary for this relationship to be observed. Martins and colleagues found an association between improvement of CRP concentrations as a result of

either aerobic or strength training in forty-five sedentary men and women over 64 years of age who did not have CRP concentrations of more than 15 mg/L. Hypertension, diabetes, and angina pectoris were the most common conditions noted among the study group. After assignment to either a normal activity control group, an aerobic training group, or a strength training group for 16 weeks, participants were asked to discontinue activity for another 16 weeks. Aerobic and strength training groups exercised for 45 minutes, 3 times a week. Aerobic training intensity progressively increased from 40 to 70-85% heart rate reserve throughout the 16 week training period and strength training sessions consisted of calisthenics and elastic band exercises for 8 major muscle groups. Small correlations between CRP and, both, waist circumference and BMI values were noticed at baseline. The aerobic training group experienced a 3.9 cm reduction in waist circumference, a 0.9 kg decrease in body mass, and a 0.42 mg/ L reduction in log CRP, from baseline to 32 weeks. Log CRP concentrations the strength training group decreased by 0.21 mg/L during this time as well. A significant correlation between waist circumference and CRP concentration ($r = 0.552$) was also noticed at week 32 for the strength training group. CRP was unchanged in the control group. From baseline, 51% and 31% reductions in CRP concentrations were experienced by the aerobic and strength training groups, respectively. From these findings, the researchers concluded that exercise resulting in increased muscular strength and adiposity loss was anti-inflammatory in this population (Martins, Neves, Coelho-Silva, Veríssimo, & Teixeira, 2010).

Similarly, in a group of 82 men and women with type II diabetes and metabolic syndrome that performed no exercise, aerobic training only, or mixed aerobic and strength training, reductions in CRP differed from non-exercisers only for those prescribed mixed aerobic and strength training. Lean and obese 40-75 year old participants were placed into either sedentary

(A), low-intensity aerobic exercise counseling (B), aerobic activity only (C), or mixed aerobic and strength training (D) groups for 12 months. The aerobic training only group (C) exercised aerobically for 60 minutes at 70-80% VO_2 max each session, while the mixed training group (D) performed 40 minutes of aerobic exercise at 70-80% VO_2 max, as well as 20 minutes of strength training at 80% 1RM. Standard diabetes care, including continuation of pharmacologic medication, was continued throughout the study for all patients. VO_2 max increased in groups C and D, regardless of weight loss, while increases in strength and flexibility were only noted in group D. Compared to baseline measures, CRP decreased by 28% and 54% in groups C, and D, respectively, at follow-up, though only group D participants were shown to experience a significantly higher 12 month reduction in CRP concentrations than participants who did not exercise. The reduction in CRP for participants in group D was nearly twice that of group A. The combination of exercise type, exercise duration, VO_2 max, and waist circumference change was identified as a significant "predictor" CRP concentration change ($r^2 = 0.73$), though BMI and fat mass were not. The researchers concluded that exercise seemed to produce an anti-inflammatory effect in men and women with diabetes and metabolic syndrome, regardless of weight loss, and, that specific exercise type, volume, and duration, specifically, high intensity mixed training that increased levels of daily physical activity, were needed to see these results (Balducci, et al., 2010). These results are significant, since other studies have indicated reductions in CRP with lifestyle modification were mediated through weight loss (Ounis, et al., 2010; Nemet, Oren, Pantanowitz, & Eliakim, 2013; Garanty-Bogacka, et al., 2011).

Interestingly, a difference in the response of CRP concentrations to strength training has been noted between 39 young African American and Caucasian men. All participants were healthy, were asked to avoid aerobic training, participated in an hour of resistance training 3

times per week for 6 weeks, then detrained for 4 weeks. Baseline concentrations of CRP were increased in African American, as compared to Caucasian men, with African American men having CRP concentrations of 4.84 $\pm$ 0.9 mg/L and Caucasian men having CRP concentration of 1.34 $\pm$ 0.9 mg/L. Decreases in CRP concentration with training were only seen for the African American participants, as their CRP concentration was reduced to 2.34 $\pm$ 0.5 mg/L after 6 weeks. This decrease remained significantly lower than baseline CRP concentrations after detraining. From these findings, it was concluded that resistance training may be appropriate for lowering cardiovascular disease risk in men (Heffernan, et al., 2009). Not only do these studies provide evidence for modality specific decreases in CRP concentration with exercise training in adults, but also suggest race as a viable factor for inflammatory responses.

Physical Activity and CRP in Children. Physical activity has also been found to be associated with CRP concentration in children. Childhood CRP values have been shown to be significantly related to predicted VO_2 max and BMI, but not with self-reported physical activity levels in a group of 10.25 $\pm$ 0.75 year old boys (Sadeghipour, Rahnama, Salesi, Rahnama, & Mojtahedi, 2010). Among a group of 44 male school aged children from Shiraz, Iran, Sadeghipour et al. studied the relationship between serum CRP concentrations and physical fitness, physical activity, obesity, and cardiovascular risk factors. A significant, negative correlation was noticed between CRP and relative VO_2 max ($r = -0.45$), while a significant, positive, correlation was noticed between CRP and BMI ($r = 0.55$). No significant correlations were seen between CRP and physical activity ($r = -0.31$; $p = 0.08$). From these findings, it was concluded that BMI was a strong predictor of CRP values in young children, and that body weight control may mediate associations between CRP and physical fitness. Researchers acknowledged, however, that the small sample size and potential inaccurate physical activity

reflections may have skewed the results (Sadeghipour, Rahnama, Salesi, Rahnama, & Mojtahedi, 2010).

On the other hand, Isasi et. al found an association between physical fitness level and CRP concentration that was independent of body composition measures and ethnicity in boys. Participants consisted of 205 healthy boys and girls, ages 6-24 years whose ethnicity was determined to be Hispanic, black without Hispanic descent, white without Hispanic descent, or Asian or Pacific Islander from the mother's self-report. Mean CRP concentrations did not differ for boys and girls, though physical fitness was moderately associated with CRP values only in boys ($r = -0.35$). CRP concentrations were positively associated with measurements of BMI in boys and girls ($r = 0.23$ and 0.24, respectively), sum of skinfolds in boys and girls ($r = 0.34$ and 0.31, respectively), and waist/hip ratio in boys ($r = 0.27$), while CRP concentrations were negatively associated with age ($r = -0.12$). Low, middle, and high physical fitness values in boys were significantly associated with graded decreases in CRP concentrations, with average CRP concentrations of 2.0 mg/L, 1.6 mg/L, and 1.1 mg/L for low, middle, and high fitness levels, respectively. Furthermore, physical fitness remained significantly inversely associated with CRP concentration in boys ($r = -0.32$) after adjustments for age, ethnicity, family history, recruitment site, and BMI or sum of skinfolds. Though smoking status and Tanner stage were not assessed, the authors concluded that the relationship between CRP and physical fitness was more evident in boys than girls, and that higher physical fitness may be helpful in modifying increased CRP concentrations seen with low-grade inflammation (Isasi, et al., 2003). Interestingly, CRP was significantly associated with physical fitness in boys, regardless of ethnicity or body composition measures.

Associations between CRP concentration and exercise training have also been found in the literature (Ounis, et al., 2010; Roberts, Chen, & Barnard, 2007). Ounis et al. showed that an 8 week exercise program leading to weight loss in obese children also led to a decrease in CRP levels (Ounis, Elloumil and Zouhal) and one group of researchers found that a 2 week diet and physical activity program lead to a reduction in CRP concentrations in overweight children (Roberts, Chen and Barnard).

While examining the effects of an 8 week lifestyle intervention on metabolic parameters in 28 obese children with an average age of 13.2 years, Ounis et al determined that exercise training, coupled with diet modification and improved body composition was significantly associated with improvements in inflammatory markers, including CRP. Participants were matched for age, anthropometric measurements, and pubertal status before the intervention, and were placed into either a diet/exercise group or a normal activity control group for 8 weeks. The diet/exercise group received a calorically reduced diet, exercise instruction, and educational sessions for the duration of the intervention, while participants in the control group were instructed to maintain their normal behaviors. Training consisted of 4 supervised, 90 minute, exercise sessions per week at a heart rate corresponding to the maximum level of lipid oxidation. Sessions involved warming up, running, jumping, and playing with a balloon. Caloric intake, body weight, percent body fat, and CRP concentrations were reduced in the diet/exercise group, while no changes for any of these measurements were observed in the control group. Coupled with a 9.4 ml/kg/min increase in VO_2 max was a 0.9 mg/L decrease in CRP concentration experienced by the diet/exercise group, again, without any significant change for these parameters in the control group. Changes in BF% were positively associated with changes in CRP values, while changes in VO_2 max were negatively correlated with changes in CRP values

(Ounis, et al., 2010).From these findings, it was concluded that exercise, in combination with diet modification and improved body composition, may significantly affect the improvement of inflammatory markers, such as CRP, in obese children. Though changes in both body composition and aerobic fitness levels were correlated with lower CRP values, the researchers indicated that their results contradicted the idea that exercise interventions alone, aside from weight loss, are able to lower levels of inflammation (Ounis, et al., 2010).

Similarly, Roberts and colleagues reported reductions in CRP levels of 19 overweight 8-17 year old children who all participated in a diet and exercise program lasting only 2 weeks. The program consisted of a qualitatively modified diet and 2-2.5 hours per day of supervised activities such as tennis, beach games, and gym based physical activities. Intensity was unmonitored since purpose of the intervention was to increase physical activity levels, not improve fitness. On average, the participants experienced a 4.3% reduction in BMI and a serum CRP reduction of 1.47 mg/L, from 3.61 mg/L to 2.14 mg/L (Roberts, Chen, & Barnard, 2007). These authors concluded that, in obese children without disease, reductions in cardiovascular risk factors and inflammation levels may be achieved through vigorous diet and exercise intervention in a relatively short time frame. Researchers recognized that these participants were very motivated and that generalizations of their adherence to the study protocol may not be extrapolated to other populations, and that the study was not designed to measure which part of the intervention may have been responsible for improvements, or to what extent these intervention components impacted the results (Roberts, Chen, & Barnard, 2007).The lack of a control group, and identification of 7 participants with metabolic syndrome in a previous article from the same intervention (Chen, Roberts, & Barnard, 2006), may further limit the credibility

and generalizability of the findings. Never the less, the study showed that improvements in CRP concentration are possible with 2 weeks of diet and exercise in motivated, overweight children.

Though interventions including physical activity have been shown to lower C-reactive protein values, authors of some of these studies have attributed the improvements to improvements in body composition (Nemet, Oren, Pantanowitz, & Eliakim, 2013; Garanty-Bogacka, et al., 2011). A study performed with 50 obese Polish children ages 8-18 years old, indicated that inflammatory biomarkers as well as blood lipid profiles were improved after a 6 month diet and physical activity intervention leading to weight loss. All children were provided instruction for including exercise into everyday life, reducing tv time, and reducing fat and sugar intake. Thus, there was no control group. Significant positive correlations (r values between 2.0 and 4.0) were noticed between CRP and BMI z-scores, percent body fat, and waist circumference z-scores at baseline. CRP was reduced from 1.5 $\pm$ 0.8 mg/L at baseline to 0.7 $\pm$ 0.5 mg/L at follow-up, and changes in both percent body fat and HOMA-IR were positively correlated with changes in CRP (r = 0.33 and 0.29, respectively).The authors concluded that measures of adiposity and insulin resistance were significantly associated with inflammatory markers, and that the half year diet/physical activity lifestyle intervention leading to weight reduction and improved lipid profiles was sufficient to improve insulin resistance and lower inflammatory markers, such as CRP, in obese children (Garanty-Bogacka, et al., 2011).

Another 3 month life-style intervention lead to improvements in weight, body composition, physical activity levels, physical fitness, inflammation, and insulin resistance in a group of 21 obese 6-13 year old children (Nemet, Oren, Pantanowitz, & Eliakim, 2013). Participants in the diet/physical activity intervention group were matched for age and sex with participants in the control group, who were referred to at least 1 nutritional consult and were

instructed to perform physical activity on their own at least 3 days per week. The diet/exercise group met 4-6 times with a dietician, received hypo-caloric diets, was encouraged to limit sedentary time, participated in supervised physical activity twice per week, and was encouraged to participate in 30-45 minutes of walking/weight bearing activities at least one more day per week. Exercise was completed in age groups and mimicked type and intensity of that typically performed by elementary and middle school children (Nemet, Oren, Pantanowitz, & Eliakim, 2013).

The intervention group experienced significant reductions in BMI percentile (–0.96 $\pm$ 1.29), waist circumference (–2.1 $\pm$ 2.7 cm), fasting CRP (–0.06 $\pm$ 0.29 mg/L), and HOMA-IR score (–0.15 $\pm$ 0.57) from baseline to follow-up, none of which were experienced by the control group. Though CRP concentrations decreased only slightly during the intervention in the diet/physical activity group, CRP concentrations doubled in the control group (+ 0.5 $\pm$ 0.86 mg/L). Interestingly, CRP reductions were found to be associated only with self-reported physical activity levels ($r = -0.421$), and no other parameters. It was concluded that the multidisciplinary intervention resulted in significant favorable effects in body weight, BMI percentiles, waist circumference, physical activity, physical fitness, insulin resistance, and inflammatory markers in obese children, and that the preventive role of exercise in obesity is at least partially mediated through the accompanying attenuation of inflammatory response (Nemet, Oren, Pantanowitz, & Eliakim, 2013).

Though these findings seem to present a fairly stable argument for the association of CRP with physical activity, not all studies examining the two variables reached the same conclusions (Nassis, et al., 2005; Kelly, Steinberger, Olson, & Dengel, 2007). Nassis et al and Kelly et al both reported changes in fitness level, yet not CRP concentrations after aerobic exercise

interventions. In a group of 21 overweight and obese girls from Athens, Greece, Nassis et al found that a 12 week exercise program improved fitness and insulin sensitivity, yet not CRP concentrations, while Kelly et al reported improvements in fitness, without changes in CRP concentration or body composition after 8 weeks of exercise in a group of 19 overweight children. Though all participants of the Nassis et al study were placed in the exercise group, Kelly et al placed children into an exercise or control group. Exercise sessions of both studies were supervised and aerobic in nature, consisting of a variety of activities for children in the Nassis et al study, and cycle ergometry only for those in the study by Kelly et al. The participants in the Nassis et al study met 3 times per week for 40 minutes, while those in the Kelly et al study met 4 times per week for 30-50 minutes. The intensity of training sessions were measured via heart rate for both studies, as Nassis et al encouraged children to keep hear rates above 150 beats per minute and Kelly et al facilitated exercise to elicit heart rates corresponding to 50-80% VO2 max as the program progressed (Nassis, et al., 2005; Kelly, Steinberger, Olson, & Dengel, 2007).

Neither weight nor BMI changed in any group of participants in either study, though aerobic fitness increased in exercise groups of both studies, and was reported to decrease in the control group studied by Kelly et al. No significant changes in baseline CRP were reported between exercise and control groups of the Kelly et al study, and CRP was not found to be significantly different in any groups after the exercise intervention of either study (Nassis, et al., 2005 Kelly, Steinberger, Olson, & Dengel, 2007). It can be concluded from these studies that exercise without changes to body composition may not be a means by which to improve CRP concentrations in overweight and children.

There is evidence to suggest similar arithmetic CRP concentrations in 3-19 year old African American (0.4 mg/L) and Caucasian (0.4 mg/L) children, differences in geometric mean

CRP concentrations between African American (0.10 mg/L) and Caucasian (0.9 mg/L) children, and increased median CR P concentrations in African American (0.4 mg/L) and Caucasian (0.3 mg/L) children (Ford, et al., 2001). One study indicated that the association between physical fitness and CRP ($\beta = -0.02$, SE = 0.01) was independent of ethnicity in 95 boys, ages 6-24 years old (Isasi, et al., 2003). However, the reviewed literature was lacking in terms of comparing change in CRP concentration during physical activity interventions in African American and Caucasian children.

Summary

CRP has been linked with numerous disease states in adults and children and there is evidence to suggest associations between reductions in CRP concentration and exercise training in these populations. However, the inconsistency of these findings with respect to intervention design, race, and body composition, leave room for further study of the effect of exercise training on CRP concentrations in children.

Chapter III: Methods

Participants

8-11 year old African American and Caucasian prepubescent children from Eastern North Carolina were included in the study. Pubertal status was assessed with criteria from Tanner staging. Children were further grouped by race and baseline body composition status. Children with African American parents and grandparents were classified as African American, while children with Caucasian parents and grandparents were classified as Caucasian. Body composition was categorized as lean (BMI<85^{th}%tile), or non-lean (BMI$\geq$$85^{th}$ %tile).

Inclusion criteria

Blood samples from participants that were ages 8-11 years, African American or Caucasian, who had Tanner stage I or II pubertal status, were healthy, were not taking medication other than for attention deficit hyperactivity disorder or seasonal allergies, were not participating in purposeful physical activity or sports, and whose baseline and follow-up CRP concentrations were < 10 mg/L were included in the analysis.

Exclusion criteria

Blood samples from children who were races other than African American or Caucasian, had pubertal status > Tanner stage II, diabetes (fasting blood glucose > 125 mg/dL), blood pressure values > 95^{th} %tile for age and sex, were taking medications other than for attention deficit hyperactivity disorder or seasonal allergies, were participating in regular, purposeful physical activity or sports, had orthopedic injury limiting physical activity participation, or who had CRP concentrations $\geq$ 10 mg/L at baseline or follow-up were excluded from the analysis.

Recruitment

Participants were recruited from Pitt County, North Carolina through local pediatricians, physical education teachers, as well as through e-mail and newspaper advertisements. The population in Pitt County was approximately 35% African American at the time of recruitment.

Measurements

Tanner staging was assessed by the children's parents. Anthropometric and aerobic fitness measurements were taken at baseline and after the 16 week intervention, as were blood samples.

Anthropometric measurements included height and weight, as well as waist and hip circumference measurements. Height was assessed using a standard stadiometer to the nearest cm and weight measurements were gathered using an electronic scale and recorded to the nearest 0.1 kg. BMI was calculated from height and weight information and percent body fat was estimated from fat-free mass (lean mass + bone mineral content) and fat mass (android + gynoid) values that were assessed using dual-energy x-ray absorptiometry (DXA; GE Lunar Prodigy Advance, Madison, WI).

Aerobic fitness was evaluated using peak VO2 values ascertained from maximal treadmill exercise testing. Each participant completed two tests to assure that there was adequate effort during maximal treadmill testing and to determine day-to-day variability in the test. If peak VO2 values from the initial two tests were not within ±5%, a third test was conducted. Peak aerobic capacity was defined as the average oxygen consumption of all tests performed. Relative peak aerobic oxygen consumptions were calculated using the average of all weights recorded for exercise testing sessions performed.

Exercise testing sessions began with a submaximal 5-minute walk at 2.5 miles per hour (mph) at 0% grade to determine submaximal VO_2 at a given absolute exercise intensity, followed by a graded maximal run/walk test. Once steady state heart rate was achieved during the submaximal session, the test transitioned to a maximal protocol. During the maximal protocol, treadmill speed increased (0.5 mph) each minute up to 5 mph, at which point, the speed remained constant (5 mph) and the grade increased 3% per minute thereafter. Participant heart rate and Rating of Perceived Exertion (RPE) were monitored and recorded each minute, while oxygen consumption was determined and averaged every 20 seconds by indirect calorimetry with a Parvomedics metabolic cart. Peak oxygen consumption and total time on the treadmill were the outcome variables used for analyses of changes in peak aerobic capacity.

Participant groups

Children were placed into either a normal activity control group or a physical activity intervention group. Participants in the physical activity group completed 16 weeks of supervised physical activity while those in the normal activity group were instructed to continue their normal activity patterns during this time.

Physical Activity Intervention

The 16 week physical activity intervention was led by undergraduate physical activity leaders (PALs), and was designed to provide a noncompetitive, supportive environment for children to develop confidence in physical activity participation. Participants were encouraged to come to the program 4 days per week and were required to attend at least 3 days per week for a minimum of 1 hour per session. Participants were allowed to choose activities, such as tennis, racquetball, kickball, tag, stair climbing, and treadmill, cycle ergometry, or elliptical exercise.

Physical activities were performed in a way that average heart rates during the sessions were140 bpm. Heart rates were monitored during each session in order to evaluate mean heart rate. PALs were responsible for leading groups of three to five participants, keeping children involved and active. Dropouts were defined as those who attended less than 75% of the exercise sessions (which would be less than 3 days per week on average) or performed less than 75% of the total accumulated minutes of physical activity.

CRP analysis

Blood samples were drawn from an antecubital or hand vein, the morning after a 12 hour overnight fast and were frozen between 2-6 years at -80 degrees Celsius until analysis. Pre and post intervention blood samples from 60 participants in the physical activity and control groups were retrieved for the analysis of CRP concentrations.

CRP was analyzed with a MSD 96-Well MULTI-SPOT Vascular Injury Panel II Assay kit from Meso Scale Discovery (Rockville, Maryland). The multi-spot 96 well plate was blocked with 5% Blocker A Solution overnight at 4 degrees C. The plate was washed with phosphate buffered saline with 0.05 % Tween-20 (PBS-T). An eight point standard curve consisting of calibrators ranging from 0-1000 ng/mL of CRP was applied to the plate. Plasma samples were diluted 200x using diluent provided with the kit and applied to the plate. After the appropriate incubation time plates were washed with PBS-T and detection antibody was applied. After the appropriate incubation time, the plates were washed and a 1x read buffer was applied. The plate was immediately read in the MSD SECTOR Imager 2400 (model #1250). Plasma concentrations of CRP were derived using MSD's Data Analysis Toolbox for software version 3.0.

Statistical Analysis

Demographic data were analyzed with non-paired t-tests. Between groups comparisons for baseline and follow-up BMI, BMI %tile, WHR, body fat%, absolute peak VO_2, relative peak VO_2, time to exhaustion, and CRP concentration were analyzed with t-tests. Differences in BMI, BMI %tile, WHR, body fat%, absolute peak VO_2, relative peak VO_2, time to exhaustion, CRP concentration during the intervention were calculated by subtracting pre from post measurements, and analyzed with t-tests. Comparisons of difference in CRP for African Americans in the physical activity and control groups, Caucasians in the physical activity and control groups, African Americans and Caucasians in the physical activity group, as well as African Americans and Caucasians in the control group were also analyzed with t-tests. Comparisons of difference in CRP for females in the physical activity and control groups, males in the physical activity and control groups, males and females in the in the physical activity group, as well as males and females in the control group were also analyzed with t-tests. Comparisons of difference in CRP for lean participants in the physical activity and control groups, non-lean participants in the physical activity and control groups, lean and non-lean participants in the physical activity group, as well as lean and non-lean participants in the control group were also analyzed with t-tests. Similar t-tests were used to compare African Americans and Caucasians by lean and non-lean status.

Pearson correlations were carried out to evaluate the relationship between baseline CRP and CRP change with other variables. Data are presented as mean ± standard deviation. The significance level was set as $p<0.05$.

Chapter IV: Results

Baseline Measures

Table 1 shows the baseline characteristics of the physical activity and control group. There were no statistical differences in age, race, BMI percentile, percent body fat, waist to hip ratio, CRP, exercise time to exhaustion, or relative and peak aerobic oxygen consumption found between physical activity and control groups at baseline. There was also no difference in baseline CRP concentration between African American and Caucasian participants.

Correlations. Figures 1-4 display significant correlations with baseline CRP. Baseline plasma CRP values were significantly correlated with baseline age ($r = 0.356$; $p = 0.006$) (not shown), BMI percentile ($r = 0.397$; $p = 0.002$) (Figure 1), and percent body fat ($r = 0.603$; $p = 0.000$) (Figure 2), yet not with sex, race, or baseline waist-to-hip ratio. Baseline CRP values were also correlated to absolute ($r = 0.314$; $p = 0.016$) and relative ($r = -0.455$; $p = 0.000$) measures of baseline peak oxygen consumption (Figure 3 and Figure 4, respectively).

Follow-up Measures

Table 2 shows characteristics by group at follow-up, while Table 3 shows the change in these characteristics by group during the 16 week intervention.

Aerobic Fitness. Absolute peak oxygen consumption was found to be higher after, compared to before, the 16-week physical activity program in both the exercise and control groups (Table 2). No significant differences were seen for pre and post peak oxygen consumption in either group when expressed relative to body weight. No significant differences were seen for changes in absolute or relative peak oxygen consumption between the exercise and

control group. Exercise time to exhaustion increased in the exercise group ($p = 0.001$), yet not the control group (Table 2). A difference in the magnitude of change in exercise time to exhaustion was seen between the physical activity and control group ($p = 0.012$), such that the physical activity group increased time to exhaustion to a greater extent than did the control group (Table 3).

Body Composition. BMI percentile significantly increased ($p = 0.001$) during the 16 weeks in the control group (Table 2), while no significant difference between pre and post percent body fat or waist to hip ratio was found for participants in this group. No significant differences in BMI percentile, percent body fat, or waist to hip ratio were detected in the exercise group pre versus post training. Changes in percent body fat ($p = 0.023$) and waist to hip ratio ($p = 0.037$) during the intervention were different between the exercise and control group (Table 3). Differences in percent body fat change were seen between the exercise and control group, such that percent body fat tended to decrease from pre to post intervention in the exercise group and decrease pre to post intervention in the control group (Table 3). Waist to hip ratio decreased more in the control group than in the exercise group (Table 3). There was no significant difference for the change in BMI percentile during the intervention between the exercise and control group.

C - reactive protein. No significant changes in average pre and post CRP concentrations were seen for the exercise or control group. There were no significant differences found for change in CRP concentration between exercise and control groups.

No difference was seen in CRP change between males in the physical activity (-0.47 ± 1.74 mg/L) and control (-0.04 ± 0.92 mg/L) groups, ($p = 0.48$) or between females in the

physical activity (-0.35 ± 2.40 mg/L) and control (-0.03 ± 0.95 mg/L) groups (p = 0.68). Neither were there differences for CRP change between males (-0.47 ± 1.74 mg/L) and females (-0.35 ± 2.40 mg/L) in the physical activity group (p = 0.864) or between males (-0.04 ± 0.92 mg/L) and females (-0.03 ± 0.95 mg/L) in the control group (p = 0.993).

No differences in CRP change were seen between African American children of the physical activity (-0.72 ± 2.16759 mg/L) and control (-0.41 ± 1.07 mg/L) groups (p = 0.69), or between Caucasians in the physical activity (-0.12 ± 2.07 mg/L) or control (+0.2474 ± 0.68 mg/L) groups (p = 0.56). Similarly, no differences in CRP change were seen between African Americans (-0.72 + 2.16759 mg/L) and Caucasians (-0.12 + 2.07 mg/L) in the physical activity group (p = 0.38), or between African Americans (-0.41 + 1.07 mg/L) and Caucasians (+0.2474 + 0.68 mg/L) in the control group (p = 0.10).

No differences for change in CRP were seen between lean children in the physical activity (-0.24 ± 0.70 mg/L) and control (+0.02 ± 0.12 mg/L) groups (p = 0.40), or between non-lean children of the physical activity (-0.46 ± 2.47 mg/L) and control (-0.06 ± 1.17 mg/L) groups (p = 0.58). Also, no differences for change in CRP were seen between lean (-0.24 ± 0.70 mg/L) and non-lean (-0.46 ± 2.47 mg/L) children in the physical activity group, or between lean (+0.02 ± 0.12 mg/L) and non-lean (-0.06 ± 1.17 mg/L) children in the control group.

Furthermore, no differences in CRP change were seen between lean African Americans in the physical activity (+0.28 ± 0.48 mg/L) and control (-0.04 + 0.06 mg/L) groups (p = 0.44), or between lean Caucasians in the physical activity (-0.43 + 0.69 mg/L) and control (+0.04 + 0.14 mg/L) groups (p = 0.21). Similarly, no differences in CRP change were seen between lean African Americans (+0.28 + 0.48 mg/L) and lean Caucasians (-0.43 + 0.69 mg/L) in the physical

activity group (p = 0.14), or between lean African Americans (-0.04 + 0.06 mg/L) and lean Caucasians (+0.04 + 0.14 mg/L) in the control group (p = 0.47).

Also, no significant differences in CRP change were seen between non-lean African Americans in the physical activity (-0.93 + 2.33 mg/L) and control (-0.60 + 1.31 mg/L) groups (p = 0.75), or between non-lean Caucasians in the physical activity (+0.07 + 2.60 mg/L) and control (+ 0.39 + 0.89 mg/L) groups (p = 0.76). Similarly, no difference in CRP change were seen between non-lean African Americans (-0.93 + 2.33 mg/L) and non-lean Caucasians (+0.07 + 2.60 mg/L) in the physical activity group (p = 0.29), or between non-lean African Americans (-0.60 + 1.31 mg/L) and non-lean Caucasians (+ 0.39 + 0.89 mg/L) of the control group (p = 0.13).

Correlations. Figures 5 and 6 show significant correlations between CRP change during the 16 week intervention and baseline variables. The overall average change in CRP during 16 weeks was negatively associated with absolute baseline peak oxygen consumption (r = -0.263; p = 0.046) and baseline CRP (r = -0.599; p = 0.001). Overall CRP change over the 16 week intervention period was not significantly associated with any other aforementioned baseline parameter. Figures 7 and 8 show significant correlations between CRP change during the 16 week intervention and changes in aerobic fitness during the 16 week intervention. The change in CRP during the 16 week intervention was positively associated with the change in absolute peak oxygen consumption (r = 0.281; p = 0.046) during the same time frame, yet not with the change in any other aforementioned parameter. A significant correlation was found between the change in CRP concentration and change in absolute peak aerobic oxygen consumption for Caucasians in the exercise group (r = 0.527) (p = 0.036), yet not for African Americans in the exercise group (r = 0.22; p = 0.41). No significant correlations were found between change in CRP concentration and change in absolute fitness for African Americans (r = -0.33; p = 0.43) or

Caucasians (r = 0.04; p = 0.91) in the control group. No significant correlations were found between change in CRP concentration and change in relative fitness for African Americans (r = 0.08; p=0.76) or Caucasians (r = 0.15; p = 0.58) in the physical activity group, nor for African Americans (r = -0.18; p = 0.67) or Caucasians (r = -0.21; p = 0.60) in the control group. Likewise, no significant correlations were found between change in CRP concentration and change in BMI %tile for African Americans (r = -0.14; p = 0.59) or Caucasians (r = 0.15; p = 0.58) in the physical activity group, nor for African Americans (r = -0.18; p = 0.67) or Caucasians (r = -0.13; p = 0.73) in the control group. No significant correlations were seen between change in CRP concentration and change in WHR for African Americans (r = 0.43; p = 0.12) or Caucasians (r = 0.27; p = 0.42) in the physical activity group, nor for African Americans (r = -0.01; p = 0.99) or Caucasians (r = 0.26; p=0.50) in the control group. Finally, no significant correlations were found between change in CRP concentration and change in percent body fat for African Americans (r = 0.03; p = 0.92) or Caucasians (r = -0.30; p = 0.25) in the physical activity group, nor for African Americans (r = 0.48; p = 0.28) or Caucasians (r = -0.12; p = 0.73) in the control group.

	Physical Activity Group mean ± σ (n)	Control Group mean ± σ (n)
Age (yrs.)	9.54 ± 1.05 (n=39)	9.85 ± 1.09 (n=20)
Tanner Stage	1.15 ± 0.36 (n = 34)	1.29 ± 0.46 (n = 18)
African American (%)	46.2	42.9
Female (%)	56.4	52.4
BMI (kg/m^2)	23.39 ± 5.68 (n=39)	22.16 ± 5.03 (n=19)
BMI (%tile for age and sex)	83.38 ± 24.22 (n=39)	79.37 ± 29.27 (n=19)
WHR	0.83 ± 0.05 (n=29)	0.86 ± 0.05 (n=15)
Body fat (%)	37.17 ± 10.70 (n=37)	31.93 ± 10.20 (n=19)
Absolute peak VO_2 (L/min)	1.67 ± 0.36 (n=39)	1.71 ± 0.32 (n=19)
Relative peak VO_2 (ml/kg/min)	33.76 ± 7.71 (n=39)	37.74 ± 7.63 (n=19)
Time To Exhaustion (sec)	359 ± 89 (n = 39)	373 ± 114 (n = 20)
CRP (mg/L)	1.72 ± 1.93 (n=39)	0.95 ± 1.24 (n=21)

Table 1: Baseline Characteristics (*p<0.05 between groups difference).
BMI = Body Mass Index; WHR = Waist-to-Hip Ratio; CRP = C - reactive protein.

	Physical Activity Group mean ± σ (n)	Control Group mean ± σ (n)
BMI (kg/m^2)	24.28 ± 5.86 (n = 36)	22.65 ± 4.98* (n = 19)
BMI (%tile for age and sex)	84.81 ± 24.07 (n = 36)	81.29 ± 27.15* (n = 17)
WHR	0.76 ± 0.26 (n = 31)	0.83 ± 0.05 (n = 15)
Body Fat (%)	35.65 ± 11.62 (n = 34)	32.47 ± 10.52 (n = 19)
Absolute Peak VO_2 (L/min)	1.81 ± 0.38* (n = 33)	1.85 ± 0.40* (n = 18)
Relative Peak VO_2 (ml/kg/min)	33.68 ± 9.67 (n = 34)	34.73 ± 15.15 (n = 19)
Time To Exhaustion (sec)	441 ± 96 (n = 34)	410 ± 128 (n = 18)
CRP (mg/L)	1.32 ± 1.84 (n = 39)	0.91 ± 0.92 (n = 21)

Table 2: Post-Intervention Characteristics (*p<0.05 difference from pre).
Note: BMI = Body Mass Index; WHR = Waist-to-Hip Ratio; CRP = C - reactive protein.

	Physical Activity Group mean ± σ (n)	Control Group mean ± σ (n)
BMI (kg/m^2)	0.32 ± 1.15 (n = 36)	0.59 ± 1.00 (n = 18)
BMI (%tile for age and sex)	-0.97 ± 11.76 (n = 36)	3.65 ± 6.28 (n = 17)
WHR	0.00 ± 0.05 (n = 26)	-0.03 ± 0.04* (n = 14)
Body fat (%)	-0.75 ± 2.46* (n = 32)	0.83 ± 1.95 (n = 18)
Absolute peak VO_2 (L/min)	0.12 ± 0.19 (n = 33)	0.13 ± 0.13 (n = 18)
Relative peak VO_2 (ml/kg/min)	1.08 ± 4.13 (n = 33)	1.08 ± 3.17 (n = 17)
Time To Exhaustion (sec)	72 ± 66* (n = 34)	21 ± 71 (n =18)
CRP (mg/L)	-0.40 ± 2.11 (n = 39)	-0.04 ± 0.91 (n = 21)

Table 3: Change (post-pre) in Characteristics during 16 week intervention (*p<0.05 between groups).
Note: BMI = Body Mass Index; WHR = Waist-to-Hip Ratio; CRP = C - reactive protein.

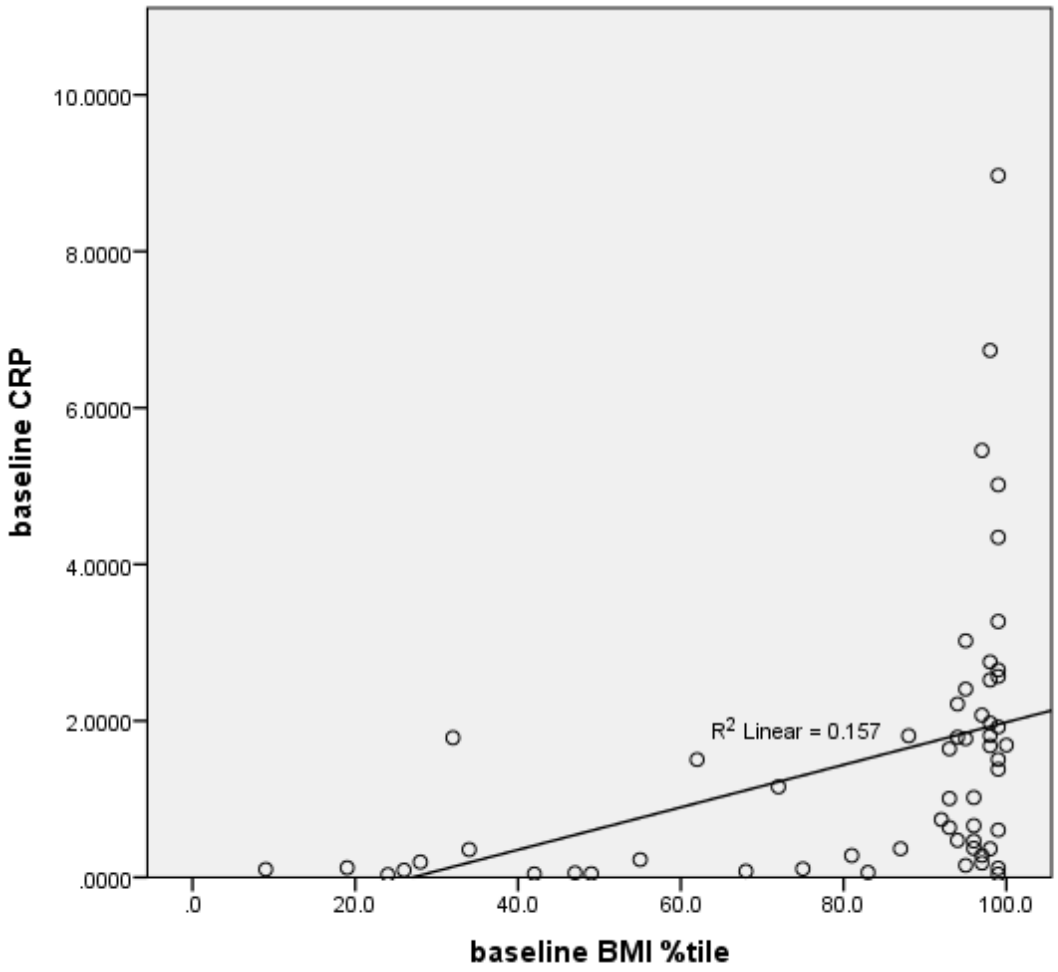


Figure 1: Correlation between C - reactive protein at baseline and Body Mass Index percentile at baseline.
Note: CRP measured in mg/L; BMI measured as kg/m^2.

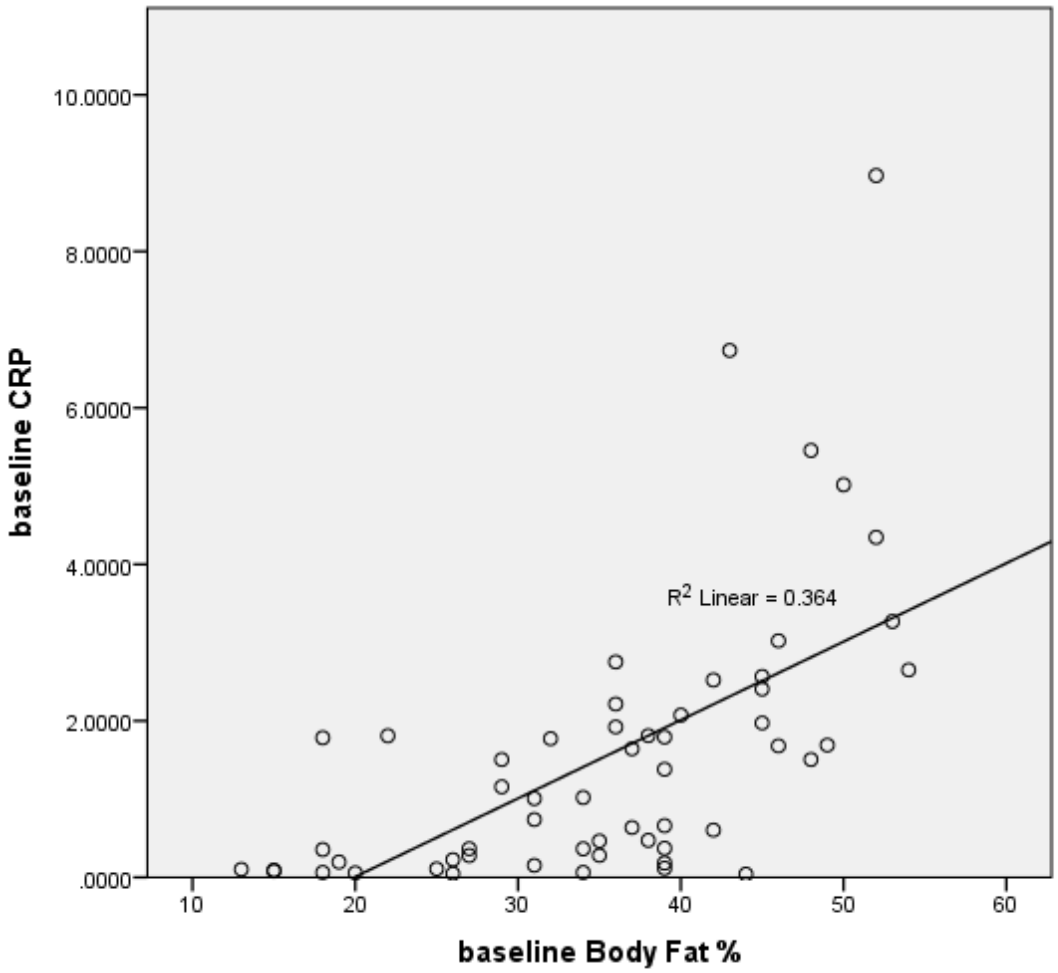


Figure 2: Correlation between C –reactive protein at baseline and body fat percentage at baseline.
Note: CRP measured in mg/L.

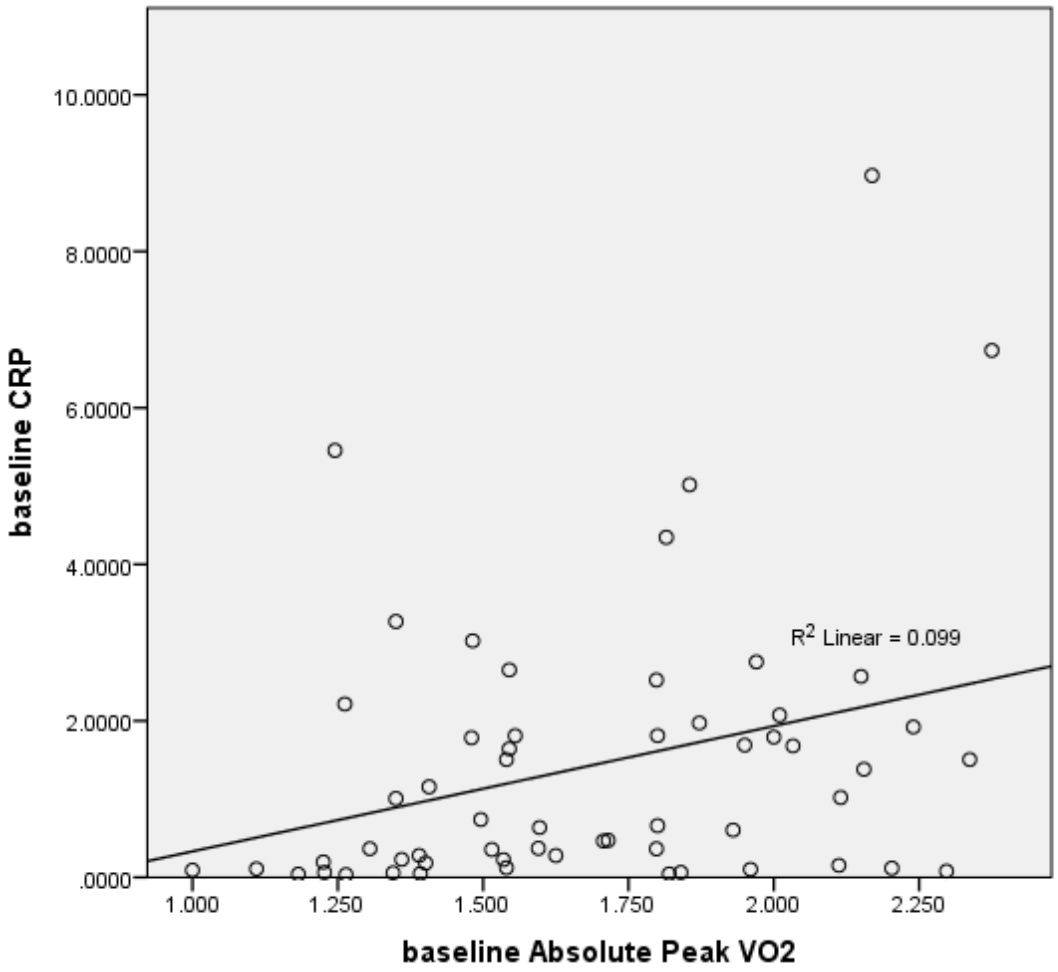


Figure 3: Correlation between C –reactive protein at baseline and absolute fitness at baseline. Note: CRP measured in mg/L; Absolute fitness measured in L/min.

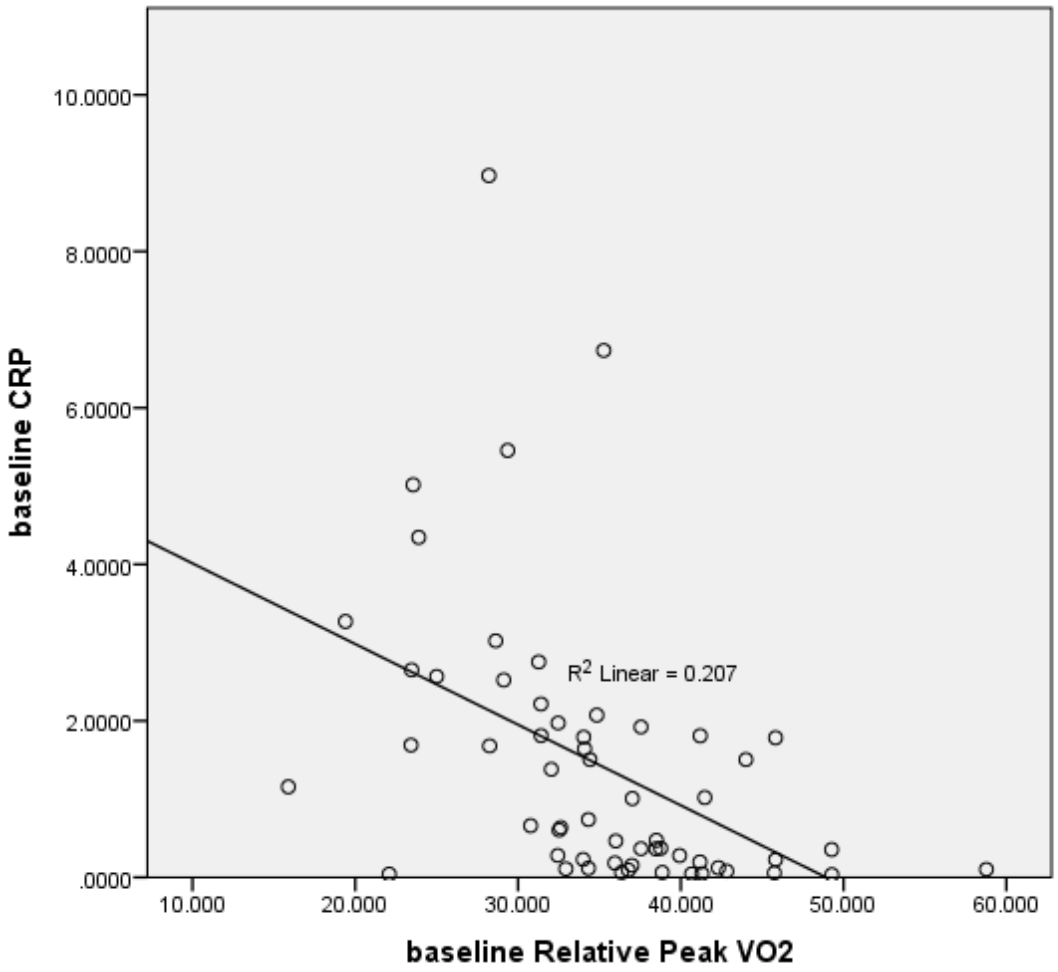


Figure 4: Correlation between C –reactive protein at baseline and relative fitness at baseline. Note: CRP measured in mg/L; Relative fitness measured in mL/kg/min.

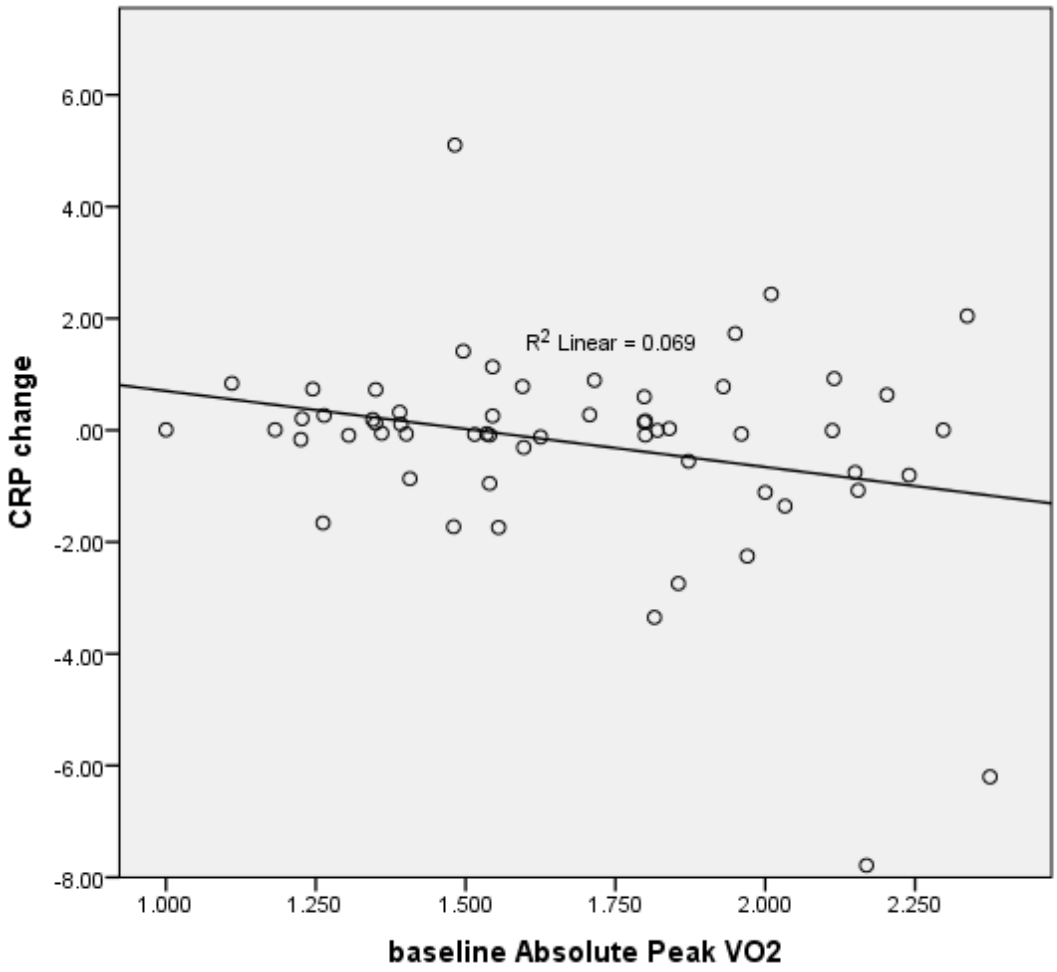


Figure 5: Correlation between change in C –reactive protein during the 16 week intervention and absolute fitness at baseline.
Note: CRP measured in mg/L; Absolut fitness measured in L/min.

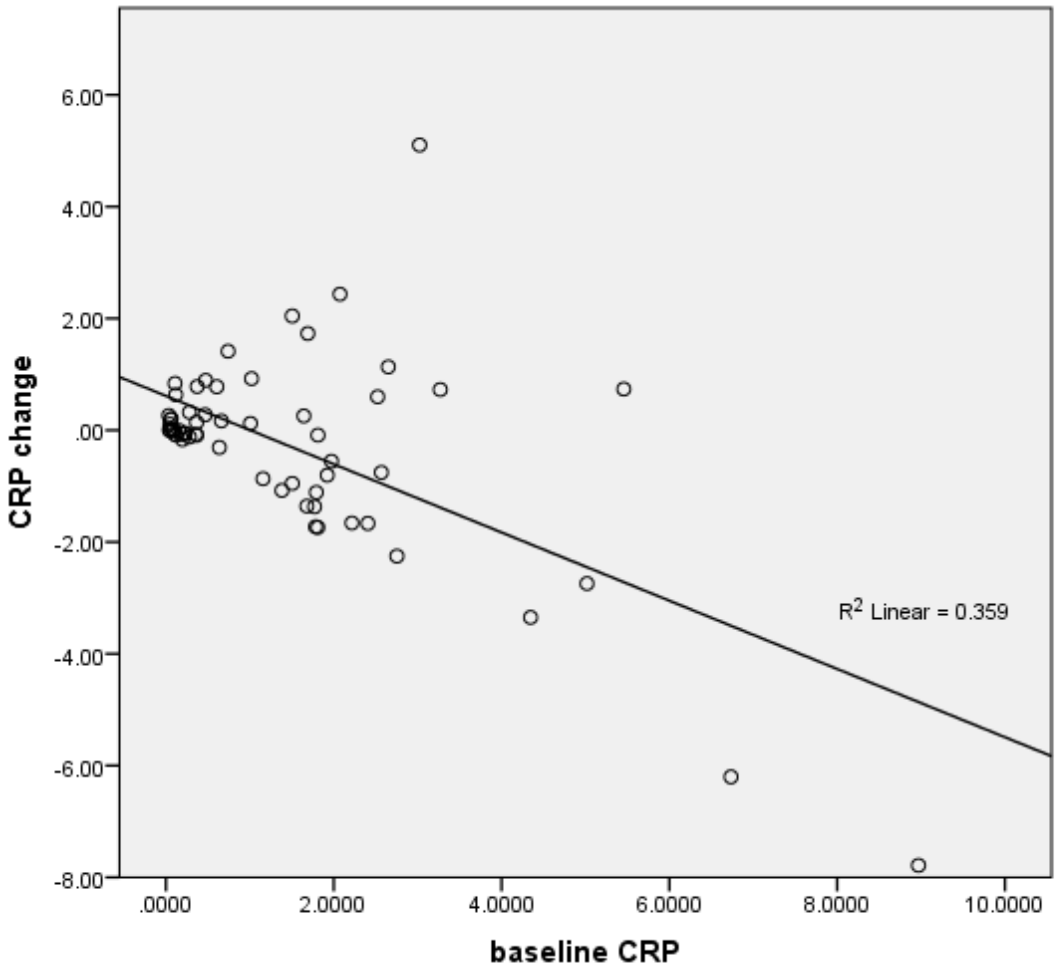


Figure 6: Correlation between change in C –reactive protein during the 16 week intervention and C –reactive protein at baseline.
Note: CRP measured in mg/L.

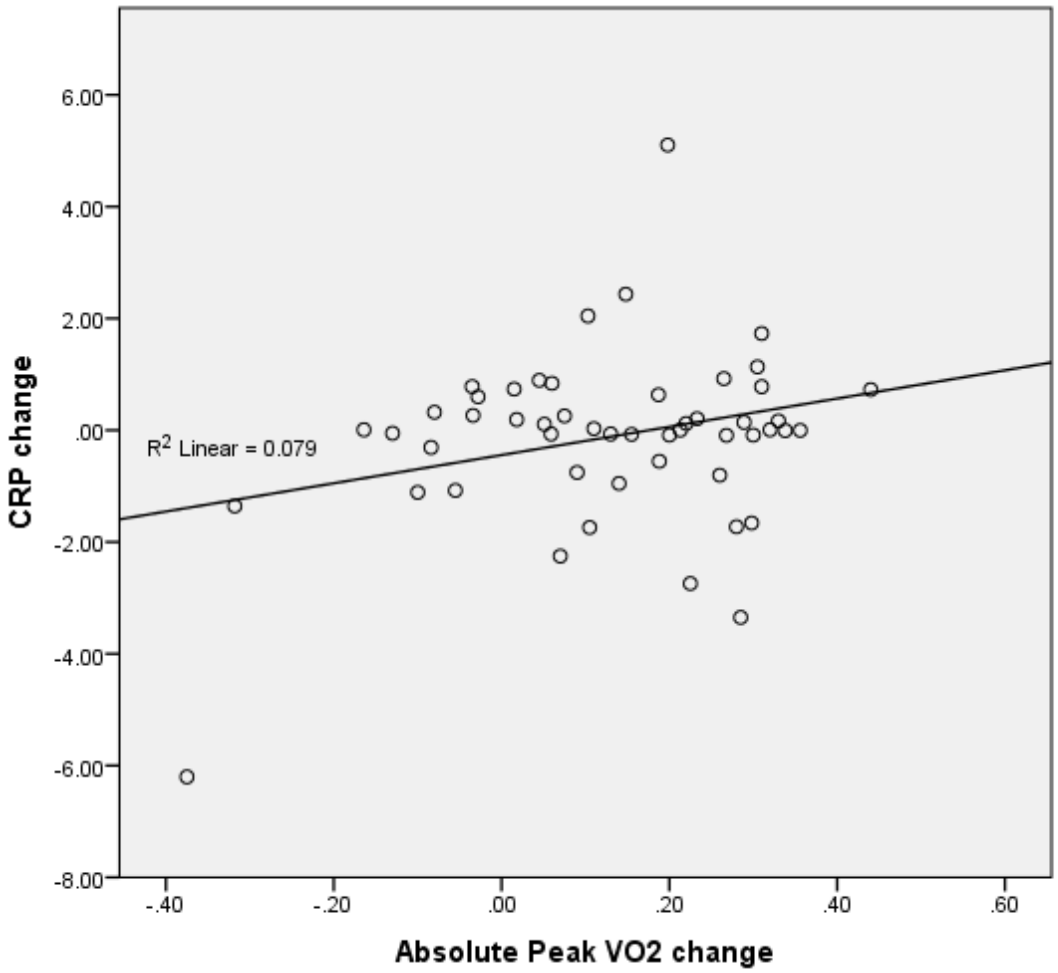


Figure 7: Correlation between change in C –reactive protein during the 16 week intervention and change in absolute fitness during the 16 week intervention.
Note: CRP measured in mg/L; Absolute fitness measured in L/min.

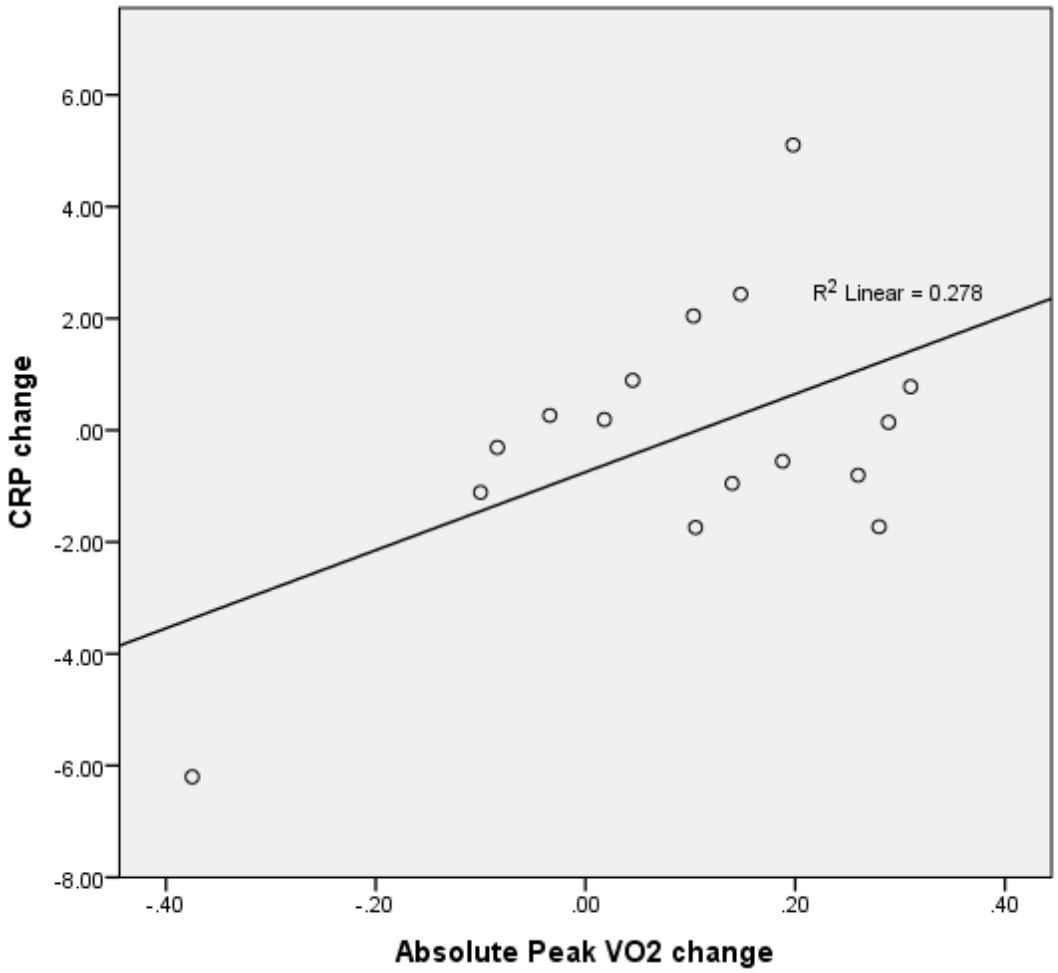


Figure 8: Correlation between change in C –reactive protein during the 16 week intervention and change in absolute fitness during the 16 week intervention for Caucasians in the exercise group. Note: CRP measured in mg/L; Absolute fitness measured in L/min.

Chapter V: Discussion

The purpose of this study was to determine the effects of a 16-week aerobic activity intervention on serum CRP concentrations in 8-11 year old African American and Caucasian children, and to evaluate the extent to which body composition influenced this outcome. Serum CRP concentrations were not significantly changed during the 16 week intervention for participants in the exercise or control group. These findings contradict results from other studies that reported significant decreases in CRP concentrations for participants in exercise, yet not control groups during diet/exercise interventions of various durations in children (Ounis, et al., 2010) (Roberts, Chen, & Barnard, 2007; Garanty-Bogacka, et al., 2011; Nemet, Oren, Pantanowitz, & Eliakim, 2013). Findings from the current study support others that reported no changes in CRP concentration during exercise interventions of similar duration (Nassis, et al., 2005; Kelly, Steinberger, Olson, & Dengel, 2007). It should be mentioned that absolute aerobic fitness improved to similar extents in children of the exercise and control groups during the 16 week intervention. Though these findings weaken the argument that the activity intervention influenced aerobic fitness levels of children, it should also be noted that treadmill time to exhaustion was increased only in the exercise group, suggesting the intervention lead to some improvement in the ability to perform treadmill exercise. The emphasis of this discussion will be to expound upon the relevance of these findings.

It could be that reductions in CRP with exercise training reported from other studies are due to longer, 6 month and 3 month, interventions (Garanty-Bogacka, et al., 2011; Nemet, Oren, Pantanowitz, & Eliakim, 2013); however, this is unlikely because other physical activity interventions lasting 8 weeks and 2 weeks have been associated with reductions in CRP concentration in children (Ounis, et al., 2010; Roberts, Chen, & Barnard, 2007).

It is also possible that the nature of intervention used in the current study was not suitable to reduce inflammation in children of this age group. Balducucci et al showed that the response of CRP concentrations to exercise training may be contingent on exercise modality in older adults, with those participating in mixed training experiencing greater reductions in CRP than those with aerobic training only (Balducci, et al., 2010). The exercise interventions in the aforementioned childhood studies that reported reductions in CRP also included diet components designed to encourage improvements in body composition, further supporting the argument that intervention type may influence changes in CRP concentration (Ounis, et al., 2010; Roberts, Chen, & Barnard, 2007; Garanty-Bogacka, et al., 2011; Nemet, Oren, Pantanowitz, & Eliakim, 2013). Characteristics of these diet components included caloric reduction (Nemet, Oren, Pantanowitz, & Eliakim, 2013; Ounis, et al., 2010), less sugar and fat intake (Garanty-Bogacka, et al., 2011), as well as macronutrient ratios of approximately 12-15% protein, 55-70% carbohydrate, and 12-30% fat (Ounis, et al., 2010; Roberts, Chen, & Barnard, 2007). Two of these studies provided educational sessions as well as meals consisting of high fiber whole grains (5 servings/day), vegetables (4 servings/day), fruit (3 servings/day), plant protein, non-fat diary (< 2 servings/day), fish/fowl (3-4 days/week), soups and casseroles (2 days/week), no caffeine, and limited sodium intake (<1600 mg/day) (Ounis, et al., 2010; Roberts, Chen, & Barnard, 2007). Furthermore, studies showing no change in CRP did not include diet components designed to improve body composition, though Nassis et al. employed a high carbohydrate diet to encourage the maintenance of body weight during the intervention (Nassis, et al., 2005; Kelly, Steinberger, Olson, & Dengel, 2007). Also of note is that two of the four childhood studies reviewed showing reductions in CRP with exercise training included activity sessions that were 1.5-2.5 hours in duration (Ounis, et al., 2010; Roberts, Chen, & Barnard, 2007), whereas the current study, and

others not showing changes in CRP, involved training programs consisting of an hour or less of activity per session (Nassis, et al., 2005; Kelly, Steinberger, Olson, & Dengel, 2007).

The finding of no significant change in CRP in the current study, despite mean decreases of 0.04 mg/L and 0.4 mg/L in the control and exercise groups, respectively, is consistent with other reports in the literature indicating that decreases in CRP of no less than 0.8 mg/L were needed to achieve statistical significance (Roberts, Chen, & Barnard, 2007; Garanty-Bogacka, et al., 2011; Ounis, et al., 2010). Other studies that reported no significant changes in CRP displayed average reductions similar to those in the current study (Nassis, et al., 2005; Kelly, Steinberger, Olson, & Dengel, 2007). Nonetheless, though statistical significance for the change in CRP concentrations from pre to post intervention was not seen, the question remains as to whether reductions in CRP of this magnitude are physiologically relevant, particularly considering some studies have cited normal values to be 0.3 mg/L for children of similar age and sex (Ford, et al., 2001).

Findings that baseline CRP concentration was positively correlated with age, baseline BMI percentile, and baseline percent body fat were comparable to findings of previous studies (Ford, et al., 2001; Garanty-Bogacka, et al., 2011). Thus, the present study adds additional support for the hypothesis that serum CRP concentration increases with age and percent body fat in children. Also in agreement with other studies, was the finding that baseline CRP concentrations were negatively associated with relative fitness. Sadenpour et al and Isasi et al have described an inverse relationship between aerobic fitness levels and CRP concentrations in children (Sadeghipour, Rahnama, Salesi, Rahnama, & Mojtahedi, 2010; Isasi, et al., 2003). However, contrary to these findings, baseline CRP concentrations in the current study were positively associated with baseline aerobic fitness expressed in absolute terms, indicating that

children with higher fitness levels may also display increased CRP concentrations compared to those who are less aerobically fit when body weight is not accounted for. Though baseline CRP was positively associated with baseline absolute aerobic capacity, the change in CRP with 16-weeks of exercise was negatively correlated with baseline absolute aerobic capacity. This negative correlation between CRP change and baseline absolute fitness is baffling considering CRP did not change in the exercise or control group over 16 weeks, though absolute aerobic fitness levels improved in each. This may suggest a plateau effect in which CRP values do not improve beyond a certain level of absolute fitness, though it is unlikely the untrained children in this study would have been at this level of fitness at baseline.

Baseline CRP measurements revealed no significant differences in CRP concentration between African American and Caucasian participants, contradicting other literature that suggests higher CRP concentrations may be expected in African Americans as compared to Caucasians (Wong, Pio, Valencia, & Thakal, 2001; Heffernan, et al., 2009). Finding no difference for the change in serum CRP with a 16 weeks intervention among African American and Caucasian participants further supports the idea that the two races may not differ with respect to inflammatory characteristics. Further analysis revealed a positive correlation between CRP change and baseline aerobic capacity to be significant only for Caucasians in the exercise group. This is unexpected and could be investigated with further study.

Changes in CRP were not associated with baseline measures of body composition or the changes in body composition during the intervention. This is in contrast to findings in other studies showing positive correlations between changes in CRP concentration and changes in body composition, or changes in body composition and follow-up CRP concentration (Garanty-Bogacka, et al., 2011; Ounis, et al., 2010). Though it is unexpected that BMI percentile and CRP

would be significantly correlated at baseline, yet not as they change, it should also be noted that average BMI percentile values in the current study did not change in the exercise group from baseline to follow up. This finding is contrary to literature where reductions in BMI are reported with exercise training in children (Ounis, et al., 2010; Garanty-Bogacka, et al., 2011). Also worth mentioning is that, of the studies reviewed where CRP concentration decreased in children with physical activity interventions, either body fat percentage (Ounis, et al., 2010; Garanty-Bogacka, et al., 2011) or BMI percentile (Roberts, Chen, & Barnard, 2007; Nemet, Oren, Pantanowitz, & Eliakim, 2013) decreased as well. Body fat percentage was unmeasured in the 2 studies not indicating changes in this variable. In the current study, BMI percentile increased only in the control group and remained unchanged in the physical activity group, while body fat percentage did not change for either group. Furthermore, in agreement with findings from the current study, other studies reviewed, where no difference in CRP concentration was found with physical activity interventions in children, did not report decreases in body fat percentage either (Nassis, et al., 2005; Kelly, Steinberger, Olson, & Dengel, 2007), . Taken together, this suggests that in the absence of decreases in body composition, specifically decreases in BMI percentile or percent body fat, a change in CRP concentration may not be expected.

The present study provides evidence to support the hypothesis that there is a positive relationship between body composition and C-reactive protein concentrations in children; however, this study contradicts other studies reporting reductions in body composition or CRP concentration with exercise intervention. Though exercise modalities and exclusion of a dietary intervention component may have influenced the outcome of the physical activity intervention with regard to changes in CRP in the current study, it is more likely that changes in CRP with physical activity interventions in children may be a result of changes in body fat percentage or

BMI percentile experienced during the training. Further studies with larger sample sizes, differing exercise modalities, with and without diet components, may be warranted to further investigate the relationship between exercise training and CRP concentrations in children.

References

Alvarez, J. A., Higgins, P. B., Oster, R. A., Darnell, B. E., & Gower, B. A. (2009). Fasting and postprandial markers of inflammation in lean and overweight children. *The American Journal of Clinical Nutrition*, 1138-1134.

Balducci, S., Zanuso, S., Nicolucci, A., Fernando, F., Cavallo, S., Cardelli, P., . . . Pugliese, G. (2010). Anti-inflammatory effect of exercise training in subjects with type 2 diabetes and the metabolic syndrome is dependent on exercise modalities and independent of weight loss. *Nutrition, Metabolism, and Cardiovascular Disease*, 608-617.

Berenson, G. S. (2002). Childhood Risk Factors Predict Adult Risk Associated with Subclinical Cardiovascular Disease: The Bogalusa Heart Study. *The American Journal of Cardiology*, 3L-7L.

Chen, A. K., Roberts, C. K., & Barnard, R. J. (2006). Effect of a short-term diet and exercise intervention on metabolic syndrome in overweight children. *Metabolism Clinical and Experimental*, 871-878.

Cole , T., Bellizzi , M., Flegal , K., & Dietz , W. (2000). Establishing a standard definition for child overweight and obesity worldwide: international survey. *British Medical Journal*, 1240-1243.

Cole, T., Freeman, J., & Preece , M. (1998). British 1990 growth reference centiles forweight, height, body mass index and head circumference fitted by maximum penalized likelihood. *Statistics in Medicine.*, 407-429.

Cook, D. G., Mendall, M. A., Whincup, P. H., Carey, I. M., Ballam, L., Morris, J. E., . . . David , S. P. (2000). C-reactive protein concentration in children: relationship to adiposity and other cardiovascular risk factors. *Atherosclerosis*, 139-150.

Ford, E. S. (1999). Body Mass Index, Diabetes, and C-Reactive Protein Among U.S. Adults. *Diabetes Care*, 1971-1977.

Ford, E. S., Galuska, D. A., Gillespie, C., Will, J. C., Giles, W. H., & Dietz, W. H. (2001). C-reactive protein and body mass index in children: Findings from the Third National Health and Nutrition Examination Survey, 1988-1994. *The Journal of Pediatrics*, 486-492.

Garanty-Bogacka, B., Syrenicz, M., Goral, J., Krupa, B., Syrenicz, J., Walczak, M., & Syrenicz, A. (2011). Changes in inflammatory biomarkers after successful lifestyle intervention in obese children. *Polish Journal of Endocrinology*, 499-505.

Heffernan, K. S., Jae, S. Y., Vieira, V. J., Iwamoto, G. A., Wilund, K. R., Woods, J. A., & Fernhall, B. (2009). C-reactive protein and cardiac vagal activity following resistance exercise training in young African-American and white men. *American Journal of Physiology - Regulatory, Integrative and Comparative Physiology*, 1098–1105.

Henauw, S. D., Verbestel, V., Marild, S., Barba, G., Bammann, K., Eiben, G., . . . Pigeot, I. (2011). The IDEFICS community-oriented intervention programme: a new model for childhood obesity programme: a new model for childhood obesity. *International Journal of Obesity*, S16–S23.

Isasi, C., Deckelbaum, R., Tracy, R., Starc, T., Lars, B., & Lars, S. (2003). Physical Fitness and C-Reactive Protein Level in Children and Young Adults: The Columbia University BioMarkers Study. *Pediatrics*, 332-338.

Kelly, A. S., Steinberger, J., Olson, T. P., & Dengel, D. R. (2007). In the absence of weight loss, exercise training does not improve adipokines or oxidative stress in overweight children. *Metabolism Clinical Experimental*, 1005–1009.

Laaksonen, D. E., Niskanen, L., Nyyssönen, K., Punnonen, K., Tuomainen, T. -P., Valkonen, V. -P., . . . Salonen, J. T. (2004). C-reactive protein and the development of the metabolic syndrome and diabetes in middle-aged men. *Diabetologia*, 1403-1410.

Li, A. M., Chan, M. H., Yin, J., So, H. K., Ng, S. K., Chan, I. H., . . . Ng, P. C. (2008). C-Reactive Protein in Children With Obstructive Sleep Apnea and the Effects of Treatment. *Pediatric Pulmonology*, 34–40.

Martins, R. A., Neves, A. P., Coelho-Silva, M. J., Veríssimo, M. T., & Teixeira, A. M. (2010). The eVect of aerobic versus strength-based training on high-sensitivity C-reactive protein in older adults. *European Journal of Applied Physiology*, 161-169.

Miller, M., Zhan, M., & Havas, S. (2005). High Attributable Risk of Elevated C-Reactive Protein Level to Conventional Coronary Heart Disease Risk Factors. *Archives of Interna Medicine*, 2063-2068.

Morrison, J. A., Friedman, L. A., Wang, P., & Glueck, C. J. (2008). Metabolic Syndrome in Childhood Predicts Adult Metabolic Syndrome and Type 2 Diabetes Mellitus 25 to 30 Years Later. *The Journal of Pediatrics*, 201-206.

Nappo, A., Iacoviello, L., Fraterman, A., Gonzalez-Gil, E. M., Hadjigeorgiou, C., Marild, S., . . . Russo, P. (2013). High-sensitivity C-reactive Protein is a Predictive Factor of Adiposity in Children: Results of the Identification and prevention of Dietary and lifestyle-induced health Effects in Children and InfantS (IDEFICS) Study. *Journal of the American Heart Association*.

Nassis, G. P., Papantakou, K., Skenderi, K., Triandafillopoulou, M., Kavouras, S. A., Yannakoulia, M., . . . Sidossis, L. S. (2005). Aerobic exercise training improves insulin sensitivity without changes in body weight, body fat, adiponectin, and inflammatory markers in overweight and obese girls. *Metabolism Clinical and Experimental*, 1472-1479.

Nemet, D., Oren, S., Pantanowitz, M., & Eliakim, A. (2013). Effects of a Multidisciplinary Childhood Obesity Treatment Intervention on Adipocytokines, Inflammatory and Growth Mediators. *Hormone Research in Paediatrics*, 325–332.

Ounis, O. B., Elloumi, M., Zouhal, H., Makni, E., Denguezli, M., Amri, M., . . . Tabka, Z. (2010). Effect of Individualized Exercise Training Combined with Diet Restriction on Inflammatory Markers and IGF-1/IGFBP-3 in Obese Children. *Annals of Nutrition and Metabolism*, 260-266.

Rikder, P. M., Cushman, M., Stampfer, M. J., Tracy, R. P., & Hennekens, C. H. (1997). Inflammation, Aspirin, and the Risk of Cardiovascular Disease in Apparently Healthy Men. *The New England Journal of Medicine*, 973-979.

Roberts, C. K., Chen, A. K., & Barnard, R. J. (2007). Effect of a short-term diet and exercise intervention in youth on atherosclerotic risk factors. *Atherosclerosis*, 98-106.

Sadeghipour, H. R., Rahnama, A., Salesi, M., Rahnama, N., & Mojtahedi, H. (2010). Relationship between C-reactive protein and physical fitness, physical activity, obesity, and selected cardiovascular risk factors in shcool-children. *International Journal of Preventive Medicine*, 242-246.

Santos, A.-C., Lopes, C., Guimara˜es, J. T., & Barros, H. (2005). Central obesity as a major determinant of increased high-sensitivity C-reactive protein in metabolic syndrome. *International Journal of Obesity*, 1452–1456.

Siervo, M., Ruggiero, D., Sorice, R., Nutile, T., Aversano, M., Iafusco, M., . . . Ciullo, M. (2012). Body mass index is directly associated with biomarkers of angiogenesis and inflammation in children and adolescents. *Nutrition*, 262-266.

Steiropoulos, P., Tsara, V., Nena, E., Fitili, C., Kataropoulou, M., Froudarakis, M., . . . Bouros, D. (2007). Effect of Continuous Positive Airway Pressure Treatment on Serum Cardiovascular Risk Factors in Patients With Obstructive Sleep Apnea-Hypopnea Syndrome. *CHEST*, 843–851.

Sun, S. S., Liang, R., Huang , T. T.-K., Daniels, S. R., Arslanian, S., Liu, K., . . . Siervogel, R. M. (2008). Childhood Obesity Predicts Adult Metabolic Syndrome: The Fels Longitudinal Study. *The Jounral od Pediatrics*, 191-200.

TJ, C., KM, F., D, N., & AA, J. (2007). Body mass index cut offs to define thinness in children and adolescents: international survey. *British Medical Journal*, 194.

Volankis, J. E. (2001). Human C-reactive protein: expression, structure, and function. *Molecular Immunology*, 189-197.

Wee, C. C., Mukamal, K. J., Huang, A., Davis, R. B., McCarthy, E. P., & Mittleman, M. A. (2008). Obesity and C-reactive Protein Levels Among White, Black, and Hispanic US Adults. *Obesity*, 875-880.

Wijnstok, N. J., Twisk, J. W., Young, I. S., Woodside, J. V., McFarlane, C., McEney, J., . . . Boreham, C. A. (2010). Inflammation Markers are Associated with Cardiovascular Diseases Risk in Adolescents: The Young Hearts Project 2000. *Journal of Adolescent Health*, 346–351.

Wong, N. D., Pio, J., Valencia, R., & Thakal, G. (2001). Distribution of C-Reactive Protein and Its Relation to Risk Factors and Coronary Heart Disease Risk Estimation in the National Health and Nutrition Examination Survey (NHANES) III. *Preventive Cardiology*, 109-114.

Yokoe, T., Minoguchi, K., Matsuo, H., Oda, N., Minoguchi, H., Yoshino, G., . . . Adachi, M. (2003). Elevated Levels of C-Reactive Protein and Interleukin-6 in Patients With Obstructive Sleep Apnea Syndrome Are Decreased by Nasal Continuous Positive Airway Pressure. *Circulation*, 1129-1134.

Yuan, G., Zhou, L., Tang, J., Yang, Y., Gu, W., Li, F., . . . Mingdao Chen. (2006). Serum CRP levels are equally elevated in newly diagnosed type 2 diabetes and impaired glucose tolerance and related to adiponectin levels and insulin sensitivity. *Diabetes Research and Clinical Practice*, 244-250.

Yudkin, J. S., Stehouwer, C. D., Emeis, J. J., & Coppack, S. W. (n.d.). C-Reactive Protein in Healthy Subjects: Associations With Obesity, Insulin Resistance, and Endothelial Dysfunction : A Potential Role for Cytokines Originating From Adipose Tissue?

Appendix A: Study Approval

EAST CAROLINA UNIVERSITY
University & Medical Center Institutional Review Board Office
4N-70 Brody Medical Sciences Building· Mail Stop 682
600 Moye Boulevard · Greenville, NC 27834
Office **252-744-2914** · Fax **252-744-2284** · www.ecu.edu/irb

Notification of Continuing Review Approval: Expedited

From: Biomedical IRB
To: Robert Hickner
CC:

Date: 8/30/2013
Re: CR00001294
UMCIRB 05-0384
[IMPORTED] Reduction in CVD Risk in Children Through Physical Activity

The continuing review of your expedited study was approved. Approval of the study and any consent form(s) is for the period of 8/29/2013 to 8/28/2014. This research study is eligible for review under expedited category #8. The Chairperson (or designee) deemed this study no more than minimal risk.

Changes to this approved research may not be initiated without UMCIRB review except when necessary to eliminate an apparent immediate hazard to the participant. All unanticipated problems involving risks to participants and others must be promptly reported to the UMCIRB. The investigator must submit a continuing review/closure application to the UMCIRB prior to the date of study expiration. The Investigator must adhere to all reporting requirements for this study.

Approved consent documents with the IRB approval date stamped on the document should be used to consent participants (consent documents with the IRB approval date stamp are found under the Documents tab in the study workspace).

The approval includes the following items:

Document	Description
3-day food records.doc(0.01)	Surveys and Questionnaires
Assent(0.01)	Consent Forms
flyer black and white with tabs (2).doc(0.01)	Recruitment Documents/Scripts
HPCS.doc(0.01)	Surveys and Questionnaires
Infcon 061410.doc(0.01)	Consent Forms
Informed consent-parent(0.01)	Consent Forms
Medical Form.doc(0.01)	Surveys and Questionnaires
MSPCS.doc(0.01)	Surveys and Questionnaires
PAlog Form.doc(0.01)	Surveys and Questionnaires
PedsQL4-0Ch.doc(0.01)	Surveys and Questionnaires

Appendix B: Informed Consent Form

Reduction in CVD risk in children through physical activity

INFORMED CONSENT

Principal Investigator: Robert C. Hickner, Ph.D.
Institution: Human Performance Laboratory
Address: 371 Ward Sports Medicine Building
Telephone Number: (252) 328-4677

TITLE OF PROJECT: Reduction in CVD risk in children through physical activity

INTRODUCTION
Your child has been asked to participate in a research study being conducted by Robert C. Hickner and colleagues. This research is designed to determine the effect of physical activity on cardiovascular disease risk in children.

We will study lean and overweight preadolescent children. Studies will take place in the Human Performance Laboratory of East Carolina University and in Minges Coliseum.

PLAN AND PROCEDURES
Prior to testing, you, as a guardian(s) will read and sign this Informed Consent for research, as well as fill out a medical history questionnaire pertaining to your child.

Your child's participation will involve:

- You will fill out a personal history form that pertains to your child. Your child will fill out forms consisting of a youth risk behavior survey, leisure time exercise questionnaire, a personal history form, a medical form, a 30-day physical activity recall, pediatric quality of life inventory, and a physical self-perception profile and children's attraction toward physical activity scale

- Determination of **body composition** using body mass index (BMI), waist-to-hip ratio (WHR), skinfolds, and a DEXA Scan will be conducted at the Human Performance Laboratory. To calculate BMI, height and weight will be measured. Circumference measures will be taken at the waist and hip to calculate WHR. Finally, skinfold thickness of the tricep, subscapular (shoulder blade), abdomen, thigh, suprailium (hip bone), and calf will be taken on the right side of the body, in duplicate, with a skinfold caliper. Your child will undergo a test of body composition called a DEXA scan. It is like an x-ray of your entire body. During this test your child will be asked to wear minimal clothing (e.g., swimsuit, or shorts and a shirt, or a gown), and to remove all jewelry. He/she will lie still on a padded table for the length of the scan (approximately 6 minutes). The table will move across and up and down to scan his/her body. Your child will not feel anything and can breathe normally during the scan. If your child has metal in his/her body, then your child will not be able to participate in the DEXA scan. Radiation exposure from a DEXA scan is approximately 0.04 mrem. The effective radiation exposure that your child would receive in this study is less than 0.6% of the radiation exposure an individual receives from natural background sources in one year.

- Determination of **Aerobic Capacity**

  Two maximal treadmill tests will be completed to evaluate initial aerobic capacity. Two tests will be performed to assure that there is adequate effort by the children during the maximal treadmill test and to determine day-to-day variability in the test. If these two tests are not very similar, a third test may need to be conducted, so it is important that your child put out a maximal effort for this test. For this test, your child will walk or run on a treadmill for approximately 10-15 minutes. During this test, your

child will wear a mouthpiece so the air they breathe out can be collected for analysis of oxygen. At first, your child will walk leisurely on the level treadmill, but the speed and level of hill climbing will become harder until your child can no longer continue.

You will be asked to complete the n-3 FFQ at baseline, 4 weeks, 8 weeks, 12 weeks, and 16 weeks. This will allow us to determine 1) what your child eats over time and 2) seasonal variations in your childs age group and population.

- Your child will complete a 3 or 4 day food record at baseline (before microdialysis), 4 weeks, 8 weeks, 12 weeks and 16 weeks.

- Your child will wear a **physical activity monitor** (RT3 Triaxial Accelerometer) and a pedometer (Yamax, Japan) five days prior to microdialysis and during the microdialysis portion of the study. Additionally, your child will wear the accelerometer and pedometer for 3 days at 4 weeks, 8 weeks, and 12 weeks.

- A **fasting blood sample** will be obtained from an arm or hand vein. This will involve one to three small needle sticks. The procedure will take place in the Human Performance Laboratory.

- Your child will be given a cotton swab to test for **salivary cortisol levels** and will be instructed to chew on it for 45-60 seconds. Samples will be collected at 7 a.m. (fasting), 1:45 (prior to a standardized 2 p.m. lunch), and 30, 45, and 60 minutes after lunch. Additional samples will be collected every hour on the hour from 9 a.m. to 3 p.m. The collection of samples will take place in the Human Performance Lab.

- At the Human Performance Lab, the insertion of up to three small probes to determine glycerol levels and rates of lipolysis will take place. This probe, called a **microdialysis probe**, is a small flexible piece of plastic tubing (about an inch long and the width of a needle) that is inserted through the skin, and then through the subcutaneous fat about 1/8 to 1/4 inch below the skin of the stomach. First, to numb the area of insertion, a topical cold spray (ethyl chloride) or a numbing creme (Emla creme) will be applied to the skin. A needle surrounded by plastic tubing will be inserted into the subcutaneous fat of your child's stomach. The needle will be taken out of the fat and replaced with the small piece of tubing. The tubing will not be located in a blood vessel but between fat cells. A Ringer solution (a saline/salt-water solution) will be pumped through the piece of tubing to monitor blood flow and fat break down in the fat tissue. The pumped fluid will be harmless to your child since it is similar to the fluid already present between the cells of the body. The Ringer solution will be pumped at a rate of no more than 5 microliters per minute (equivalent to a very tiny drop). Your child will not feel the presence or effects of this solution. Samples will be collected every hour while your child is at ECU and at home for the remainder of that day until the following morning. Only one overnight sample will be collected when your child wakes up the following morning.

- After the microdialysis pump is set up, your child will participate in activities pre-planned and provided by the study investigators. Activities will take place in Minges Coliseum. Possible activities will include walking on a treadmill, riding a stationary cycle ergometer, roller-skating, and jump roping. Other activities will include watching movies and playing board games. All activities will be monitored by a trained exercise physiologist who is familiar with the usage and safety precautions for each activity. By no means will your child be limited in what he/she can do during the day, except for activities that involve rough physical contact (for example, football).

Reduction in CVD risk in children through physical activity

- The full day monitoring will take place on a day that the child is already out of school (i.e. vacation, weekend).

- If your child is randomized (similar to picking groups by flipping a coin) to the 16-week physical activity program, he/she will undergo all testing described above (preliminary measurements and a separate visit of approximately 8 hours for microdialysis in the lab) before and after the 16 week program. If your child is randomized to the control group that does not participate in the 16 week physical activity program, they will be required to undergo only the preliminary measurements and another visit (microdialysis) of approximately 8 hours in the lab.

RISKS AND DISCOMFORTS

There are certain risks and discomforts that may be associated with this research. They include:

- The total amount of blood drawn for fasting blood draw is negligible. There is an extremely small risk of local hematoma or infection associated with the needle stick.

- Insertion of the microdialysis probe is associated with mild discomfort, similar to that experienced during an intramuscular injection. Your child will not feel discomfort from the substances (for example, Ringer solution) pumped through the microdialysis probe. Risks associated with this procedure are small, and include hematoma (swelling and bruising) and infection. To minimize the risk of hematoma or infection associated with the insertion of the microdialysis probes into the subcutaneous adipose tissue, these procedures will be performed using sterile techniques. The probes are also made of biocompatible materials.

- There are some risks associated with physical activity such as bumps, bruising, scrapes and other injuries associated with active children.

    - Risks associated with the maximal exercise are dizziness, ventricular arrhythmia (odd heart beats), and in very rare instances death. These risks are very small, with an incidence of fewer than 1 in 10,000 deaths in patients who are known to, or suspected of, having heart disease. The risk is expectedly much smaller than this in a group of young, healthy subjects. To further minimize the risk, faculty and students that have been extensively trained in administering maximal exercise tests will administer the assessments. If during a test a subject complains of dizziness, chest discomfort or other signs of exercise intolerance, the test will be promptly stopped. In the event of loss of consciousness, breathing or heart beat, appropriate CPR and AED administration will be initiated and Greenville Fire/Rescue will be notified via 911.

    - Risks of the body composition assessment are those associated with exposure to low levels of radiation. Risks will be minimized by using an FDA-approved bone density machine (Prodigy, GE Lunar Corp., Madison, WI). This procedure involves a minimal amount of radiation. 1-3 microSieverts) that is within an acceptable range as provided by "North Carolina Regulations for Protection Against Radiation". The amount of radiation (1-3 microSieverts) exposure of one procedure is quite minimal. For example, radiation exposure is approximately 80 microSieverts on a transatlantic airline flight of 8 hours, 50 microSieverts living in Denver, Colorado, at an elevation of 5,000 feet for approximately 4 weeks, or 30 to 40 microSieverts during a typical chest x-ray. There is a potential risk to unborn children for those who are pregnant; therefore, pregnant women must not undergo this procedure.

Reduction in CVD risk in children through physical activity

- Your child should be aware that there are unforeseen risks involved with this and all research studies.

POTENTIAL BENEFITS

Subjects will be able to participate in supervised physical activity and games. The risks are minimal relative to these benefits and the benefits of gaining knowledge with respect to the role of cortisol in childhood obesity.

TERMINATION OF PARTICIPATION

Your child's participation in this research study may be terminated without your consent if the investigators believe that these procedures will pose unnecessary risk to your child. Your child may also be terminated from the participation if your child does not adhere to the study protocol.

COST AND COMPENSATION

Your child will be paid $50.00 as well as prizes worth $25 for his/her time and inconvenience for completion of each microdialysis procedure. Each family with child/children in the exercise group will be compensated $40 for transportation related expenses at 8 weeks.

The policy of East Carolina University does not provide for the compensation or medical treatment for subjects because of the physical or other injury resulting from this research activity. However, every effort will be made to make the facilities of Brody School of Medicine, Pitt County Memorial Hospital available for treatment in the event of such physical injury.

CONFIDENTIALITY

Only the investigators associated with this study will have access to the data obtained. No identifying information will be released. Numeric coding, which only the primary investigator will have access to, will protect the identity of your child and other subjects. Data will be secured in a locked filing cabinet in the office of the primary investigator in the Human Performance Laboratory. The data will be kept for 7 years. Samples will be stored in freezers at the Human Performance Laboratory for a maximum of 7 years. Your child can request destruction (discarded into biohazard containers and disposed of by ECU biohazard personnel) of his/her samples at any time.

VOLUNTARY PARTICIPATION

Your child understands that his/her participation in this study is voluntary. Refusal to participate will involve no penalty or loss of benefits to which your child is otherwise entitled. Furthermore, your child may stop participating at any time he/she chooses without penalty, loss of benefits, or without jeopardizing his/her continuing medical care at this institution.

RESEARCH PARTICIPANT AUTHORIZATION TO USE AND DISCLOSE INFORMATION

Federal laws require that researchers and health care providers protect your identifiable health information. Federal laws also require that researchers get your permission to use collected health information for research. The identifiable information we will collect from subjects in this research project will include:

*General Medical History including: Family health history, medications, nutrition, physical activity levels and body weight history.

*Body composition information, adipose tissue blood flow and metabolism, blood levels of insulin, glucose, free fatty acids, and other compounds related to cardiovascular disease risk.

The members of our research team that will have access to your information will include the Principle investigator, co-investigators, as well as technical and nursing personnel involved in this project.

Information about you will be used and released in such a way that will protect your identity as much as possible; however, confidentiality cannot be absolutely guaranteed. We will only share your information with those individuals listed above. If we need to share information with other individuals other than those listed, we will request your permission a second time.

You will be given a signed copy of your authorization to release medical information for your records. You can limit the amount and type of information that is shared and you must make this request in writing; however, the researcher is able to use any and all information collected prior to the request not to disclose information. Although you can limit the release of your medical information, withholding some information may cause you to become ineligible for this research project. Because research information continues to be looked at after a study is finished, it is difficult to say when the use of your information will stop. There is currently not an expiration date for the use and disclosure of your information for this study.

PERSONS TO CONTACT WITH QUESTIONS

If you have questions related to the sharing of information, please call Robert Hickner at 252-737-4677 (days) or 252-353-5556 nights or weekends or David Collier, M.D. at 744-1953 (days) or 353-2825 (nights and weekends). You may also telephone the University and Medical Center Institutional Review Board at 252-744-2914. In addition, if you have concerns about confidentiality and privacy rights, you may phone the Privacy Officer at Pitt County Memorial Hospital at 252-847-6545 or at East Carolina University at 252-744-2030.

Reduction in CVD risk in children through physical activity

CONSENT TO PARTICIPATE

Your child certifies that he/she has read all of the above information, asked questions, and received answers concerning areas he/she did not understand, and have received satisfactory answers to these questions. Your child willingly consents for participation in this research study. (A copy of this consent form will be given to the person signing as the subject or as the subject's authorized representative.)

Participant's Name (Print)

Authorized Representative's Name (Print) – Guardian #1

Signature of Authorized Representative – Guardian #1 Date

Authorized Representative's Name (Print) – Guardian #2

Signature of Authorized Representative - Guardian #2 Date

AUDITOR WITNESS: I confirm that the contents of this consent/assent form were orally presented.

Auditor's Name (Print)

Signature of Auditor Date

Principal Investigator's Name (Print)

Signature of Principal Investigator Date

FUTURE TESTING OF BLOOD/MICRODIALYSIS SAMPLES

Upon termination of this study, the blood and urine samples collected for this study will be stored for up to 10 years to research scientific questions specifically related to cardiovascular disease risk in children. I will continue to be the owner of the samples and retain the right to have the sample material destroyed at any time during this study by contacting the study principal investigator. During this study the samples will be stored with number identifiers only; however, the number identifier will be linked to a specific name and will be kept on file in the possession of the principal investigator. The linked file will be stored password protected on the Principal Investigator's computer with CD backup. No other individuals will have access to these identifying materials unless the principal investigator is required by law to provide such identifying information. Data will not be publicly available and participants will not be identified or linked to the samples in publication. If a commercial product is developed from this research project, I will not profit financially from such a product.

CONSENT TO PARTICIPATE IN FUTURE TESTING OF BLOOD SAMPLES

I certify that I have read all of the above, asked questions and received answers concerning areas I did not understand, and have received satisfactory answers to these questions. I willingly give my consent for participation in this research study. (A copy of this consent form will be given to the person signing as the subject or as the subject's authorized representative.)

CONSENT TO PARTICIPATE

Your child certifies that he/she has read all of the above information, asked questions, and received answers concerning areas he/she did not understand, and have received satisfactory answers to these questions. Your child willingly consents for participation in this research study. (A copy of this consent will be given to the person signing as the subject or as the subject's authorized representative.)

Participant's Name (Print)

Authorized Representative's Name (Print) – Guardian #1

Signature of Authorized Representative – Guardian #1 Date

Authorized Representative's Name (Print) – Guardian #2

Signature of Authorized Representative - Guardian #2 Date

AUDITOR WITNESS: I confirm that the contents of this consent/assent form were orally presented.

Auditor's Name (Print)

Signature of Auditor Date

Principal Investigator's Name (Print)

Signature of Principal Investigator Date

Appendix C: Parental Informed Consent Form

Reduction in CVD risk in children through physical activity

INFORMED CONSENT- PARENT

Principal Investigator: Robert C. Hickner, Ph.D.
Institution: Human Performance Laboratory
Address: 371 Ward Sports Medicine Building
Telephone Number: (252) 328-4677

TITLE OF PROJECT: Reduction in CVD risk in children through physical activity

INTRODUCTION

You and your child have been asked to participate in a research study being conducted by Robert C. Hickner and colleagues. This research is designed to determine the effect of physical activity on cardiovascular disease risk in children. It will also look at the affect of physical activity on the children's body composition as well as comparing the children's physical activity level to their parent's.

We will study lean and overweight preadolescent children and one of their parents. Studies will take place in the Human Performance Laboratory of East Carolina University and in Minges Coliseum.

PLAN AND PROCEDURES

Prior to testing, you, as a guardian(s) will read and sign this Informed Consent for research, as well as fill out a medical history questionnaire pertaining to you and your child.

You and your child's participation will involve:

- You will fill out a personal history form that pertains to your child as well as one for yourself. Your child will fill out forms consisting of a youth risk behavior survey, leisure time exercise questionnaire, a personal history form, a medical form, a 30-day physical activity recall, pediatric quality of life inventory, and a physical self-perception profile and children's attraction toward physical activity scale. You will fill out a Physical Activity Readiness Questionnaire (PAR-Q) and a 30-day physical activity recall.

- Determination of **body composition** using body mass index (BMI), waist-to-hip ratio (WHR), skinfolds, and a DEXA Scan will be conducted at the Human Performance Laboratory. To calculate BMI, height and weight will be measured. Circumference measures will be taken at the waist and hip to calculate WHR. Finally, skinfold thickness of the tricep, subscapular (shoulder blade), abdomen, thigh, suprailium (hip bone), and calf will be taken on the right side of the body, in duplicate, with a skinfold caliper. Both you and your child will undergo a test of body composition called a DEXA scan. It is like an x-ray of your entire body. During this test you will both be asked to wear minimal clothing (e.g., swimsuit, or shorts and a shirt, or a gown), and to remove all jewelry. You/he/she will lie still on a padded table for the length of the scan (approximately 6 minutes). The table will move across and up and down to scan your/his/her body. Neither you or your child will feel anything and can breathe normally during the scan. If either you or your child has metal their body, then you/they will not be able to participate in the DEXA scan. Radiation exposure from a DEXA scan is approximately 0.04 mrem. The effective radiation exposure that you and your child would receive in this study is less than 0.6% of the radiation exposure an individual receives from natural background sources in one year.

Reduction in CVD risk in children through physical activity

- Determination of **Aerobic Capacity**

  Two maximal treadmill tests will be completed to evaluate initial aerobic capacity. Two tests will be performed to assure that there is adequate effort by the children during the maximal treadmill test and to determine day-to-day variability in the test. If these two tests are not very similar, a third test may need to be conducted, so it is important that your child put out a maximal effort for this test. For this test, your child will walk or run on a treadmill for approximately 10-15 minutes. During this test, your child will wear a mouthpiece so the air they breathe out can be collected for analysis of oxygen. At first, your child will walk leisurely on the level treadmill, but the speed and level of hill climbing will become harder until your child can no longer continue. You will complete one maximal treadmill test to evaluate your aerobic capacity. This test will be done the same way as the child's maximal treadmill test.

  You will be asked to complete the n-3 FFQ for your child at baseline, 4 weeks, 8 weeks, 12 weeks, and 16 weeks. This will allow us to determine 1) what your child eats over time and 2) seasonal variations in your child's age group and population.

- Your child will complete a 3 or 4 day food record at baseline (before microdialysis), 4 weeks, 8 weeks, 12 weeks and 16 weeks.

- You and your child will wear a **physical activity monitor** (RT3 Triaxial Accelerometer) and a pedometer (Yamax, Japan) five days prior to microdialysis and during the microdialysis portion of the study. Additionally, your child will wear the accelerometer and pedometer for 3 days at 4 weeks, 8 weeks, and 12 weeks. You will not be a part of the microdialysis.

- A **fasting blood sample** will be obtained from an arm or hand vein of your child. This will involve one to three small needle sticks. The procedure will take place in the Human Performance Laboratory.

- Your child will be given a cotton swab to test for **salivary cortisol levels** and will be instructed to chew on it for 45-60 seconds. Samples will be collected at 7 a.m. (fasting), 1:45 (prior to a standardized 2 p.m. lunch), and 30, 45, and 60 minutes after lunch. Additional samples will be collected every hour on the hour from 9 a.m. to 3 p.m. The collection of samples will take place in the Human Performance Lab.

- At the Human Performance Lab, the insertion of up to three small probes to determine glycerol levels and rates of lipolysis will take place. This probe, called a **microdialysis probe**, is a small flexible piece of plastic tubing (about an inch long and the width of a needle) that is inserted through the skin and then through the subcutaneous fat about 1/8 to 1/4 inch below the skin of the stomach. First, to numb the area of insertion, a topical cold spray (ethyl chloride) or a numbing creme (Emla creme) will be applied to the skin. A needle surrounded by plastic tubing will be inserted into the subcutaneous fat of your child's stomach. The needle will be taken out of the fat and replaced with the small piece of tubing. The tubing will not be located in a blood vessel but between fat cells. A Ringer solution (a saline/salt-water solution) will be pumped through the piece of tubing to monitor blood flow and fat break down in the fat tissue. The pumped fluid will be harmless to your child since it is similar to the fluid already present between the cells of the body. The Ringer solution will be pumped at a rate of no more than 5 microliters per minute (equivalent to a very tiny drop). Your child will not feel the presence or effects of this solution. Samples will be collected every hour while your child is at ECU and at home for the remainder of that day until the following morning. Only one overnight sample will be collected when your child wakes up the following morning.

Reduction in CVD risk in children through physical activity

- After the microdialysis pump is set up, your child will participate in activities pre-planned and provided by the study investigators. Activities will take place in Minges Coliseum. Possible activities will include walking on a treadmill, riding a stationary cycle ergometer, roller-skating, and jump roping. Other activities will include watching movies and playing board games. All activities will be monitored by a trained exercise physiologist who is familiar with the usage and safety precautions for each activity. By no means will your child be limited in what he/she can do during the day, except for activities that involve rough physical contact (for example, football).

- The full day monitoring will take place on a day that the child is already out of school (i.e. vacation, weekend).

- If your child is randomized (similar to picking groups by flipping a coin) to the 16-week physical activity program, he/she will undergo all testing described above (preliminary measurements and a separate visit of approximately 8 hours for microdialysis in the lab) before and after the 16 week program. If your child is randomized to the control group that does not participate in the 16 week physical activity program, they will be required to undergo only the preliminary measurements and another visit (microdialysis) of approximately 8 hours in the lab.

RISKS AND DISCOMFORTS

There are certain risks and discomforts that may be associated with this research. They include:

- The total amount of blood drawn for fasting blood draw is negligible. There is an extremely small risk of local hematoma or infection associated with the needle stick.

- Insertion of the microdialysis probe is associated with mild discomfort, similar to that experienced during an intramuscular injection. Your child will not feel discomfort from the substances (for example, Ringer solution) pumped through the microdialysis probe. Risks associated with this procedure are small, and include hematoma (swelling and bruising) and infection. To minimize the risk of hematoma or infection associated with the insertion of the microdialysis probes into the subcutaneous adipose tissue, these procedures will be performed using sterile techniques. The probes are also made of biocompatible materials.

- There are some risks associated with physical activity such as bumps, bruising, scrapes and other injuries associated with active subjects.

    - Risks associated with the maximal exercise are dizziness, ventricular arrhythmia (odd heart beats), and in very rare instances death. These risks are very small, with an incidence of fewer than 1 in 10,000 deaths in patients who are known to, or suspected of, having heart disease. The risk is expectedly much smaller than this in a group of young, healthy subjects. To further minimize the risk, faculty and students that have been extensively trained in administering maximal exercise tests will administer the assessments. If during a test a subject complains of dizziness, chest discomfort or other signs of exercise intolerance, the test will be promptly stopped. In the event of loss of consciousness, breathing or heart beat, appropriate CPR and AED administration will be initiated and Greenville Fire/Rescue will be notified via 911.

    - Risks of the body composition assessment are those associated with exposure to low levels of radiation. Risks will be minimized by using an FDA-approved bone density machine (Prodigy, GE Lunar Corp., Madison, WI). This procedure involves a minimal amount of radiation. 1-3

Reduction in CVD risk in children through physical activity

microSieverts) that is within an acceptable range as provided by "North Carolina Regulations for Protection Against Radiation". The amount of radiation (1-3 microSieverts) exposure of one procedure is quite minimal. For example, radiation exposure is approximately 80 microSieverts on a transatlantic airline flight of 8 hours, 50 microSieverts living in Denver, Colorado, at an elevation of 5,000 feet for approximately 4 weeks, or 30 to 40 microSieverts during a typical chest x-ray. There is a potential risk to unborn children for those who are pregnant; therefore, pregnant women must not undergo this procedure.

- You and your child should be aware that there are unforeseen risks involved with this and all research studies.

POTENTIAL BENEFITS

Subjects will be able to participate in supervised physical activity and games. The risks are minimal relative to these benefits and the benefits of gaining knowledge with respect to the role of cortisol in childhood obesity.

TERMINATION OF PARTICIPATION

You and your child's participation in this research study may be terminated without your consent if the investigators believe that these procedures will pose unnecessary risk to either of you. You and your child may also be terminated from the participation if one or both of you do not adhere to the study protocol.

COST AND COMPENSATION

Your child will be paid $50.00 as well as prizes worth $25 for his/her time and inconvenience for completion of each microdialysis procedure. Each family with child/children in the exercise group will be compensated $40 for transportation related expenses at 8 weeks. You will be compensated $50.00 for your participation in the research.

The policy of East Carolina University does not provide for the compensation or medical treatment for subjects because of the physical or other injury resulting from this research activity. However, every effort will be made to make the facilities of Brody School of Medicine, Pitt County Memorial Hospital available for treatment in the event of such physical injury.

CONFIDENTIALITY

Only the investigators associated with this study will have access to the data obtained. No identifying information will be released. Numeric coding, which only the primary investigator will have access to, will protect the identity of your child and other subjects. Data will be secured in a locked filing cabinet in the office of the primary investigator in the Human Performance Laboratory. The data will be kept for 7 years. Samples will be stored in freezers at the Human Performance Laboratory for a maximum of 7 years. Your child can request destruction (discarded into biohazard containers and disposed of by ECU biohazard personnel) of his/her samples at any time.

VOLUNTARY PARTICIPATION

You and your child understand that you and his/her participation in this study is voluntary. Refusal to participate will involve no penalty or loss of benefits to which you and your child are otherwise entitled. Furthermore, you and your child may stop participating at any time you or he/she chooses without penalty, loss of benefits, or without jeopardizing your and his/her continuing medical care at this institution.

Reduction in CVD risk in children through physical activity

RESEARCH PARTICIPANT AUTHORIZATION TO USE AND DISCLOSE INFORMATION

Federal laws require that researchers and health care providers protect your identifiable health information. Federal laws also require that researchers get your permission to use collected health information for research. The identifiable information we will collect from subjects in this research project will include:

*General Medical History including: Family health history, medications, nutrition, physical activity levels and body weight history.

*Body composition information, adipose tissue blood flow and metabolism, blood levels of insulin, glucose, free fatty acids, and other compounds related to cardiovascular disease risk.

The members of our research team that will have access to your information will include the Principle investigator, co-investigators, as well as technical and nursing personnel involved in this project. Information about you will be used and released in such a way that will protect your identity as much as possible; however, confidentiality cannot be absolutely guaranteed. We will only share your information with those individuals listed above. If we need to share information with other individuals other than those listed, we will request your permission a second time.

You will be given a signed copy of your authorization to release medical information for your records. You can limit the amount and type of information that is shared and you must make this request in writing; however, the researcher is able to use any and all information collected prior to the request not to disclose information. Although you can limit the release of your medical information, withholding some information may cause you to become ineligible for this research project. Because research information continues to be looked at after a study is finished, it is difficult to say when the use of your information will stop. There is currently not an expiration date for the use and disclosure of your information for this study.

PERSONS TO CONTACT WITH QUESTIONS

If you have questions related to the sharing of information, please call Robert Hickner at 252-737-4677 (days) or 252-353-5556 nights or weekends or David Collier, M.D. at 744-1953 (days) or 353-2825 (nights and weekends). You may also telephone the University and Medical Center Institutional Review Board at 252-744-2914. In addition, if you have concerns about confidentiality and privacy rights, you may phone the Privacy Officer at Pitt County Memorial Hospital at 252-847-6545 or at East Carolina University at 252-744-2030.

CONSENT TO PARTICIPATE

You and your child certifies that you both have read all of the above information, asked questions, and received answers concerning areas you or he/she did not understand, and have received satisfactory answers to these questions. You and your child willingly consents for participation in this research study. (A copy of this consent form will be given to the person signing as the subject or as the subject's authorized representative.)

Child Participant's Name (Print)

Authorized Representative's Name (Print) – Guardian #1

Signature of Authorized Representative – Guardian #1 Date

Authorized Representative's Name (Print) – Guardian #2

Signature of Authorized Representative - Guardian #2 Date

Adult Participant's Name (Print)

Signature of Adult Participant's Name Date

AUDITOR WITNESS: I confirm that the contents of this consent/assent form were orally presented.

Auditor's Name (Print)

Signature of Auditor Date

Principal Investigator's Name (Print)

Signature of Principal Investigator Date

Reduction in CVD risk in children through physical activity

FUTURE TESTING OF BLOOD/MICRODIALYSIS SAMPLES

Upon termination of this study, the blood and urine samples collected for this study will be stored for up to 10 years to research scientific questions specifically related to cardiovascular disease risk in children. I will continue to be the owner of the samples and retain the right to have the sample material destroyed at any time during this study by contacting the study principal investigator. During this study the samples will be stored with number identifiers only; however, the number identifier will be linked to a specific name and will be kept on file in the possession of the principal investigator. The linked file will be stored password protected on the Principal Investigator's computer with CD backup. No other individuals will have access to these identifying materials unless the principal investigator is required by law to provide such identifying information. Data will not be publicly available and participants will not be identified or linked to the samples in publication. If a commercial product is developed from this research project, I will not profit financially from such a product.

CONSENT TO PARTICIPATE IN FUTURE TESTING OF BLOOD SAMPLES

I certify that I have read all of the above, asked questions and received answers concerning areas I did not understand, and have received satisfactory answers to these questions. I willingly give my consent for participation in this research study. (A copy of this consent form will be given to the person signing as the subject or as the subject's authorized representative.)

CONSENT TO PARTICIPATE

Your child certifies that he/she has read all of the above information, asked questions, and received answers concerning areas he/she did not understand, and have received satisfactory answers to these questions. Your child willingly consents for participation in this research study. (A copy of this consent will be given to the person signing as the subject or as the subject's authorized representative.)

Participant's Name (Print)

Authorized Representative's Name (Print) – Guardian #1

Signature of Authorized Representative – Guardian #1 Date

Authorized Representative's Name (Print) – Guardian #2

Signature of Authorized Representative - Guardian #2 Date

AUDITOR WITNESS: I confirm that the contents of this consent/assent form were orally presented.

Auditor's Name (Print)

Signature of Auditor Date

Principal Investigator's Name (Print)

Signature of Principal Investigator Date

Appendix D: Childhood Assent Form

Reduction in CVD risk in children through physical activity

ASSENT DOCUMENT FOR CHILDREN

Principal Investigator: Robert C. Hickner, Ph.D.
Institution: Human Performance Laboratory. 371 Ward Sports Medicine Building
Telephone Number: (252) 737-4677

TITLE OF PROJECT: Reduction in CVD risk in children through physical activity

You have been asked by Dr. Robert Hickner and workers in the Human Performance Lab to be part of a research project at East Carolina University. In this project, you will do several different things.

1. You will fill out forms about physical activity habits, including forms consisting of a youth risk behavior survey, leisure time exercise questionnaire, a medical form, a 30-day physical activity recall, pediatric quality of life inventory, and a physical self-perception profile and children's attraction toward physical activity scale

2. You will have your height, weight, and skinfolds and percent body fat measured. Skinfolds are measured by pinching different areas of fat on your body. You may feel a very light pinch. You will then go to another room where we will do a test called a DEXA Scan. It is like an x-ray of your entire body. During this test you will wear shorts and a shirt, or a gown, and you will take off any jewelry. You will then lie still on a padded table for about 6 minutes. The table will move across and up and down to scan your body, but you do not feel anything and can breathe normally during the scan.

3. You will come to the Human Performance Lab for a day (7 a.m. to 3 p.m.), where you will be able to play fun games, watch movies, and make new friends.

4. Someone at the lab will draw blood from a vein in your arm or hand. The needle stick will only hurt for a few seconds, although we may need to try up to three times if we do not get the blood on our first try.

5. You will have a small needle put into the fat under the skin of your stomach. You may feel a slight sting, but Dr. Hickner will try to make sure that this hurts as little as possible by spraying a cold spray or putting a cream on your stomach to numb your skin. A small plastic tube (as thin as a piece of thread) will be put through this needle under your skin. The needle will then be taken out after the plastic tube is in place. The plastic tube will then be hooked up to a little pump (smaller than a Walkman). You will have three of these needle sticks and plastic tubes put under the skin of the stomach. A liquid, called Ringer's solution, will be pumped through the plastic tubes. This solution will help measure the break down of fat in your tissue. You will wear the pump on a belt while you are at ECU and while you are at home on this day until the next morning. You will have this test done before and after the 16-week physical activity program.

6. You will wear activity monitors, which looks like a pager, for 5 days prior to the day visit, and during the day visit. Additionally, you will wear the activity for 3 days during weeks 4, 8, and 12. You will wear the monitors on your belt or clothes.

7. You will participate in a maximal exercise test on the treadmill. For this test, you will walk or run on a treadmill for approximately 10-15 minutes. During this test, you will wear a mouthpiece so the air you breathe out can be collected. At first, you will walk on the level treadmill, but the speed and level of hill climbing will become harder until you can no longer continue. You will go through this test on two separate days. If these two tests are not very similar, a third test may be needed, so it is important that you put out a maximal effort for this test.

Reduction in CVD risk in children through physical activity

8. You will participate in a 16-week physical activity program where you will skate, ride bicycles, and play active games. You will need to come to the activity center 3 to 4 times per week for at least one hour per time.
9. You will be asked to complete a 3 or 4 day food records at baseline (before microdialysis), 4 weeks, 8 weeks, 12 weeks and 16 weeks.
10. Your personal information and samples collected will be kept private and safe in the Human Performance Lab. Only Dr. Hickner and co-workers will have access to your data. If you decide that you want you samples thrown out, your samples will be gotten rid of properly by workers at ECU.

Child's Name (print) (Date)

Child's signature (Date)

PERSONS TO CONTACT WITH QUESTIONS

The investigators will be available to answer your (or your guardian's) questions concerning this research, now or in the future. You or your guardian(s) may contact the investigators, Robert Hickner, Ph.D. at 737-4677 (days) or Joseph Garry, M.D. at 744-1953 (days) or 353-2825 (nights and weekends). Also, if questions arise about your rights as a research subject, you or your guardian(s) may contact the Chairman of the University and Medical Center Institutional Review Board at 252-744-2914 (days).

CONSENT TO PARTICIPATE

You certify that you have read all of the above information, asked questions, and received answers concerning areas you did not understand, and have received satisfactory answers to these questions. You willingly consent for participation in this research study. (A copy of this consent form will be given to the person signing as the subject or as the subject's authorized representative.)

Authorized Representative Name (Print) – Guardian #1

Signature of Authorized Representative – Guardian #1 Date

Authorized Representative Name (Print) – Guardian #2

Signature of Authorized Representative – Guardian #2 Date

AUDITOR WITNESS: I confirm that the contents of this consent/assent form were orally presented.

Objective Third Party Witness Name (Print)

Signature of Objective Third Party Witness Date

Principal Investigator's Name (Print)

Signature of Principal Investigator Date

Appendix E: Medical History Form

PLEASE PRINT AND FILL OUT COMPLETELY

1. ID #: _____ Date: _____
   Name: _____
   Parent or Gaurdians name _____
   Address: _____
   City: _____ State _____ Zip_____
   Phone: (home)_____(cell)_____(work)_____
   E-mail:_____

2. Date of birth: _____ Age: _____ Race:_____

3. **General Medical History of your Child** Circle one

Any medical complaints presently? (if yes, explain) .... yes no
_____
_____
_____

Any major illnesses in the past? (if yes, explain) ..... (date) _____ yes no
_____
_____
_____

Any hospitalization or surgery? (if yes, explain) ..... (date) _____ yes no
_____
_____
_____

Has your child ever had an EKG (electrocardiogram) ? ..... (date) _____ yes no

Has your child ever had asthma, difficulty breathing , shortness of breath
or any respiratory illness ? (date)_____ yes no
_____
_____

Is your child diabetic? .If yes, at what age did you develop diabetes: _____ yes no

Are you currently taking any medications? ..... yes no

Medication	Dosage	Reason	Times taken per day

4. **Cardio-Respiratory History of your Child**

Any heart disease ?..... yes no

Heart murmur?..... yes no

Occasional chest pains?..... yes no

Chest pains on exertion?..... yes no

Fainting?..... yes no

Daily coughing?..... yes no

High blood pressure?.....yes no

Shortness of breath --

at rest..... yes no
lying down..... yes no
sleeping at night..... yes no
after 2 flights of stairs..... yes no

5. **Muscular History of your Child**

Any muscle injuries or illnesses now?..... yes no

Any muscle injuries in the past?..... yes no

Muscle pain at rest?..... yes no

Muscle pain on exertion?..... yes no

6. **Bone-Joint History of your child**

Any bone or joint (including spinal) injuries or illnesses now?..... yes no

Any bone or joint (including spinal) injuries or illnesses in the past?..... yes no

Ever had painful joints?..... yes no

Ever had swollen joints?..... yes no

7. **Sleeping Habits of Your Child**

Do your child ever experience insomnia (trouble sleeping)? Yes ____ No ____

If yes, approximately how often: _____
How many hours of sleep does he/she usually average per night: _____

8. **Family History of Your Child**

	Age	Age of death	Cause of death
Father	_____	_____	_____
Paternal Grandmother	_____	_____	_____
Paternal Grandfather	_____	_____	_____
Mother	_____	_____	_____
Maternal Grandmother	_____	_____	_____
Maternal Grandfather	_____	_____	_____

Does your **child** have a family history of: (Blood relatives only: give age of occurrence if applicable)

			Relationship	Age of Occurrence
--High blood pressure	yes	no	_____	
--Heart attack.....	yes	no	_____	
--By-pass surgery.....	yes	no	_____	
--Stroke.....	yes	no	_____	
--Diabetes.....	yes	no	_____	
--Gout.....	yes	no	_____	
--Obesity.....	yes	no	_____	

9. Does anyone in the household smoke? yes no
If yes do they smoke in the house or in the car? _____

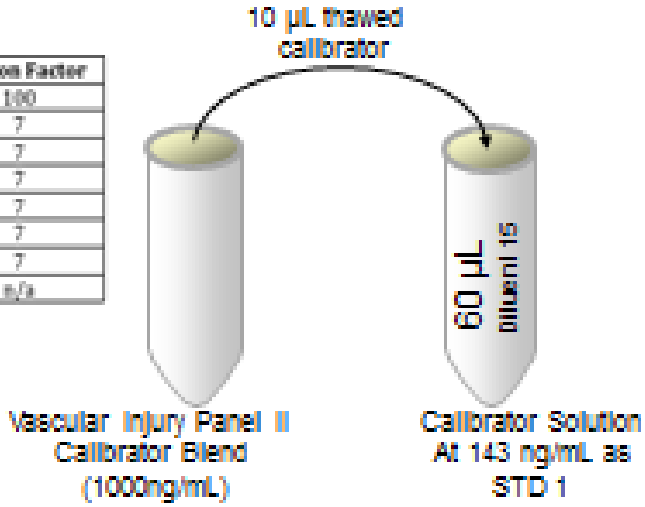
10. Is there good physical reason not mentioned here why your child should not participate in an activity program even if he/she wanted to? yes no

11. **Family Physician**
Name: _____
Address: _____
Phone: _____
Should it be necessary, may we send a copy of your results to your physician? _____
_____
Parent or Gaurdian signature: _____
Date: _____

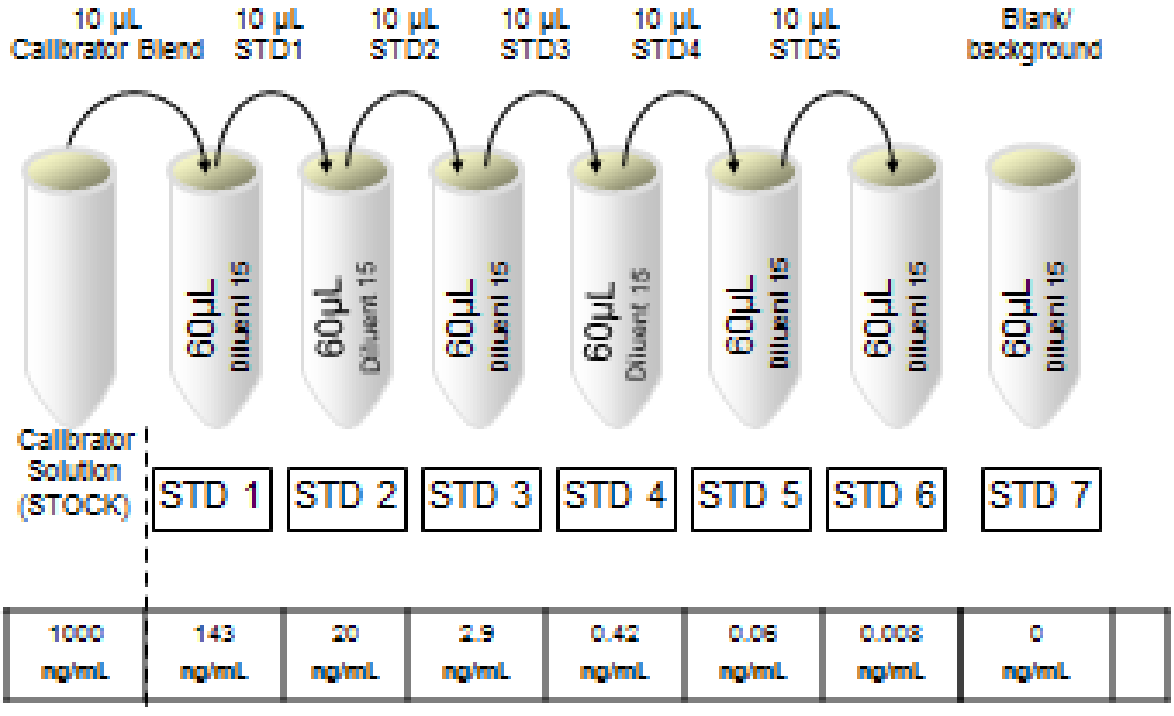
Appendix F: Assay Protocol Diagram

MSD 96-well Multi-Spot Vascular Injury Panel II Assay

Standard	Concentration (ng/mL)	Dilution Factor
Calibrator Stock	1000	100
STD-01	143	7
STD-02	20	7
STD-03	2.9	7
STD-04	0.42	7
STD-05	0.06	7
STD-06	0.008	7
STD-07	0	n/a



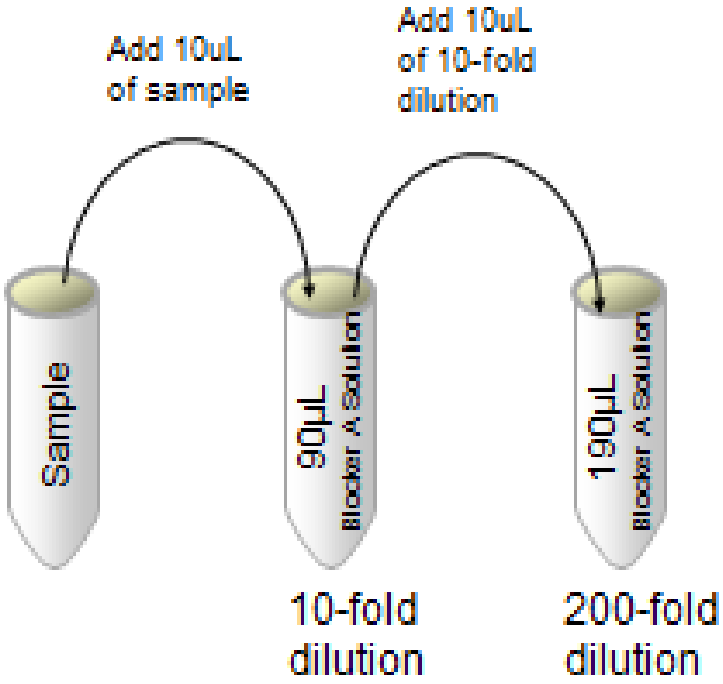
Plasma/serum curve → Diluent 15 as matrix
1:7 serial dilutions



Dilution of Samples:

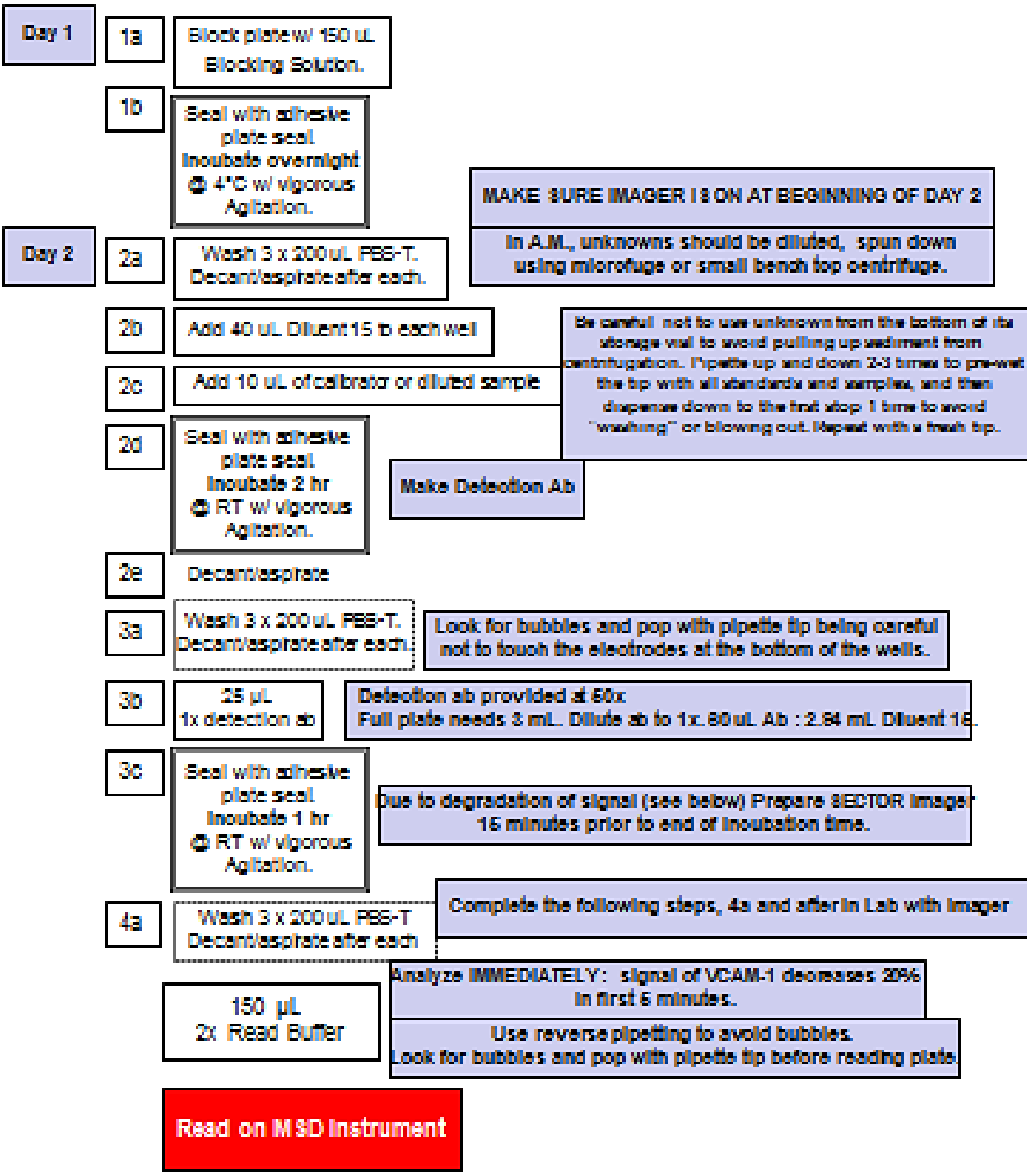
Prepare an initial 10-fold dilution by adding 10uL of sample to 90uL of Blocker A solution and mix thoroughly.

Prepare the 200X diluted sample by starting with the 10X diluted sample and diluting by a factor of 20; add 10uL of the 10X diluted sample to 190uL of Blocker A solution.



Use 0.65 mL microtubes to prepare dilutions

MSD human cytokine ultra-sensitive kits
Analysis using plasma or serum



MSD assay protocol for Vascular Injury Panel II (assay).ppx
Page 2

MSD assay recording sheet

	Plate 1	Plate 2	Plate 3	Plate 4
Assay				
Barcode				
Time (real) for step:				
1a				
1b				
2a				
2b				
2c				
2d				
2e				
3a				
3b				
3c				
4a				
Time stamp (read time)				
Notes:				