ABSTRACT

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| Title of Document: | THE EFFECTS OF LOW-VOLUME/MODERATE-INTENSITY AEROBIC TRAINING ON METABOLIC SYNDROME  COMPONENTS IN MORBIDLY OBESE MINORITY ADOLESCENTS. |
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Despite the increased prevalence of obesity and associated diseases among pediatric minorities, the intensity-specific effects of aerobic training have not been examined extensively in adolescent minorities. Fifteen morbidly obese, sedentary and insulin-resistant Black and Latino adolescents completed two-months of low-volume/moderate-intensity aerobic exercise training to examine the effects of training on three phenotypes dysregulated in obese and physically inactive states: insulin sensitivity ($S_I$); fibrinolytic potential, as indicated by plasminogen activator inhibitor type 1 (PAI-1) and tissue plasminogen activator (t-PA) antigen levels; and chronic low-grade systemic inflammation, as indicated by C-reactive protein (CRP). In response to training, $S_I$ increased ~37% ($1.00 \pm 0.15$ to $1.37 \pm 0.26$ $mU^{.}L^{-1}min^{-1}$, $p<0.05$) and t-PA antigen levels increased ~15% ($6.34 \pm 0.51$ to $7.32 \pm 0.85$ ng/mL, $p<0.05$). No significant changes in CRP or PAI-1 antigen were observed. Our findings demonstrate that aerobic training improves insulin sensitivity and fibrinolytic potential in morbidly obese minority adolescents.

THE EFFECTS OF LOW-VOLUME/MODERATE-INTENSITY AEROBIC TRAINING
ON METABOLIC SYNDROME COMPONENTS
IN MORBIDLY OBESE MINORITY ADOLESCENTS


By

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## Abbreviations:

%HRR: Percent heart rate reserve

%VO$_{2peak}$R: Percent of peak oxygen consumption elicited during treadmill testing; calculated as a percentage of peak oxygen consumption minus resting oxygen consumption

AACE: American Association of Clinical Endocrinologists

ACE: American College of Endocrinology

ACSM: American College of Sports Medicine

ADA: American Diabetes Association

AHA: American Heart Association

AUC: Area under the curve

B: Black

BMI: Body mass index

CRP: C-reactive protein

CDC: Centers for Disease Control

CDC DNPAO: Centers for Disease Control Division of Nutrition, Physical Activity and Obesity

CNMC: Children's National Medical Center

CVD: Cardiovascular disease

DEXA: Dual energy X-ray absorptiometry

ECU: East Carolina University

ELISA: Enzyme-linked immunoabsorbant assay

FSD: Fractional standard deviation

FSIVGTT: Frequently sampled intravenous glucose tolerance test

GCRC: General Clinical Research Center

GUMC: Georgetown University Medical Center

HR: Heart rate

hr: Hour

HRR: Heart rate reserve

hsCRP: High sensitivity C-reactive protein

IL-1: Interleukin 1 complex

IL-6: Interleukin 6 complex

IRB: Institutional Review Board

kcal: Kilocalories

MET: Metabolic equivalent

NIH: National Institutes of Health

L: Latino

min: Minute

mm: Millimeters

mRNA: Messenger ribonucleic acid

OGTT: Oral Glucose Tolerance Test

PAI-1: Plasminogen activator type 1

RER: Respiratory exchange ratio

ScV#1: Screening Visit #1

ScV#2: Screening Visit #2

$S_I$: Insulin sensitivity index

SLFSIVGTT: Stable isotope-labeled frequently sampled intravenous glucose tolerance
    test

STRRIDE: Studies Targeting Risk Reduction Interventions through Defined Exercise

SV#1: Study Visit #1

SV#1: Study Visit #1

TNF-α: Tumor necrosis factor, alpha

t-PA: Tissue plasminogen activator

u-PA: Urinary-type plasminogen activator

$VO_{2\ Peak}$: Peak oxygen consumption elicited during treadmill testing

$VO_{2\ Rest}$: Resting oxygen consumption

wk: week

yr: year

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**Introduction:**

Habitual levels of moderate physical activity decrease the risk of all-cause mortality and cardiovascular disease (CVD) over the lifespan (van Dam et al., 2009; Barengo et al., 2004; Blair et al., 1989). Despite public knowledge of the benefits of physical activity, Americans are becoming increasingly inactive as nearly half of all Americans do not meet minimal physical activity recommendations (CDC DNPAO a & b, 2008; CDC, 2003). Some of the most pronounced declines in physical activity are observed in the transition from childhood to adolescence (Nader et al., 2008). Adolescent-onset physical inactivity is especially prevalent in minority populations, where national physical inactivity rates are as high as 42% among Black adolescent females (AHA II, 2009; Kimm et al., 2002). Physical inactivity in youth is associated with an increased risk of obesity (Dwyer et al., 2009; Ogden et al., 2006; Raitakari et al., 1994).

The obesity-associated 'metabolic syndrome' phenotype presents as a clustering of metabolic disturbances including: insulin resistance, hypertension, dyslipidemia, low-grade systemic inflammation, endothelial dysfunction, enhanced thrombosis, and impaired fibrinolysis (Cirillo et al., 2009; Steinberger et al., 2009; Shaibi et al., 2008; ACE, 2003; Sakkinen et al., 2000; Bouchard, 1997). The clustering of these cardiometabolic risk factors was classically termed 'the insulin resistance syndrome' as insulin insensitivity was thought to be the central pathological moderator (Shaibi et al., 2008; Narayan, 2006). Recent studies, however, suggest the combination of insulin resistance and obesity-associated chronic low-grade systemic inflammation result in the clustering of these metabolic syndrome components and the subsequently increased risk of type 2 diabetes and CVD (Ridker et al., 2004).

Correlations between body mass index and metabolic syndrome phenotypes may be attributable to the disruptive endocrine and paracrine effects of excessive adiposity on multi-organ cell signaling mechanisms (Weiss, Taksali & Caprio, 2006; Weiss et al., 2004). Excessive adiposity results in the elevated secretion of a variety of pro-inflammatory cytokines and proteins, termed 'adipokines,' and increases circulating levels of free fatty acids through enhanced rates of lipolysis. An increased number of free fatty acids in the circulation can induce hepatic and peripheral insulin resistance (Ragheb et al., 2009). Elevated adipokine circulation is thought to contribute to the low-grade systemic inflammation observed in obese states and to dysregulate a variety of systemic signaling pathways (Antuna-Puente et al., 2008). The downstream effects of enhanced adipokine secretion include the disruption of peripheral and hepatic insulin signaling, blood hemostasis and angiogenic repair mechanisms, all of which may result in the presentation of metabolic syndrome phenotypes such as insulin insensitivity, diminished fibrinolysis and endothelial dysfunction (Galic, Oakhill & Steinberg, 2010; You et al., 2008).

Skeletal muscle may also behave in an endocrine-, paracrine-, and autocrine-like fashion through the release of a variety of cytokines, termed 'myokines,' which may exert anti-inflammatory, angiogenic and insulin-sensitizing effects (Mathur & Pedersen, 2008). Exercise training may attenuate metabolic syndrome components through enhanced myokine secretion and subsequent myokine-induced cross-talk between the skeletal muscle and the adipose, hepatic and vasculature tissues (Pedersen, 2009; Mathur & Pedersen, 2008). Thus, investigating the effects of aerobic training on metabolic syndrome

components is of interest as attenuation of such phenotypes suggests a transition towards a more metabolically favorable systemic phenotype (Pedersen, 2009).

Two well-established interventions used to attenuate metabolic syndrome components are dietary interventions, which primarily target the adipose and gastric systems, and exercise training, which primarily targets the skeletal muscle and adipose tissues. As such, aerobic exercise training may induce a transition towards a more metabolically favorable systemic phenotype such that it attenuates peripheral and hepatic insulin sensitivity, reduces systemic inflammation, decreases free fatty acid levels and increases fibrinolysis. Together, these positive training adaptations may reduce the risk of type 2 diabetes, CVD and associated mortality by improving insulin sensitivity and reducing pro-atherosclerotic stimuli (Ginsberg & MacCallum, 2009; AACE 2002; Berenson et al., 1998).

Large-scale longitudinal studies demonstrate that atherosclerotic processes begin in youth and increase in proportion to the number of metabolic syndrome components (Zieske, Malcom & Strong, 2002). This is particularly alarming as the relative risk of CVD is nearly doubled among individuals with metabolic syndrome which has been estimated to present in up to 50% of overweight and obese adolescents (ADA, 2008; Galassi, Reynolds & He, 2006; Weiss et al., 2004). Previously-conducted exercise intervention studies demonstrate favorable changes in metabolic syndrome phenotypes in youth populations, despite minor changes in body mass and composition (van der Heijden et al., 2009; Conwell et al., 2008; Balagopal et al., 2008; Kim et al. 2007; Bell et al., 2007; Nassis et al., 2005). However, the majority of these studies are limited to lifestyle-based rather than intensity-specific interventions in White populations. As described above, children and

adults from minority populations are at high risk, and there is a lack of literature on the effects aerobic exercise training in these populations. We thus felt it was of interest to examine the effects of aerobic exercise training on metabolic syndrome components in previously sedentary, obese and insulin-resistant minority adolescents.

**Hypothesis and Specific Aims:**

**Hypothesis: Low-volume/moderate-intensity aerobic exercise training will attenuate metabolic syndrome components in previously-sedentary, obese and insulin-resistant minority adolescents.**

*Specific Aim 1:* Determine the effects of two-months of low-volume/moderate-intensity aerobic training on insulin sensitivity in previously-sedentary and obese adolescent minorities.

>    **Aim 1A:** Quantify baseline and final glucose and insulin levels from frequently sampled intravenous glucose tolerance testing (FSIVGTT).

>    **Aim 1B:** Examine changes in insulin sensitivity index ($S_I$) in response to training as determined by MINMOD Millennium pharmacokinetic modeling software.

*Expected findings include significant improvements in $S_I$ in response to two-months of low-volume/moderate-intensity aerobic exercise training.*

*Specific Aim 2:* Determine the effects of two-months of low-volume/moderate-intensity aerobic exercise training on 'non-traditional' metabolic syndrome components.

>    **Aim 2A:** Determine the effects of training on fibrinolytic potential as determined by PAI-1 and t-PA antigen levels.

>    **Aim 2B:** Determine the effects of training on low-grade systemic inflammation as determined by C-reactive protein (CRP) levels.

*Expected findings include significant decrements in PAI-1 antigen and CRP, and significant increases in t-PA antigen in response to training.*

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# Chapter 1: Literature Review

## 1.1    Background and Significance

Washington DC youth demonstrate some of the highest rates of overweight and obesity in the United States (Trust for America's Health, 2009). Among a multitude of purported causes, the high proportion of minority populations (Black and Latino) and an obesogenic 'built' environment contribute significantly to the current childhood obesity epidemic in Washington DC (Patrick & Nicklas, 2005). Physical activity declines in the transition from childhood to adolescence are thought to significantly contribute to the current pediatric obesity epidemic (Nader et al., 2008). Although regular physical activity has been suggested to attenuate obesity and type 2 diabetes risk in adult populations, the magnitude of the intensity-specific effects of aerobic exercise training-induced improvements on type 2 diabetes risk factors has been studied very little in pediatric populations and even less in pediatric minorities.

Findings from previously-conducted pediatric studies demonstrate improvements in metabolic syndrome phenotypes in response to exercise training and lifestyle-based interventions, despite minor changes in body mass and composition; these findings suggest the ability of aerobically-trained muscle to attenuate metabolic syndrome components in obese and physically inactive states (van der Heijden et al., 2009; Conwell et al., 2008; Balagopal et al., 2005; Bell et al., 2007; Nassis et al., 2005). However, further investigation of the intensity-specific effects of aerobic exercise training is warranted as many of these previously-conducted studies comprise lifestyle-based rather than intensity-specific interventions in predominantly White populations. As such, the American Heart Association suggests that additional research is needed to develop exercise training

recommendations for the treatment of pediatric obesity, as no such exercise treatment guidelines exist specifically for the management of obesity in youth populations (Daniels et al., 2009).

Examining the effects of aerobic training independent of dietary-induced weight loss is of interest as improvements suggest the ability of aerobically-trained muscle to mitigate adiposity-associated metabolic aberrations. It is notable that most metabolic syndrome or obesity markers are age-related, and show co-morbidity with many age-related conditions. Thus, in adults, it is very difficult to determine cause-effect relationships between inflammation, obesity, physical activity, and lipid profiles when all of them are strongly correlated (Donges, Duffield & Drinkwater, 2010; Stowe et al., 2010; Kim et al., 2007; Oberbach et al., 2006; Short et al., 2003). Moving interventions to younger populations has the advantage of decreasing potentially confounding age-related co-morbid conditions, to include insulin sensitivity, fibrinolysis and chronic low grade systemic inflammation all of which show age-related impairments (Cevenini et al., 2010; Menzel & Hilberg, 2009; Ribeiro et al., 2007; Short et al., 2003; Kelly et al., 2004).

### 1.2.1   Insulin Resistance

Insulin resistance in youth is associated with obesity indices and has a largely pubertal and post-pubertal onset (Matyka, 2008; Brandou, Brun & Mercier, 2005; Weiss et al., 2004). Insulin resistance plays a major role in the development of type 2 diabetes (Bergman, 2007; Shulman, 2000). Insulin resistance is characterized by increased insulin secretion, peripheral and hepatic insulin insensitivity, and glucose intolerance (Bergman, 2007; Bergman et al., 1979). Prolonged insulin resistance commonly results in further disruptions in glucose homeostasis and lipid metabolism, β-cell deficiency, and the onset of

type 2 diabetes (Weiss & Gillis, 2008; Elder et al., 2006). Insulin resistance also contributes to the etiology of atherosclerosis and CVD (Zieske, Malcom & Strong, 2003; ACE, 2003).

Insulin resistance is commonly assessed through 'surrogate determinants' of insulin sensitivity which include total glucose and insulin area under the curve (AUC) by oral glucose tolerance testing (OGTT), homeostasis model assessment for insulin resistance (HOMA-IR) and fasting plasma insulin levels. These methods are of limited utility as they do not experimentally control for individual variations in glucose and insulin dynamics (Bergman, 2007; Yeni-Komshian et al., 2000). Experimental determination of insulin sensitivity can be quantified by frequently sampled intravenous glucose tolerance testing (FSIVGTT) or hyperinsulinemic-euglycemic clamp. FSIVGTT enables calculation of insulin-stimulated glucose uptake, or insulin sensitivity index ($S_I$) through pharmacokinetic modeling of plasma insulin and glucose levels following exogenous glucose and insulin administration. Use of mathematical modeling software enables determination of steady-state insulin-dependent glucose uptake, or $S_I$, by FSIVGTT through quantification of a series of pre-programmed peripheral and hepatic glucose and insulin rate constants (Bergman, 2007; Bergman, Beard & Chen, 1986).

### 1.2.2    Exercise Training and Insulin Resistance

Previously-conducted exercise intervention studies have demonstrated improvements in insulin sensitivity in pediatric populations (Reviewed in Table 1) (van der Heijden et al., 2009; Conwell et al., 2008; Bell et al., 2007; Kim et al., 2007; Nassis et al., 2005; Balagopal et al., 2005; Ferguson et al., 1999 I).

**Table 1:** Summary of training-induced changes on insulin sensitivity parameters in previously-conducted pediatric exercise training studies.

| Study | n | Ethnicity/ Gender | Average Age (years ± SD) | Training Type | Program Length (weeks) | Training Frequency and Duration | Intensity | Change in BMI (kg/m$^2$) | Change in Percent Body Fat | Change in Insulin Sensitivity |
|---|---|---|---|---|---|---|---|---|---|---|
| van der Heijden et al., 2009 | 15 | Latino/ 7 M; 8 F | 15.6 ± 0.4 | Aerobic (2 of 4 sessions home-based) | 12 | 30 min x 4 d/wk | ~85% VO$_{2peak}$ | 33.2 ± 0.9 to 33.0 ± 0.8 | DEXA: 38.4 ± 1.5 to 37.3 ± 1.5%* | SLFSIVGTT: 59% increase in peripheral S$_I$* |
| Conwell et al., 2008 | 15 | (n/a)/ 8 F; 6 M | 11.8 ± 0.6 | Home-based walking program | 10 | n/a | n/a | 34.5 ± 1.3 to 34.9 ± 1.5 | n/a | FSIVGTT: 32.90% increase in S$_I$* |
| Bell et al., 2007 | 14 | White/ 9 M; 6 F | 12.7 ± 2.32 | Circuit training | 8 | 1hr x 3 d/wk | HR: ~85% HR$_{Max}$ (later 5 weeks of aerobic portion of circuit) | 31.58 ± 4.36 to 31.19±4.0 | DEXA: 47.07 ± 5.23 to 46.50± 5.84% | Clamp: 22.32 % increase in M$_{(lbm)}$* |
| Kim et al., 2007 | 14 | Korean/ M | 17 ± 0.11 | Jump rope interval | 6 | 30 min (1.5-4 min exercise per 30 sec rest) x 5 d/wk | n/a | 29.6 ± 0.6 to 28.6 ± 0.6* | Bioelectrical Impedance: 31.5 ± 1.0 to 29.3 ± 1.0%* | HOMA-IR: 33.6% decrease* |
| Nassis et al., 2005 | 21 | Greek/ F | 13.1 ± 1.75 | Game-based | 12 | 40 min x 3 d/wk | HR: ~161 ± 2 bpm | 26.8 ± 3.9 to 26.7 ± 3.8 | DEXA: 41.4 ± 4.8 to 40.7 ± 5.2% | OGTT: 23.3% decrease in insulin AUC* |
| Balagopal et al., 2005 | 8 | (n/a)/ 4 M; 4 F | 15.6 ± 0.3 | Home-based, included dietary intervention | 12 | 45 min x 3 d/wk | n/a | 38.1 ± 2.1 to 37.5 ± 2.1 | DEXA: 45.5 ± 2.3 to 39.2 ± 2.3%* | HOMA-IR: ~24% decrease* |
| Ferguson et al., 1999 | 70 | Black, White, Asian/ M; F | 9.5 ± 1.0 | Aerobic training (20 min) and game-based (20 min) | 16 | 40 min x 4 d/wk | Average HR: ~157 ± 7 bpm | n/a | DEXA: 44.3 ± 0.3 to 42.6 ± 0.4%* | Fasting Insulin: 23.90 ± 1.14 to 20.24 ± 1.14 μU/mL* |
| Gutin et al., 1996 | 12 | Black/ Female | 9.2 | Mixed-modality (including circuit training) | 10 | 30 min x 5 d/wk | HR: >70% HR$_{Max}$ | n/a | DEXA: 42.8 ± 2.3 to 41.4 ± 2.5%* | Fasting Insulin: 33.17 ± 6.62 to 41.23 ± 7.10 μU/mL |

*Significance as defined as $p < 0.05$.

Nassis et al. demonstrated body mass-independent improvements in insulin sensitivity as defined by a ~23.3% decrease in total insulin area under the curve (AUC) in obese girls (age 9-15 years) in response to a 12-week game-based aerobic intervention (2005). Training sessions were supervised, administered three-times weekly, and included a 10

minute warm-up, 25 minutes of 'physical training' games, and a 5-minute cool-down (Nassis et al., 2005).

Conwell et al. demonstrated body mass-independent improvements in $S_I$ by FSIVGTT in obese youth (BMI $34.5 \pm 1.3$kg/m$^2$, age 8-18 years) after a 10-week home-based physical activity program which consisted of health education encouraging an increased number of steps per day (2008). Bell et al. demonstrated a ~22% increase in insulin sensitivity as determined by the amount of glucose required to maintain euglycemia (milligrams of glucose infused per kilogram lean body mass per minute), or $M_{(lbm)}$, by euglycemic clamp in obese youth (age 9-16 years) in response to eight weeks of circuit training (2007). The training regimen consisted of 1 hour sessions administered 3 times weekly, which included: 1) a 10 minute warm-up; 2) 40 minutes of alternating one-minute intervals of cycling ergometry (~85% maximal heart rate (HRmax) weeks 3-8) and resistance training; and 3) a 10 minute cool-down period (Bell et al., 2007). Due to the circuit training modality of the Bell et al. study, the individual effects of aerobic and resistance training cannot be distinguished.

Gutin et al. did not observe significant changes in fasting insulin in 12 obese Black girls (age 7-11 years) in response to 5 weekly 30 minute sessions of mixed-modality (aerobic and resistance) training administered over a 10 week period despite a ~1.4% reduction in total body fat as determined by DEXA (1996). In this study, subjects were encouraged to maintain their heart rate at >70% HRmax for the 30-minute training session which consisted of mixed-modality aerobic and resistance exercise training (cycle ergometry, walking/jogging, circuit training, etc.); HR was monitored during 2 of 5 weekly training sessions. A similar study by Ferguson et al. observed significant decrements in

fasting insulin in 70 obese children (age 7-11 years, percent total body fat 27-61%) in response to four months of aerobic training (1999 I). Subjects engaged in aerobic and game-based activities for ~40 min (20 min of aerobic and 20 min game-based exercise) four-times per week; average heart during such activities was recorded by use of a heart rate monitor, thus exercise intensity was not standardized. All of the aforementioned studies are, however, limited by the inclusion of children at different stages of pubertal development; insulin sensitivity decreases upon the onset of puberty and displays heterogeneous age-related changes between genders (Casazza et al., 2009; Kelly et al., 2007; Goran & Gower, 2001).

The following studies did stratify for developmental stages. Balagopal et al. enrolled obese subjects, average BMI $38.1 \pm 2.1$ kg/m$^2$, with a Tanner growth stage of 4, into a 12-week combined physical activity and dietary intervention program and observed an improvement in insulin sensitivity as defined as a ~24% decrease in HOMA-IR (2005). The training intensity of this study was not specified; subjects were encouraged to participate in aerobic activities, such as brisk walking, 3 times weekly for 45 minutes, and one of the weekly exercise sessions was monitored.

Kim et al., found a ~33.6% decrease in HOMA-IR in 14 obese (BMI $29.5 \pm 2.2$ kg/m$^2$) Korean adolescent males (age $17 \pm 0.11$ years) in response to a 6 week jump-rope-interval training program (2007). This training program consisted of six weeks of 30 minute training sessions administered five days per week. The training sessions consisted of alternating jump rope and one-minute resting intervals; the duration spent exercising (jumping rope) was increased from 1 to 4 minutes over the six-week training period (Kim et al., 2007).

A recent study by van der Heijden et al. enrolled 15 post-pubertal (Tanner growth stage >4), obese (BMI $33.2 \pm 0.9$ kg/m$^2$), Latino adolescents (age $15.6 \pm 0.3$ years) into a 12-week (30 min x 4 days/week) high-intensity (~85% $VO_{2\ Peak}$) aerobic exercise training program (2009). In response to this program, subjects demonstrated a $59 \pm 19\%$ improvement in peripheral insulin sensitivity as determined by stable isotope labeled FSIVGTT (SLFSIVGTT), despite no significant changes in body mass and a slight (1.1%) decrease in total body fat as determined by DEXA (2009). This study is, however, limited in that two of four of the training sessions were home-based.

Investigation of the insulin-sensitizing effects of low-volume/moderate intensity aerobic training is warranted as high-intensity training programs may not be feasible in previously-sedentary obese youth populations (Daniels et al., 2009). Houmard et al. suggest that low-volume/moderate-intensity and high-volume/high-intensity aerobic training result in similar improvements in insulin sensitivity in overweight/obese adults (2004). Investigation of the effects of low-volume/moderate-intensity aerobic training on insulin sensitivity is thus warranted.

The aforementioned studies (Balagopal et al., Nassis et al., Kim et al., Ferguson et al., and Gutin et al.) are limited by the use of surrogate determinants of insulin sensitivity. All of the aforementioned studies are limited in that the training protocols were comprised of mixed-modality (discontinuous and intensity-aspecific aerobic and/or resistance exercise training) or home-based training sessions. Data from these previous studies present a need for interventions controlling for training intensity and utilizing direct methods of insulin sensitivity quantification, such as $S_I$ by FSIVGTT, as surrogate methods have limited validity in children (Brandou, Brun & Mercier, 2005). Additionally, due to the increased

prevalence of physical inactivity, obesity and associated metabolic syndrome phenotypes in minority groups, investigation of the effects of intensity-controlled aerobic exercise training in pediatric minorities is warranted.

### 1.3.1   Hypofibrinolysis

Hemostatic disturbances, characterized by enhanced thrombosis and diminished fibrinolysis, comprise another metabolic syndrome phenotype. These disturbances in normal blood clotting mechanisms result in elevated blood coagulability and fibrin clot development (El-Sayed, El-Sayed & Ahmadizad, 2004). Obesity, insulin resistance and physical inactivity exaggerate these disturbances which ultimately contribute to atherosclerosis development and progression, and enhanced CVD risk (Wang, 2006; Zieske, Malcom & Strong, 2002).

Fibrinolysis, or the degradation of fibrin clots, primarily occurs through lysis by plasmin. The zymogen of plasmin, plasminogen, is activated by tissue and urinary-type plasminogen activators, t-PA and u-PA, respectively. Once activated, plasmin serves to proteolyze fibrin, leading to fibrin clot degradation (Kohler & Grant, 2000). Plasminogen activator inhibitor type 1 (PAI-1) is the primary inhibitor of t-PA and u-PA and thus increased PAI-1 to t-PA antigen levels are associated with diminished fibrinolytic activity (Kohler & Grant, 2000). PAI-1 and t-PA levels correlate with body mass, adiposity and physical activity levels in adolescent and adult populations (Balagopal et al., 2008; Kulaputana et al., 2005).

Elevated PAI-1 protein levels are thought to significantly contribute to the hypofibrinolysis observed in insulin resistant states as hyperinsulemia and hyperglycemia increase PAI-1 expression *in vivo* (Pandolfi et al., 2000; McGill et al., 1994). A variety of

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adipose tissue-derived cytokines and proteins upregulate PAI-1expression and protein levels *in vivo*, including CRP (Jag, Zavadil & Stanley, 2009; Nassis et al., 2005; Kelly et al., 2004). The exact signaling mechanisms linking CRP and other metabolic syndrome components to hypofibrinolysis are not well characterized.

### 1.3.2  Exercise Training and Fibrinolysis

Previously-conducted aerobic exercise training studies demonstrate favorable changes in t-PA and PAI-1 mRNA and protein expression in the skeletal muscle in response to aerobic training (Hittel, Kraus & Hoffman, 2004). This suggests that aerobically-trained muscle may attenuate the fibrinolytic impairments observed in obese and physically inactive states. However, the effects of aerobic training on fibrinolysis in obese youth have been infrequently investigated and are limited to lifestyle-based interventions, often examining the combined effects of dietary modifications and exercise training (Balagopal et al., 2008; Conwell et al., 2008; Ferguson et al., 1999 II).

In obese pediatric lifestyle intervention groups Balagopal et al. observed no significant changes in PAI-1 and t-PA antigen levels, or the molar ratio of PAI-1 to t-PA in obese (BMI-for-age percentile 98.5 ± 0.9) adolescents (age 15-18 years) in response to a 12-week lifestyle and dietary intervention, which consisted of one supervised session per 3 weekly 45 minute training sessions (2008). Conwell et al. also found no significant changes in PAI-1 antigen levels following a 10-week home-based walking program in obese youth (BMI 34.5 ± 1.3kg/m$^2$, age 8-18 years) (2008). Ferguson et al. did not observe changes in PAI-1 antigen levels in obese (BMI 26.8 ± 4.9 kg/m$^2$) children (age 9.5 ± 1.0 years) in response to 4 months of aerobic and game-based training administered for 40 minutes for ~4 days per week (1999 II). In this study subjects were encouraged to keep their heart rate

above 150 bpm while playing aerobic exercise-based games (20 minutes per session) and using aerobic exercise equipment (20 minutes per session). Despite no significant changes in PAI-1antigen levels in response to training, decrements in PAI-1 antigen levels in response to training were more pronounced when elevated at baseline (1999). Ferguson et al. and Conwell et al. did not investigate changes in t-PA antigen levels in response to training (2008; 1999 II). Thus, it is of interest to investigate the effects of moderate-intensity aerobic exercise training on t-PA antigen levels as its measurement may serve as an additional indicator of changes fibrinolytic potential in response to training (Morris et al., 2003). An acute bout of exhaustive exercise has been demonstrated to improve fibrinolytic potential in healthy male adolescents as indicated by increases in t-PA antigen levels and decreases in PAI-1 antigen levels (Ribeiro et al., 2007). Due to the documented intensity-specific responses of aerobic exercise training on fibrinolysis in adult populations and the potential confounding influences of diet and unsupervised training (Wang, 2006; Fumeron et al., 1991; Marckmann et al., 1991), it is of interest to examine the effects of supervised and intensity-controlled aerobic training on plasma fibrinolysis without substantial modifications in dietary intake.

### 1.4.1   Metabolic Syndrome and Systemic Inflammation

Studies demonstrating significant associations between metabolic syndrome phenotypes and body composition suggest that excessive adiposity acts as a central regulator in the pathological manifestations of the syndrome (Sell & Eckler, 2009; Katagiri, Yamada & Oka, 2007). A purported mechanism by which adipose tissue contributes to the presentation of metabolic syndrome phenotypes is through the increased expression of pro-

inflammatory cytokines (Goldberg, 2009; Bray & Ryan, 2006; Kelly et al., 2004; Yudkin et al., 1999).

CRP is a reactive acute-phase protein expressed in response to a variety of pro-inflammatory stimuli including interleukin 1 complex (IL-1), interleukin 6 complex (IL-6), resistin and tumor necrosis factor α (TNF-α) (Calabrò, Golia & Yeh, 2009). CRP is primarily expressed in the liver and to a lesser degree in adipocytes in response to a variety of cytokines; its expression is, therefore, increased in obese states where excessive adiposity contributes to chronic low-grade systemic inflammation through the elevated secretion of pro-inflammatory cytokines (Yudkin et al., 2000; Tosi et al., 2009; Gabay & Kushner, 1999). Increased circulating levels of CRP and its pro-inflammatory stimulators are positively associated with body mass index, adiposity, insulin insensitivity and fibrinolytic impairments in both pediatric and adult populations (Balagopal et al., 2008; Qi et al, 2007; Nassis et al., 2005; Bastard et al., 2002; Vozarova et al., 2001). Numerous mechanisms linking circulating adipokines to metabolic syndrome-associated pathophysiology have been identified and are currently under investigation. IL-6, for example, has been shown to induce lipolysis which may lead to impairments in insulin sensitivity and fibrinolytic activity (Qi et al., 2007). Due to the increased half life of CRP relative to that of its inducers, it is commonly used as a marker of chronic systemic inflammation (Ridker, 2003). Elevated CRP are levels are associated with an increased risk of developing CVD (AHA I, 2009).

### 1.4.2 Exercise Training and Systemic Inflammation

Exercise training has been documented to decrease systemic inflammation, yet the dietary-independent and intensity-specific effects have been infrequently studied in youth

populations (van der Heijden et al., 2009; Balagopal et al., 2005; Kim et al. 2007; Nassis et al., 2005). Aerobic training has been demonstrated to decrease CRP levels in adult populations (Campbell et al., 2009; Obisesan et al., 2006). In youth populations, a study by Balagopal et al. demonstrated a ~30% decrease in CRP in 8 obese youth (BMI $38.1 \pm 2.1$ kg/m$^2$, age $15.6 \pm 0.3$ years) in response to a 12-week home-based physical activity and dietary education program (2005). Balagopal et al., additionally demonstrated correlations between decrements in fibrinogen, fasting insulin and systemic markers of inflammation in obese youth in response to this lifestyle intervention program (2005). However, other studies investigating the effects of training on systemic inflammation have not observed significant changes in CRP in obese youth populations (van der Heijden et al., 2009; Kim et al. 2007; Nassis et al., 2005). As described previously, Nassis et al. found no significant differences in CRP in response to 12-week game and aerobic exercise-based physical activity program (2005). Kim et al. did not observe changes in CRP levels in 14 obese (BMI $29.5 \pm 2.2$ kg/m$^2$) Korean male adolescents (age $17 \pm 0.11$ years) in response to a 6-week jump rope interval training program, despite improvements in body mass and composition (2007). Findings from these studies may be limited by the methodological approaches of the exercise programs (interval-type and lifestyle-based).

Overall, circulating levels of CRP correlate negatively with fibrinolytic activity, insulin sensitivity and physical activity (Campell et al., 2009; Balagopal et al., 2008; Qi et al., 2007; Bastard et al., 2002). Studies examining associations between the attenuation of metabolic syndrome phenotypes in response to aerobic exercise training have yielded inconsistent findings in pediatric populations (van der Heijden et al., 2009; Balagopal et al., 2005; Conwell et al., 2007; Kim et al, 2007; Nassis et al., 2005). This may be due to not

monitoring exercise intensity, and differences between training protocols and

characteristics of enrolled subjects.

## Chapter 2: Experimental Design and Methods

### 2.1    Experimental Design

The following describes a previously-conducted pediatric training study, conducted at Children's National Medical Center (CNMC). Collected data and stored blood samples were used for post-hoc investigation by the degree candidate (Ms. Many).

#### 2.1.1    Subject Recruitment

Subjects were recruited in the DC Metropolitan Area via radio and newspaper advertisements and locally-posted flyers. Recruitment methods specified inclusion criteria of obesity, age (14 to 18 years), and sedentary behavior. Subjects were incentivized to call through potential qualification for a free diabetes screening and enrollment in an exercise intervention study. Inquiring subjects underwent eligibility determination via telephone screening questionnaire. Subjects reported as sedentary, BMI-for-age $\geq 95^{th}$ percentile, nonsmoker, no known history of illness documented to affect metabolism and/or preclude exercise, neither pregnant nor lactating, not taking medications known to affect metabolism, and no known history of diabetes were invited to Screening Visit #1 for further eligibility determination and the consent process.

#### 2.1.2    Screening Visit #1 (ScV#1)

During the consent process, protocol design, potential risks and benefits, compensation and participation alternatives were explained to subjects and their legal guarantor. All participating subjects and their guarantor gave written consent and assent as approved by CNMC and the National Institute of Health (NIH) Institutional Review Boards (IRBs). Height and weight were measured on a calibrated stadiometer and digital scale in the CNMC General Clinical Research Center (GCRC). Subjects completed self-

administered medical history and physical activity questionnaires. A validated *Modifiable Activity Questionnaire for Adolescents* was used to assess past-year physical activity at baseline in order to exclude physically active individuals (ACSM, 1997). Subjects meeting the aforementioned inclusion criteria from ScV#1 were deemed eligible for ScV#2.

### 2.1.3   Screening Visit #2 (ScV#2)

Oral glucose tolerance testing (OGTT) was performed to screen for insulin resistance and undiagnosed type 2 diabetes. Subjects arrived at the CNMC GCRC after a 12-hour overnight fast for baseline blood withdrawal. Within five minutes of venipuncture, 1.75g/kg of glucola to a maximum of 75g was administered orally and repeat venipuncture was obtained 2 hours later. Urine pregnancy testing was administered on all female subjects as means of study exclusion. All subjects received a physical examination for further eligibility determination. One-hundred-and-fifty-two Black and Latino adolescents underwent OGTT testing and 21% (n=32) met inclusion criteria for insulin resistance as defined as having any of one of the following: fasting insulin > 17 µU/mL, 100mg/dL < fasting plasma glucose < 126 mg/dL, and/or 140 mg/dL < 2-hour OGTT plasma glucose < 200 mg/dL. No subjects were excluded based on contradictory medical findings (illnesses know to affect metabolism or preclude aerobic training) upon physical examination.

### 2.1.4   Dietary Monitoring Period

Eligible subjects were given 3-day food record instructional guidelines in order to complete two 3-day food records each week over a two-week dietary monitoring period to establish baseline dietary intake. Intake was subsequently monitored by three weekly (2 weekday and 1 weekend-day) 24-hr dietary recalls each week for the duration of the study to assess

any substantive changes in dietary intake patterns. Subjects were encouraged to maintain current intake to control for dietary influences on outcome variables.

### 2.1.5 Study Visit #1 (SV#1)

Body composition assessment was performed using a DEXA Hologic QDR 4500A (Hologic, Bedford, MA). Exercise testing was performed using a modified Balke treadmill protocol (Rowland, 1996). Peak oxygen consumption ($VO_{2peak}$) was quantified through use of a Medgraphics Ultima Metabolic Cart (Medical Graphics Corp., St. Paul, MN). The criteria for reaching $VO_{2peak}$ included volitional exhaustion and at least one of the following: 1) plateau or decrease in $VO_2$ with increased workload; 2) RER >0.99; 3) a heart rate of $\geq$195 bpm (Rowland, 1996; Armstrong et al., 1994). Subjects were enrolled in the study if they met the aforementioned criteria and were free of cardiac pathology, as defined by: 1) ST elevation or depression >2mm during rest, exercise or recovery; 2) arrhythmias contradicting aerobic exercise; 3) ventricular tachycardia; 4) signs of poor perfusion; 5) history or presentation of angina pectoris; 6) hypertensive response to exercise (systolic blood pressure >250 mmHg and/or a diastolic blood pressure of >115 mmHg); 7) a drop in systolic or diastolic blood pressure with increasing work load (>10 mmHg); and/or 8) Stage II Clinical Hypertension (ACSM, 2000).

### 2.1.6 Study Visit #2 (SV#2)

Subjects were admitted to the Georgetown University Medical Center (GUMC) GCRC after a 12-hour overnight fast, between the hours of 7 and 8 a.m. for FSIVGTT administration. Weight and height were measured upon arrival for glucose and insulin dosing. All female subjects were in the follicular stage of menstruation to control for the influence of endocrine fluctuations on insulin sensitivity. An intravenous catheter was

inserted in the brachial or radial artery for insulin and glucose administration. All blood samples were drawn from a catheter placed in the brachial or radial artery of the contralateral arm to prevent contamination at the injection site. After a 30-minute equilibration period, blood pressure, heart rate, glucose levels (by glucometer), and body temperature were monitored at 10-minute intervals. Fifty-percent glucose (300 mg glucose/kg as 50% dextrose) was administered over a 2-minute period; the initiation of glucose infusion was set as time zero. Blood samples for glucose and insulin measurement were collected at time points: −10, -1, 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 14, 16, 20, 22, 24, 25, 27, 30, 40, 50, 60, 70, 80, 90, 100, 120, 160 and 180 minutes. Elapsed time was monitored by the study coordinator and a GUMC nurse to decrease testing errors. Humulin insulin (0.05 U/kg) was injected at 20-minutes to augment the insulin response. Samples were immediately stored on ice and centrifuged at 4°C within 2 hours of being drawn for plasma isolation; all samples were subsequently stored at -70°C until assay. During this visit, fasting blood samples were also obtained for the measurement of CRP and fibrinolytic markers.

### 2.1.7   Exercise Training Period

Our study was designed and powered from the low-volume/moderate-intensity STRRIDE study cohort (Houmard et al., 2004; Kraus et al., 2002). In the STRRIDE study, sedentary, overweight/obese individuals displaying characteristics of the metabolic syndrome (n=154) were randomly assigned to one of four exercise training groups: 1) low-volume/moderate-intensity (~12 miles walking/wk at 40-55% $VO_{2peak}$); 2) low-volume/high-intensity (~12 miles jogging/wk at 65-80% $VO_{2peak}$); 3) high-volume/high-intensity (~20 miles jogging/wk at 65-80% $VO_{2peak}$); and 4) non-exercise control (Houmard

et al., 2004; Kraus et al., 2001). Dosages were achieved by training ~115 min/wk (low-volume/high-intensity) or ~170 min/week (low-volume/moderate-intensity and high-volume/high-intensity) with training frequencies of 3 to 4 sessions/week. Similar improvements in $S_I$, ~85%, were observed in both the low-volume/moderate-intensity and high-volume/high-intensity training groups (Houmard et al., 2004). Due to similar improvements in $S_I$ in the low-volume/moderate-intensity and high-volume/high-intensity training groups and a greater adherence and retention rate in the low-volume/moderate-intensity training group, we modeled our pediatric intervention study after the low-volume/moderate-intensity STRRIDE cohort.

Training intensity was calculated as percent peak oxygen reserve (%$VO_{2peak}$ R) as determined by treadmill testing, where $VO_{2peak}$ R=$VO_{2peak}$ − $VO_{2rest}$ (Wilmore, Costill & Kenny, 2008). Exercise prescription by this method is based upon the classic Karvonen method of exercise intensity prescription which uses a percentage of the physiological heart rate range (maximal - resting) or percent of heart rate reserve (%HRR) (Wilmore, Costill & Kenny, 2008). Exercise prescription based on this method accounts better accounts for individual variation at submaximal exercise intensities (Swain & Leutholtz, 1997). Use of this method is supported by a recent study which found that %HRR and %$VO_{2peak}$R are positively correlated with observed HR and $VO_2$ in obese individuals during treadmill exercise, while absolute values of %HR and %$VO_{2peak}$ do not display a linear relationship (Pinet et al., 2008).

The observed heart rate corresponding to %$VO_{2peak}$R of the desired intensity during treadmill testing was used for exercise prescription. All exercise sessions were monitored by trained personnel and through the use of a heart rate monitor (Polar Electro, Inc;

Woodbury, NY). Subjects underwent a one-month pre-training period to adjust to exercise and minimize musculoskeletal injury risk. During the adjustment period, a 5-10% increase in $VO_{2peak}R$ occurred each week for 4 weeks until an intensity of 45-55% $VO_{2peak}$ R was reached. Subjects exercised at target intensity 2-4 days/week for ~180 min/week in order to expend ~1200 kcal/week for eight consecutive weeks. Caloric expenditure was calculated based on the metabolic equivalent (MET) attributed to the intensity at % $VO_{2peak}R$ as measured by indirect calorimetry during baseline exercise testing. Thus, the energy cost was based upon body mass at baseline.

The specified exercise dosage (1200 kcal/week) was chosen based upon recommendations from the Surgeon General's Report (CDC, 1996). Currently, the specified exercise dosage (~1200 kcal/week) and moderate-intensity meet ADA recommendations for exercise training prescription for the *initial* treatment of type 2 diabetes components in previously sedentary adults (Marwick et al., 2009).

Exercise training sessions were monitored by a trained exercise science student and/or exercise physiologist. Exercise modes included cycle ergometer, elliptical trainer, stair stepper and treadmill in order to enhance variety, adherence and retention. Body weight was assessed once weekly in conjunction with a questionnaire designed to assess medical and lifestyle changes warranting study exclusion. The protocol was designed to reduce potential confounding variables by excluding subjects with $\geq$ 5% change in body mass, changes in dietary intake, initiation of exercise training outside of the study, and initiation of medications known to affect metabolism.

### 2.1.8 Study Visit #3 (SV#3)

Upon exercise training completion, female subjects were given a urine pregnancy test no more than 24 hours prior to final FSIVGTT testing as a means of exclusion. FSIVGTT testing, as previously described (2.1.6: SV#2), was administered 24-48 hrs after the last bout of exercise. During this visit, fasting blood samples for CRP and fibrinolytic markers were also collected.

### 2.1.9 Study Visit #4 (SV#4)

Subjects were given a repeat DEXA scan and an exercise stress test to examine changes in body composition and cardiorespiratory fitness following exercise training.

*Figure 1: Exercise Training Protocol Design Overview*
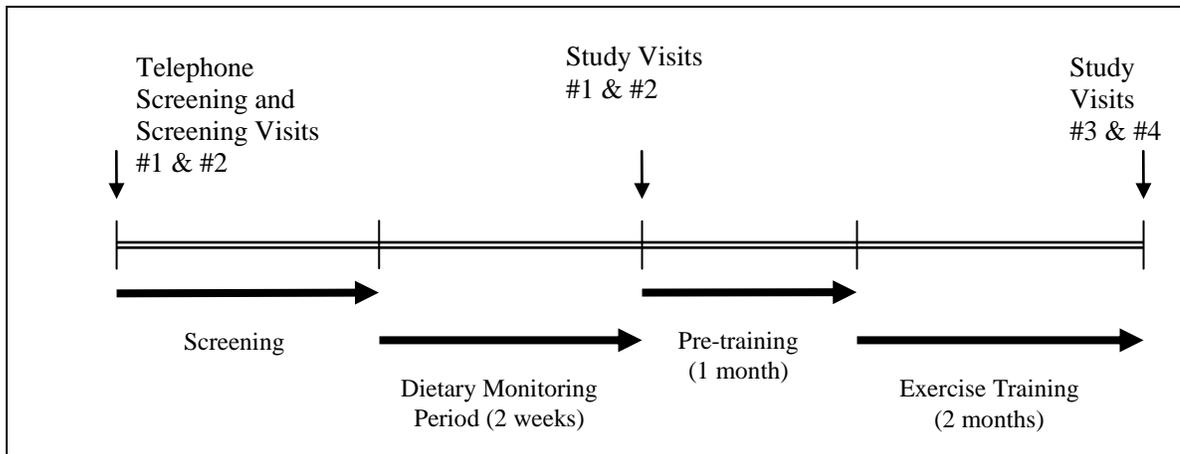


## 2.2 Experimental Methods:

### 2.2.1 Data Collection

Stored serum samples from the previously-described pre-existing pediatric minority study at Children's National Medical Center (CNMC); Principal Investigator: Eric Hoffman; CNMC Protocol #3290, were used for assay. All data were de-indentified prior to assay and analysis in compliance with the regulations of the CNMC and NIH IRBs.

## 2.2.2     Aim 1: Determination of the Effects of Aerobic Exercise Training on Insulin Sensitivity

Despite the clinical utility of OGTT testing, glucose and insulin homeostasis is more accurately measured through frequently-sampled intravenous glucose tolerance testing (FSIVGTT) (Nittala et al., 2006). FSIVGTT enables quantification of the severity of insulin resistance through calculation of the insulin sensitivity index ($S_I$). $S_I$, as calculated by incremental glucose disappearance under the insulin curve, represents steady-state insulin-dependent glucose uptake (Bergman, Beard & Chen, 1986).

### *2.2.2.a*     Aim 1A: Insulin and Glucose Quantification

Samples for insulin quantification were assayed in duplicate by use of a Mercodia insulin enzyme-linked immunoabsorbant assay (ELISA) (Mercodia AB, Uppsala, Sweden). In this procedure, insulin in the aliquoted serum sample binds to a pre-coated insulin antibody plate; subsequent addition of an enzyme-linked antibody binds to another insulin antigen. The addition of a chromogenic substrate binds to the antibody- insulin-antibody complex and the sample concentration of insulin is subsequently quantified by light absorption of bound chromogen through use of microplate reader at an absorbance of 450 nm (Mercodia, 2008). Control samples ranging from 0 to 500 μU/ml of insulin were included in each assay plate to control for inter- and intra-assay testing errors. Duplicate samples with a coefficient of variation ≥15% were reran. The reliability of this assay is high, yielding within and between-assay coefficients of variation of 3.4 and 3.6%, respectively. A recent American Diabetes Association-sponsored study found that 97% of Mercodia insulin assays were within 15.5% of clinically manufactured insulin concentrations, confirming its validity (Miller et al., 2009).

All blood samples for glucose measurement were assayed in duplicate by use of YSI 2300 STAT Plus glucose analyzer (YSI Inc., Yellow Springs, OH) according to the manufacturer's instructions. The reliability of the YSI 2300 is ±2% or 2.5 mg/dL per sample, r=0.999, for glucose concentrations between 0-900 mg/dL (YSI, 2008). Glucose control samples were ran daily and pre-programmed auto-calibration was performed every 15 minutes as required by the Food and Drug Administration to control for assay validity and reliability (FDA, 2009). The YSI 2300 shows high concurrent validity when compared to samples of standardized glucose concentration, r=0.99 (FDA, 2009).

### *2.2.2.b*    **Aim 1B: Insulin Sensitivity Quantification**

Early insulin resistance is mostly commonly characterized by increased insulin production in response to peripheral and hepatic insulin insensitivity; this response maintains blood glucose within or only slightly above normal physiologic ranges. As insulin sensitivity continues to decline, the pancreatic β-cell production of insulin deteriorates. Eventually, the β-cells cannot produce enough insulin to regulate blood glucose levels, resulting in the clinically-defined onset of type 2 diabetes (Bergman, 2007; Shulman, 2000). The overall state of glucose and insulin homeostasis can be quantified through determination of insulin-stimulated glucose uptake, or insulin sensitivity ($S_I$). Here, a reduced $S_I$ indicates decreased insulin sensitivity. Normal $S_I$ values average $2.62 \pm 2.21$ $mU^.L^{-1.}min^{-1}$, values of $1.27 \pm 1.20$ $mU^.L^{-1.}min^{-1}$ are considered impaired glucose tolerance and thus indicate insulin insensitivity, and $S_I$ values of type 2 diabetics average $0.57 \pm 0.82$ $mU^.L^{-1.}min^{-1}$ (Bergman, 2007). Thus, increases in $S_I$ in response to training represent improvements in insulin sensitivity (Bergman, 2007; Bergman, Beard & Chen, 1986).

Insulin sensitivity was determined by FSIVGTT using the minimal model methods of Bergman et al. (1979). $S_I$ was calculated by use of MINMOD Millennium pharmacokinetic modeling software (MinMod, Inc., Pasadena, CA). Pharmacokinetic modeling was performed in the Department of Health of Human Performance at East Carolina University (ECU) by Chuck Tanner and Joseph Houmard, PhD. FSIVGTT analysis was performed as a collaborative effort between the degree candidate (Ms. Many), Dr. Houmard, and Mr. Tanner at ECU.

MINMOD Millennium pharmacokinetic modeling software quantifies $S_I$ through use of a series of pre-programmed differential equations which integrate time-dependent plasma and injected glucose and insulin concentrations over 33 time points. This enables derivation of hepatic and muscular rate constants of insulin-mediated and insulin-independent glucose uptake. Integration of these rate constants quantifies $S_I$ (Nittala et al., 2006). FSIVGTT demonstrates strong predictive validity, where the relative risk of type 2 diabetes is 31.1 between the 90th and 10th $S_I$ percentiles (Lillioja, 1993).

Baseline and final FSIVGTT testing were performed to assess changes in $S_I$ in response to training. Increases in $S_I$ represent improvements in insulin sensitivity (Bergman, 2007). FSIVGTTs producing $S_I$ values with a fractional standard deviation (FSD) > 35% and/or >80% difference between peak insulin levels (at time points 20-22 minutes, after exogenous insulin infusion) between baseline and final testing were excluded from analysis in order to enhance intrasubject validity upon retesting.

### 2.2.2 Aim 1: Determination of the Effects of Aerobic Exercise Training on 'Non-traditional' Metabolic Syndrome Components.

### *2.2.3.a* Aim 2A: Determination of the Effects of Aerobic Exercise Training on Fibrinolytic Potential

Fibrinolytic activity was assessed by use of assays standardized for the clinical diagnosis of fibrinolytic impairments (Devin et al., 2007; Juhan-Vague et al., 1996). Assays for PAI-1 and t-PA activity and antigen levels are commonly used to assess for fibrinolytic impairments. Elevated PAI-1 antigen levels characterize impaired fibrinolysis and are associated with increased stroke and CVD risk (Sakkein et al., 2000; Kohler & Grant, 2000). Reduced t-PA antigen levels also represent fibrinolytic impairments and serve as a clinical tool for assessing heart attack and stroke risk (Kinlay et al., 2009). Overall, fibrinolytic imbalances are characterized by elevated PAI-1 and decreased t-PA antigen and activity levels.

Fasting plasma samples for selected fibrinolytic markers were collected between the hours of 7:30 and 9 a.m. in order to control for diurnal variations in fibrinolytic activity (Stratton et al., 1991). All samples were collected into sodium citrate tubes which protect against the spontaneous inactivation of t-PA and inactive PAI-1 release upon venipuncture. Samples were centrifuged for plasma isolation and subsequently frozen at -70°C to prevent platelet release of PAI-1 (Kulaputana et al., 2005).

*PAI-1 and t-PA Antigen Assays*

t-PA antigen levels were assessed by enzymatic immunoassay (TintElize, Trinity Biotech/Biopool). This t-PA assay is based on the double antibody principal; t-PA in the aliquoted sample binds to precoated anti-tPA antibodies. Subsequent addition of HRP-

labeled Fab fragments bind to the t-PA antigen on the well during an incubation step. Light absorbance is read on a spectrometer at an absorbance of 490 nm to determine sample concentration by the least-squares standard curve method. The intra-assay variation for this assay for t-PA standards of 6 and 15 ng/mL has been documented to be 5.5 and 4.9%, respectively (Trinity Biotech I, 2009). The inter-assay variation for t-PA standards of 6 and 15 ng/mL has been documented to be 3.5 and 5.4 %, respectively (Trinity Biotech I, 2009). The linear detection limits are between 0-30 ng/mL t-PA for undiluted samples.

Total PAI-1 antigen was quantified by use of enzymatic immunoassay (Tintelize, Trinity Biotech/Biopool); this method quantifies active, inactive and complexed forms of circulating PAI-1 by using a double antibody principal similar to that of the aforesaid t-PA assay. Additionally, an adjacent well containing soluble PAI-1 conjugate antibodies served to rule out non-specific plate binding and, thus, protect against falsely elevated readings. The intra-assay variation for this assay for PAI-1 standards of 20 and 40 ng/mL has been documented to be 1.9 and 2.9%, respectively (Trinity Biotech II, 2009). The inter-assay variation for PAI-1 standards of 20 and 40 ng/mL has been documented to be 2.4 and 3.3%, respectively (Trinity Biotech II, 2009). The lower detection limit of PAI-1 is 0.5 ng/mL.

Quantification of the molar ratio of PAI-1 to t-PA serves as an indicator of fibrinolytic balance; lower levels represent an increased capacity for fibrinolysis (Balagopal et al., 2008). The molar ratio of PAI-1 to t-PA was calculated by dividing plasma concentrations of each (ng/mL) by their respective molar weights (70,000 g/mol for t-PA, 50,000 g/mol for PAI-1) (Balagopal et al., 2008).

### *2.2.3.b* **Aim 2B: Determination of the Effects of Aerobic Exercise Training on CRP Levels**

All samples for quantitative CRP assay were processed at Quest Diagnostics in Chantilly, VA. Samples were analyzed by use of CardioPhase hsCRP reactivity kit (Dade Behring, Newark, DE, USA). All samples were centrifuged and aliquoted prior to processing to prevent sample lipemia from interfering with CRP detection. In this assay, polystyrene-bound monoclonal anti-CRP antibodies bind specifically to human CRP in the aliquoted plasma sample. The concentration of CRP is proportional to the intensity of scattered light as detected by immunonephelometry on a BN Nephelometer (Dade Behring, Newark, DE, USA). Serum controls were included in each assay to assure validity. The assay exhibits no cross reactivity; sensitivity of this assay is 0.175 mg/L. The intra-assay variation produced by this assay for samples within 0.5-15 mg/L has been documented to be 3.1-4.0%, respectively. The inter-assay variation for samples within 0.5-14 mg/L has been documented to be 2.5-2.6%, respectively (Sander et al., 2007).

Unlike many circulating cytokines, including its primary inducers, IL-6 and TNF-$\alpha$, CRP exhibits little diurinal variation. Due to its increased stability, a single determination of CRP is thought to provide an accurate assessment of CVD risk provided recorded CRP levels are <10 mg/L (Ridker, 2003). Measurement of CRP in large adult populations typically results in a rightward skewed distribution curve which is typically normalized upon logarithmic transformation (Rifai, Tracy & Ridker, 1999). CRP levels were thus log transformed prior to analysis; due to our small sample size, we were unable to validly assess statistical normality. Subjects presenting with CRP levels >10mg/L were excluded from analysis as these CRP values indicate underlying acute inflammation. Risk category

classifications were based upon the CDC/AHA standards, as follows: 1) low risk, CRP levels <1 mg/L; 2) moderate/average risk, CRP 1-3 mg/L; 3) high risk, >3 CRP <10 mg/L (AHA I, 2009).

### 2.2.4    Statistics

The chosen exercise training program was modeled after the low-volume/moderate-intensity STRRIDE cohort which utilized a training intensity of 40-55% $VO_{2peak}$, a duration of ~170 min/week, and a frequency of 3-4 days/week (Houmard et al., 2004; Kraus et al., 2001).Our *a priori* power calculations determined the sample size required to detect significant changes in the initial primary protocol aim: insulin sensitivity. A sample size of n=11 was determined ($\alpha$=0.05, $\beta$=0.80) based upon an observed ~85% improvement in $S_I$ in the low-volume/moderate-intensity STRRIDE cohort.

Fibrin plate assays serve as an indirect indicator of total fibrinolytic capacity. An improvement in total fibrinolysis area, as indicated by sample euglobin clot lysis area on a fibrin plate, suggests underlying improvements in fibrinolytic activity (Pandolfi et al., 2000). An increase in total clot lysis area suggests improvements in fibrinolytic activity. Based on an observed two-fold improvement in total fibrin lysis area, as determined by fibrin plate assay, of subjects enrolled in the STRRIDE study, the required sample size (n=11) determined to detect significant changes in $S_I$ additionally provides sufficient power, $\beta$=0.80, to detect an increase in fibrinolytic activity, using a significance level $\alpha$=0.05. Here, the anticipated effect size is large, Cohen's d=2.84 (Hittel, Kraus & Hoffman, 2003).

Previous findings from Stewart et al. demonstrate significant reductions in CRP levels after 12-weeks of aerobic training in physically inactive subjects aged $25 \pm 5$ years (2007). Based upon these results, a sample size of 20 yields sufficient power ($\beta=0.80$) to detect a significant CRP response to training, yielding a Cohen's d of 0.74. Despite our under-powered sample size (n=11), we intend to investigate changes in CRP due to a large anticipated effect size. Our reasoning behind expecting a greater effect size includes: 1) the staff-monitored and intensity-specific nature of this protocol will minimize modality-specific variables confounding detectable changes in CRP; 2) the high BMI and minority status of enrolled subjects will result in high baseline CRP levels; and 3) a larger expected decrease in CRP levels with training is anticipated as CRP has been shown to decrease when initially elevated (Stewart et al., 2007).

Data analyses were performed by use of SAS software (SAS Inst., Cary, NC). Comparison of baseline and final measurements was made using paired t-tests; statistical significance was defined at $p<0.05$.

## Chapter 3: Results

*Subjects*

Of the 32 subjects who initially qualified for study participation, 25 subjects completed all baseline testing visits, 15 subjects completed the entire study, and 4 of these 15 subjects were excluded from data analysis. Three subjects were excluded from analysis due to abnormal dietary intake patterns and/or $\geq$5% increase in body mass, and one subject was excluded due to the FSIVGTT being administered >48 hours after the last bout of exercise. Of the 11 subjects eligible for analysis, 2 were male (1 Black/1 Latino) and 9 were female (7 Black/2 Latina). All of these subjects were post-pubertal (Tanner growth stage >IV); the average age at enrollment was $15.09 \pm 0.31$ years. The exercise adherence rate was high at 87%; no subjects missed more than 3 exercise sessions. No significant changes in $VO_{2peak}$ were observed in response to training (Table 2).

*Anthropometrics*

Changes in anthropometric variables in response to training are presented in Table 2. Subjects presented with morbid obesity at baseline as defined as a BMI $\geq$40 kg/m$^2$ (Morgan et al., 2010). In response to the training program, significant reductions in body mass (~2 kg) and BMI (~0.8 kg/m$^2$) were observed. Favorable, yet minor, changes in body composition including a ~3.5% reduction in both percent total body fat and percent truncal body fat ($p<0.05$), and a ~2.0% increase in lean body mass ($p<0.05$) were observed in response to training.

**Table 2:** Changes in Selected Variables in Response to Training

| Phenotype | n | Baseline Mean ± SEM | Final Mean ± SEM | P-value |
|---|---|---|---|---|
| *Body Mass and Composition* | | | | |
| BMI (kg/m$^2$) | 11 | 41.4 ± 1.83 | 40.6 ± 1.81* | 0.008* |
| Body mass (kg) | 11 | 116.2 ± 7.56 | 114.2 ± 7.47* | 0.002* |
| Lean body mass (kg) | 10 | 61.7 ± 4.05 | 62.9 ± 4.17* | 0.032* |
| Total Body Fat (%) | 10 | 43.4 ± 1.99 | 41.9 ± 1.85* | <0.001* |
| Truncal Fat (%) | 10 | 43.3 ± 2.01 | 41.7 ± 2.15* | 0.043* |
| *Cardiorespiratory Fitness* | | | | |
| VO$_{2peak}$ (mL/kg/min) | 10 | 22.7 ± 1.48 | 23.3 ± 1.84 | 0.437 |
| VO$_{2peak}$ (L/min) | 10 | 2.87 ± 0.14 | 2.62 ± 0.21 | 0.127 |
| RER $_{peak}$ | 10 | 1.13 ± 0.04 | 1.01 ± 0.02 | 0.007* |
| HR $_{peak}$ (bpm) | 9 | 187 ± 4 | 178 ± 4 | 0.032* |
| METs $_{peak}$ (kcal/kg/hr) | 10 | 6.48 ± 0.42 | 6.67 ± 0.52 | 0.435 |
| *Selected Metabolic Syndrome Markers* | | | | |
| S$_I$ (mU·L$^{-1}$·min$^{-1}$) | 7 | 1.00 ± 0.15 | 1.37± 0.26* | 0.026* |
| Fasting glucose (mg/dL) | 11 | 85.6 ± 4.46 | 82.1 ± 3.98 | 0.161 |
| Fasting insulin (µU/mL) | 11 | 20.6 ± 2.86 | 20.2 ± 2.80 | 0.873 |
| CRP (mg/L) | 10 | 2.53 ± 0.86 | 2.09 ± 0.54 | 0.303 |
| t-PA (ng/mL) | 10 | 6.34 ± 0.51 | 7.32 ± 0.85 | 0.035* |
| PAI-1 (ng/mL) | 10 | 25.75 ± 4.46 | 22.79 ± 3.02 | 0.472 |
| Molar ratio of PAI-1 to t-PA | 10 | 5.47 ± 0.79 | 4.46 ± 0.52 | 0.261 |

Data are presented as means ± SEM; **\***p<0.05 between baseline and final paired comparisons. *One subject was unable to complete final DEXA and treadmill testing and was thus excluded from body composition and 'Cardiorespiratory Fitness' analysis. Four subjects were excluded from S$_I$ analysis due to an inability to maintain venous access during FSIVGTT testing and/or FSIVGTT data not meeting inclusion criteria (Methods Section 2.2.2.b); all of the subjects remaining eligible for FSIVGTT analysis were female. One subject was excluded from CRP analysis due to baseline CRP levels >10 mg/L; another was excluded from t-PA and PAI-1 analysis due to severely hemolyzed samples at baseline.*

*Insulin Sensitivity*

A ~37% improvement in S$_I$ was observed in response to training (Table 2). At

baseline, subjects presented with normal fasting glucose as defined as fasting glucose

<100 mg/dL, and elevated fasting insulin as defined as fasting insulin >15 µU/mL

(Steinberger et al., 2009; Freemark & Bursey, 2001). Fasting insulin and glucose levels did not significantly change in response to training.

*C-Reactive Protein*

On average, subjects presented with CRP levels in the moderate risk category at baseline, defined as values between 1-3 mg/L by the CDC/AHA (AHA I, 2009). Three subjects presented with CRP levels in the CDC/AHA high risk category (>3 CRP <10 mg/L) at baseline. CRP levels did not change significantly in response to training (Table 2).

*Fibrinolysis*

In response to training a ~15% increase in t-PA antigen was observed. PAI-1 antigen levels did not significantly change in response to training. A non-significant ~18% decrease in the molar ratio of PAI-1 to t-PA was observed (Table 2).

**Chapter 4: Discussion**

Pediatric obesity is associated with a multitude of negative health outcomes including type 2 diabetes, early-onset atherosclerosis, non-alcoholic fatty liver syndrome, sleep apnea, and polycystic ovarian syndrome (Ginsberg & MacCallum, 2009; Daniels et al., 2009). The rates of childhood obesity have nearly tripled in the past 30 years (Institute of Medicine of the National Academies, 2006). The pediatric obesity epidemic has caused diseases like type 2 diabetes, historically presenting in adulthood, to become increasingly diagnosed in youth (Kaufman, 2002). The epidemiological and economic impacts of pediatric obesity are projected to increase in magnitude as obese youth become obese adults, requiring early life-long treatment for obesity-associated comorbidities (Lee, 2008; Levine & Stein, 2008). Some estimates project that the pediatric obesity epidemic will lead to the first reduction in lifespan demonstrated in recent years (Olshansky et al., 2005). It is thus necessary to study the intensity-specific effects of aerobic training in obese pediatric groups in order to develop treatment guidelines as no current exercise treatment guidelines exist specifically for this high-risk population (Daniels et al., 2009; Marwick et al., 2009; Matyka et al., 2008).

Pediatric minority groups display higher rates of obesity and associated comorbidities. Pediatric minorities additionally display higher rates of morbid obesity (Wang et al., 2010). Morbid obesity in youth requires aggressive treatment strategies as it is associated with a heightened risk of CVD, type 2 diabetes and early mortality (Wang et al., 2010; Lender et al., 2009). Thus, there is a critical need for studying the effects of intensity-specific aerobic exercise training in morbidly obese and insulin-resistant youth in order to develop exercise prescription recommendations, as continued weight gain and

worsening of insulin sensitivity warrants more aggressive treatment methods such as pharmacotherapy and weight loss surgery (Lenders et al., 2009; Nadler et al., 2009; Daniels et al., 2009; Marwick et al., 2009; Matyka et al., 2008).

### 4.1    Aim 1: The Effects of Aerobic Exercise Training on Insulin Sensitivity

Our research findings indicate that moderate-intensity aerobic training (45-55% $VO_{2peak}R$) performed ~180 min/week improves insulin sensitivity in morbidly obese adolescent minority females. These findings agree with those of previously-conducted training studies in pediatric populations which demonstrate improvements in insulin sensitivity measures (van der Heijden et al., 2009; Balagopal et al., 2008; Conwell et al., 2008; Kim et al., 2007; Bell et al., 2007; Nassis et al., 2005). Minority groups display higher rates of insulin resistance, morbid obesity and different insulin dynamics relative to their White peers (Casazza et al., 2009; CDC, 2009; Narayan et al., 2003). Our findings add to this literature as this is the first study conducted in a morbidly obese minority cohort. Exercise training has been demonstrated to improve surrogate measures of insulin sensitivity (Table 1) (Balagopal et al., 2008; Nassis et al., 2005; Kim et al., 2008; and Ferguson et al., 1999 I). However, investigating the effects of aerobic exercise training on direct measurements of insulin sensitivity is of interest as surrogate determinants do not accurately assess insulin resistance in youth populations (Brandou, Brun & Mercier, 2005). Our findings add to the current literature as all previous studies conducted in pediatric populations have utilized lifestyle-based training protocols (involving a combination of discontinuous aerobic and resistance exercise training) and/or home-based training sessions which do not reliably control for training intensity

(van der Heijden et al., 2009; Balagopal et al., 2008; Conwell et al., 2008; Kim et al., 2007; Bell et al., 2007; Nassis et al., 2005; Ferguson et al., 1999 I; Gutin et al., 1996).

In minority populations, Gutin et al. did not observe significant changes in fasting insulin in 12 obese Black girls (age 7-11 years) after 5 weeks of mixed-modality aerobic training, despite a ~1.4% reduction in total body fat as determined by DEXA (1996). Our findings may be different than those of Gutin et al. in that training intensity was not standardized and heart rate was monitored only in 2 of 5 weekly training sessions. Additionally, Tanner growth stage was not standardized and the girls were younger than subjects enrolled in the present study (average age ~9 versus ~15 years). These factors could have confounding effects on findings from this study as insulin sensitivity tends to stabilize in late puberty and surrogate determinants of insulin sensitivity show limited validity in youth during puberty (Casazza et al., 2009; Kelly et al., 2007; Goran & Gower, 2001; Brandou et al., 2005). A recent study by van der Heijden et al. demonstrated a $\sim 59 \pm 19\%$ improvement in peripheral insulin sensitivity as determined by SLFSIVGTT in obese post-pubertal Latino adolescents (age $15.6 \pm 0.4$ years) in response to 12 weeks of 120 min/week (4 x 30 min/week) of high intensity (~85% $VO_2$ $_{Peak}$) aerobic exercise training (2009). A greater observed improvement in insulin sensitivity by van der Heijden et al. may be due to different FSIVGTT administration methods. SLFSIVGTT enables accurate quantification of both peripheral and hepatic insulin sensitivity; $S_I$ measurements produced by this method are typically higher and more precise than standard FSIVGTT testing (Avogaro et al., 1989). Thus, $S_I$ analysis by this method in the van der Heijden et al. study may have resulted in a greater observed change in insulin sensitivity as the skeletal muscle (periphery) is responsible for the

majority of insulin-stimulated glucose uptake and training results in insulin-sensitizing adaptations in the skeletal muscle (Kelley, 2005; Hawley & Lessard, 2008). Subjects enrolled in this study were put on a 7-day low carbohydrate diet prior to SLFSIVGTT testing which may have resulted in greater observed improvements in insulin sensitivity as low carbohydrate diets prior to IVGTT testing have been shown to differentially effect observed changes in insulin sensitivity in response to exercise training (Cartee et al., 1989). Our findings also extend those of van der Heijden et al. to a morbidly obese cohort that includes both Black and Latina adolescents. Our findings of a ~37% improvement in insulin sensitivity in response to moderate-intensity aerobic training (~55% $VO_{2\,peak}$) are of interest as the attainability of such high-intensity training programs may not be feasible in previously-sedentary and obese pediatric populations (Daniels et al., 2009).

No significant changes in fasting insulin or fasting glucose were observed in response to the intervention. This is in contrast to findings from Ferguson et al. who demonstrated decrements in fasting insulin, but not glucose in Black, White and Asian children (age 7-11 years) in response to 4 months of aerobic game-based training (1999). Our subjects presented with hyperinsulemia at baseline (fasting insulin $20.6 \pm 2.86$ µU/mL) and very low insulin sensitivity indices ($S_I\,1.00 \pm 0.15$ mU$^.$L$^{-1.}$min$^{-1}$). Reduced fasting insulin in response to training signifies enhanced insulin sensitivity as less insulin is required to maintain euglycemia. Despite improvements in insulin sensitivity, our subjects did not normalize $S_I$ in response to training which may contribute to the preservation of hyperinsulemic states. Aerobic training has been documented to improve insulin sensitivity through a variety of intracellular mechanisms such as increased insulin receptor activation, increased expression and activation of phosphorylation cascades

downstream of the insulin receptor, and increased GLUT4 transcription and translocation (Short et al., 2003; Zierath, 2002; Shulman, 2000; Cox et al., 1999). The insulin-sensitizing benefits of aerobic training on the skeletal muscle may be best assessed through quantification of insulin sensitivity rather than fasting insulin levels as changes in fasting insulin levels in response to training have been demonstrated to underestimate changes in insulin sensitivity (Schmitz et al., 2002; Short et al., 2003). Additionally, improvements in fasting insulin levels in response to training have been shown to correlate to reductions in total body fat (Short et al., 2003). Subjects enrolled in this study presented with extreme adiposity; despite an observed ~3.5% reduction in total body fat, this degree of adiposity may have additionally contributed to hyperinsulemic states after the intervention.

**4.2 Aim 2A: The Effects of Aerobic Exercise Training on Fibrinolytic Potential**

The effects of aerobic training on fibrinolysis have not been studied extensively in pediatric populations. Of the few studies conducted, most are limited to lifestyle-based, rather than intensity-specific training programs (Conwell et al., 2008; Balagopal et al., 2008; and Ferguson et al., 1999 II). Studies in adult populations have demonstrated improvements in fibrinolytic activity and potential in response to training, though there is substantial heterogeneity and even conflicting results in the literature (Jahangard et al., 2009; Van Guilder et al., 2005; Kulaputana et al., 2005; Hittel, Kraus & Hoffman, 2003; van den Burg et al., 1997; Chandler et al., 1996; Stratton et al., 1991). Different types of training modalities in addition to a variety of confounding variables, such as age and the

hormonal and health status of enrolled subjects, may contribute to such discrepancies in the literature (Kulaputana et al., 2005).

Prior to the present study, the effects of aerobic training on fibrinolytic potential had not been characterized in a morbidly obese or exclusively minority pediatric population. Our findings demonstrate that low-volume/moderate-intensity aerobic training induces favorable, albeit minor, changes in fibrinolytic potential, as indicated by elevated t-PA antigen levels in response to training. This is in contrast to findings from Balagopal et al. who observed no significant changes in t-PA antigen levels in response to a 12-week lifestyle-based training program in obese adolescents (2008). The training protocol of Balagopal et al. primarily consisted of home-based exercise sessions and did not control for exercise intensity. The effects of aerobic training on fibrinolysis are largely intensity-specific (Wang, 2006). Thus, discrepant findings among studies may be attributable to differences in training protocols.

t-PA is a critical regulator of fibrin clot degradation (Van Guilder et al., 2005). Decreased t-PA antigen levels have been associated with an increased risk of recurrent myocardial infarction (Gram et al., 1987). Obesity is associated with a variety of fibrinolytic impairments that include diminished endothelial t-PA release, which results in elevated thrombotic potential (Van Guilder et al., 2005). In studies in adults, aerobic training has been documented to increase t-PA antigen levels, enhance endothelial t-PA release, and increase skeletal muscle t-PA mRNA and protein expression (Jahangard et al., 2009; Van Guilder et al., 2005; Hittel, Kraus & Hoffman, 2003). These changes in t-PA regulation appear to occur in conjunction with other improvements in fibrinolysis including decreases in PAI-1 activity and antigen levels and increases in t-PA activity.

The effect of aerobic training on t-PA antigen levels appears to differ between genders. Training studies in men exhibiting improvements in fibrinolytic activity (enhanced t-PA and diminished PAI-1 activity) often report decrements in t-PA antigen levels in response to training, while women have been shown to increase t-PA antigen levels in response to training (Jahangard et al., 2009; Chandler et al., 1996). Differences in t-PA antigen levels between genders are also observed with diurnal variations in fibrinolytic activity, where peak t-PA antigen levels correlate with peak t-PA activity levels in women, but not in men (Chandler et al., 1996). Kulaputana et al. observed slight (~0.5 ng/mL) decrements in t-PA antigen levels in women in response to training which correlated with positive changes in t-PA and PAI-1 activity (2005). In the Kulaputana et al. study, decrements in t-PA antigen and PAI-1 activity were significantly greater in men versus women despite identical training protocols and similar decrements in t-PA activity. Despite heterogeneous findings among studies, together these data suggest that the mechanisms regulating fibrinolytic balance may differ between genders. Eighty-percent of participants in our study were female which may explain the observed increase in t-PA antigen levels in response to training. Future studies investigating gender-stratified training responses in fibrinolysis in obese pediatric populations are thus warranted.

In contrast to our original hypothesis, no significant changes in PAI-1 antigen levels were observed in response to the training program. These findings agree with those of Conwell et al., Balagopal et al., and Ferguson et al. who found no significant changes in PAI-1 antigen levels in obese youth in response to lifestyle-based aerobic training programs (2008; 2008; 1999 II). PAI-1 antigen and activity levels have been documented

to decrease in response to aerobic training in adult populations (Jahangard et al., 2009; Kulaputana et al., 2005; Hittel, Kraus & Hoffman, 2003; van den Burg et al., 1997; Stratton et al., 1991). Stratton et al., demonstrated improvements in fibrinolytic activity and potential in older (60-82 years), but not younger (24-30 years) individuals in response to a high-intensity aerobic training program. These findings suggest that aerobic training may regulate PAI-1 expression and activity differently among age groups.

Additionally, discrepant findings between training studies in adult and youth populations may be due to differences in fasting insulin levels at baseline. Hyperinsulemia and hyperglycemia have been demonstrated to increase PAI-1 expression levels (Pandolfi et al., 2000; Morange et al., 1999; McGill et al., 1994). Though the present training program induced improvements in insulin sensitivity, subjects remained hyperinsulinemic after the intervention. The presence of hyperinsulemia among our cohort may explain differences from that of Hittel et al. and others who observed decrements in PAI-1 levels among subjects with normal insulinemia at baseline and in response to the intervention (2003). Additionally, obesity-associated inflammation is thought to increase PAI-1 expression levels. Our subjects presented with moderate CRP levels at baseline which did not significantly change in response to training. Retention of hyperinsulinemic and inflammatory status may have contributed to the observation of no significant changes in PAI-1antigen levels in response to training.

### 4.3 Aim 2B: The Effects of Aerobic Exercise Training on CRP Levels

No significant changes in CRP were observed in response to training. In pediatric populations, aerobic training has been documented to reduce CRP levels in response to programs using combined dietary and home-based physical activity intervention methods

(Balagopal et al., 2005). Other studies have reported no significant changes in CRP in response to training (van der Heijden et al., 2009; Kim et al., 2007; Nassis et al., 2005). None of these previously-conducted pediatric exercise training studies have investigated the effects of aerobic exercise training on CRP levels in morbidly obese minority youth populations. Nassis et al. did not observe significant changes in CRP levels in overweight and obese girls in response to a 12-week game-based aerobic intervention, despite favorable changes in insulin sensitivity as defined by total glucose and insulin area under the curve (2005). Kim et al. did not observe significant changes in CRP in obese Korean adolescent males in response to a six-week jump rope interval training program (2007). In contrast to these findings, Balagopal et al. demonstrated a ~30% decrement in CRP levels in response to a 12-week lifestyle and dietary intervention program (2005). Differences between studies may be due to lack of congruity between training modalities. Studies by Nassis et al. and Kim et al. investigated the effects of lifestyle-based and interval-type training protocols, respectively, and did not control for training intensity, all of which may limit comparisons between studies (2007; 2005). van der Heijden et al. did not observe significant changes in CRP levels in response to 12 weeks of high-intensity aerobic exercise training in obese adolescents (BMI $33.2 \pm 0.9 \text{kg/m}^2$, age $15.6 \pm 0.4$ years), despite improvements in peripheral and hepatic insulin sensitivity as determined by SLFSIVGTT (2009). Additionally, our findings and others may differ from those of Balagopal et al. as this study included a dietary intervention component; CRP levels are positively correlated to dietary fat intake, thus findings from Balagopal et al., may be due to the combined effects of diet and exercise on CRP levels (Kalogeropoulos et al., 2010).

The effects of aerobic training on CRP and other inflammatory markers are similarly conflicting in adult intervention studies (Church et al., 2009; Huffman et al., 2008; Obisesan et al., 2006; Kadoglou et al., 2006). Several factors may hinder causal inferences made in human training studies. Despite the use of CRP levels >10 mg/L as means of exclusion based upon the assumption of underlying acute inflammation, numerous factors may affect the state of acute and chronic systemic inflammation. Despite the predictive utility of CRP-based CVD risk assessment, it is an acute phase protein. Thus, its levels may be affected by a variety of potential confounding variables including tobacco and environmental smoke exposure, recent dietary intake variables, sex hormones, sleep duration, and exposure to viruses, bacteria and environmental toxins (Kalogeropoulos et al., 2010; Patel et al., 2009; Wander et al., 2008; Kuo et al., 2007).

CRP exhibits moderate with-in subject reliability (r=0.59). Thus, large-scale intervention studies quantifying changes in multiple inflammatory cytokines may better serve to investigate the effects of aerobic training on metabolic syndrome-associated systemic inflammation (Danesh et al., 2004).

### 4.4  Study Limitations

The major limitation of this is study is lack of a sedentary control group. A sedentary control group was not recruited as the risks associated with FSIVGTT testing were thought to outweigh participation benefits and deemed unethical by the CNMC and NICHD IRBs. Recruitment of a more homogenous population (i.e. stratifying for race and gender) may provide additional insight into gender and ethnicity-stratified training responses among morbidly obese pediatric minorities.

Findings of non-significant changes in peak oxygen consumption may be an additional limitation of the current study. However, the findings of significant reductions in RER and HR, combined with findings of no significant changes in metabolic equivalent units (METs) at $VO_{2peak}$, suggest that 'peak' exertion was lower upon retesting as subjects were able to exercise at nearly equivalent METs at a lower HR and RER. These findings suggest that changes in $VO_{2peak}$ cannot be validly assessed as subjects level of exertion was lower upon retesting.

Many children do not give maximal exertion during peak treadmill testing and, thus, do not meet $VO_{2peak}$ testing criteria (Kang et al., 2002; Ferguson et al., 1999 I). Obese youth experience a high degree of peripheral fatigue during exercise, which may lead to premature feelings of exhaustion (Daniels et al., 2009). In unfit youth populations, peak oxygen consumption is usually elicited at the maximal workload obtained and does not typically plateau or decrease with increasing workload (Kang et al., 2002). This suggests that '$VO_{2peak}$' testing in unfit youth is a 'test of maximal effort' rather than maximal exercise capacity (Kang et al., 2002). However, some have suggested that $VO_{2peak}$ treadmill testing protocols are feasible, valid and accurate in obese youth populations, though the reliability of such testing protocols has not been established in morbidly obese subjects (Loftin et al., 2004; Owens & Gutin, 1999). Loftin et al. suggest that cycle ergometry, rather than treadmill testing, may be the optimal testing protocol for severely overweight youth as treadmill testing may lead to more subjective feelings of fatigue than cycling protocols due to greater biomechanical loading forces elicited during locomotion (2004). There is, thus, a need to optimize cardiorespiratory testing protocols in morbidly obese pediatric populations as the test-retest reliability of graded exercise

testing protocols has not been established for morbidly obese and previously-sedentary youth. Thus, the chosen criteria for $VO_{2peak}$ testing (volitional exhaustion and at least one of the following criteria: 1) plateau or decrease in $VO_2$ with increased workload; 2) RER >0.99; 3) a heart rate of $\geq$195 bpm) may have not been appropriate for our morbidly obese subject population. Treadmill testing protocols assessing $VO_2$ or heart rate during a standardized submaximal workload may better serve to assess changes in cardiorespiratory fitness in morbidly obese youth as such testing is not contingent upon subjective feelings of exhaustion at 'peak' workloads (Ferguson et al., 1999 I).

### 4.5 Study Conclusions

Examination of the effects of intensity-controlled aerobic training on obesity-associated comorbidities is necessary to develop exercise recommendations for obese youth populations (Daniels et al., 2009). Inclusion of minorities in such studies is especially critical as the prevalence of physical inactivity, obesity and related pathologies are highest in minority groups (CDC, 2009). When developing exercise prescription guidelines for obese youth populations, training programs must be started by gradually increasing exercise intensity and duration in order to enhance program feasibility, attainability, and maintenance (Daniels et al., 2009). Our subject population represents a high-risk group requiring aggressive treatment methods to prevent and delay further obesity-associated pathologies. Investigating the effects of aerobic training on metabolic syndrome components is especially important as these individuals may require pharmacological or surgical treatment methods with continued weight gain or worsening of insulin sensitivity (Pratt et al., 2009; Nadler et al., 2009). Our study does not rule out the effects of weight and fat loss on the observed improvements in these metabolic

syndrome components. However, changes in anthropometric variables were minor, especially when considering the subjects' degree of morbid obesity at baseline.

Our study provides evidence that low-volume/moderate-intensity aerobic training improves insulin sensitivity and fibrinolytic potential in morbidly obese minority adolescents. Thus, low-volume/moderate-intensity aerobic exercise training may suffice as an *initial* treatment therapy, in combination with dietary modifications, for the treatment of obesity-associated comorbidities in morbidly obese and insulin-resistant adolescent minorities. Our findings support the use of training recommendations set forth by the ADA to *initially* treat type 2 diabetes components in previously-sedentary adults (ADA, 2009). Despite the observed improvements in these metabolic syndrome components, training programs incorporating dietary-induced weight loss in combination with higher training intensities and volumes may be required to attenuate other metabolic syndrome components, lead to greater improvements in fibrinolytic potential and insulin sensitivity, and further reduce the risk of obesity-associated disease. Thus, the effects of gradual-onset aerobic exercise training programs of higher intensities and volumes should be further investigated in morbidly obese youth in order to develop lifestyle treatment guidelines which may delay more intensive treatment alternatives such as pharmacotherapy and weight loss surgery.

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