INTERLEUKIN-6 IN RESPONSE TO HIGH INTENSITY AND MODERATE INTENSITY EXERCISE TRAINING IN AFRICAN AMERICAN INDIVIDUALS

by

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Skeletal muscle has been identified as an endocrine organ that releases myokines with contraction. These myokines have been defined as cytokines and peptides that are produced, expressed, and released by muscle fibers that can have either an autocrine, paracrine, or endocrine action[1]. Interleukin-6 (IL-6) has been identified as a prototypic exercise myokine and can be measured in plasma after a single, acute bout of exercise[2]. IL-6, however, has implications in both the development and prevention of metabolic disease. The effect of exercise duration on IL-6 has been outlined in the literature[3–5], but the effect of exercise intensity has yet to receive the same attention. In addition, racial disparities in the development of metabolic disease have been shown with African Americans having a significantly greater prevalence of CVD and type 2 diabetes than Caucasians in the United States[6].

PURPOSE: The purpose of the present study was to determine the effects of moderate and high intensity aerobic exercise training on basal, plasma IL-6 in overweight and obese African American individuals.

METHODS: A randomized controlled trial was performed on overweight and obese (body mass index of 25-45 kg/m^2), sedentary African American individuals (35-65 years) (n=24).

Participants were randomly assigned to either a non-exercise control group or a moderate or high intensity aerobic exercise training group for 24 weeks. Supervised exercise was performed at a heart rate associated with 45-55% of VO_2 max for the moderate intensity group or 70-80% of VO_2 max for the high intensity group for a total exercise dose of 600 metabolic equivalents of task (MET-) minutes per week. Pre- and post-exercise intervention 12-hour fasted intravenous glucose tolerance tests (IVGTT) were performed. IVGTT samples were analyzed for insulin, glucose, lactate, and IL-6 using the Beckman Coulter clinical analyzer system. Bergman's Minimal Model[7] was used to assess insulin and glucose kinetics of the IVGTT. Additional blood samples were also drawn and sent to a clinical laboratory (LabCorp Inc., Burlington, NC) to be analyzed for a complete lipid and metabolic profile.

RESULTS: In the control group, there were 11 participants, 6 participants in the moderate intensity exercise group, and 7 participants in the high intensity exercise group. No significant differences were found between the randomization groups for age, gender, weight, BMI, waist circumference, body fat percentage, glucose, insulin, and IL-6. Baseline BMI was significantly associated with baseline HOMA-IR ($r=786$, $p<0.01$) and baseline insulin ($r=0.784$, $p<0.01$). There was no significant change in IL-6 with the moderate or high intensity exercise group compared to the control group ($p=0.8364$). In addition, there was no significant change in SI ($p=0.233$), DI ($p=0.422$), or HOMA-IR ($p=0.653$). There was no significant correlation between relative fold change of IL-6 and SI ($p=0.772$), DI ($p=0.545$), or HOMA-IR ($p=0.165$).

CONCLUSION: The present study did not find that moderate intensity aerobic exercise training nor high intensity aerobic exercise training decreased basal, plasma IL-6 in sedentary, obese and overweight African Americans. Insulin sensitivity was not improved in either exercise group when compared to the non-exercise control group. A broader scope on IL-6 is suggested for

future studies and should include not only plasma but adipocyte IL-6 production as well as mRNA for IL-6. In addition, allowing enrollment of Caucasian individuals will allow for a direct comparison with African American individuals to further understand racial differences in the exercise response.

Interleukin-6 in Response to High Intensity and Moderate Intensity Exercise Training in African American Individuals

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Chapter I: Introduction

In the past two decades, skeletal muscle has been found to be an endocrine organ that releases myokines. These myokines have been defined as cytokines and peptides that are produced, expressed, and released by muscle fibers that can have either an autocrine, paracrine, or endocrine action[1]. They play a particularly important role in crosstalk with various tissues, such as the liver, adipose tissue, brain, and bone[3], and are largely secreted in response to muscle contraction[8]. Through mRNA sequencing, up to 250 myokines have been identified[3], but interleukin-6 (IL-6) is one of the most extensively researched. Because myokines are secreted through skeletal muscle contraction, IL-6 has been suggested to play a role in the benefits of physical activity[9].

The amount of IL-6 produced and released is dependent on both the intensity and duration of physical activity[2], though studies have shown that duration is the biggest factor in regard to the magnitude of IL-6 released[4,5]. With prolonged exercise bouts, plasma IL-6 increases of up to 100-fold of resting values can be consistently seen. Studies have also shown a positive correlation between exercise intensity and plasma IL-6[5,10], but attribute IL-6 increases to the increase in muscle mass used at higher intensities. Differences in IL-6 increases with exercise may be due to sampling methods. Many studies measure IL-6 concentrations in plasma, but there exists a high variance between blood sampling methods. Other studies measure IL-6 changes with mRNA instead and have seen similar, yet less pronounced increases with exercise[11]. Inconsistencies on the magnitude of plasma IL-6 increases with exercise have also been noted when trained athletes are compared to sedentary participants[12]. Overall, changes in IL-6 due to exercise are variable and depend highly on the population studied, the sampling method, and the nature of the exercise.

Decreased plasma IL-6 concentrations at rest and in response to exercise are now understood to be a normal training adaptation, with higher levels of physical activity reflecting lower resting plasma IL-6[13–15]. Physical activity is also known to increase insulin sensitivity for both non-insulin resistant and insulin resistant populations. It is understood that physical activity plays an essential role in the treatment and prevention of insulin resistance. However, it is unknown if the change in insulin sensitivity with exercise is correlated to the change in basal IL-6 or these two adaptations occur independently of each other.

IL-6 is a highly researched myokine, yet there still seems to be limited information looking at the effect exercise intensity has on plasma IL-6, especially in sedentary individuals. There also exists an inconsistency on the magnitude of changes in IL-6. The present study will lead to a better understanding of myokine changes in response to exercise.

Hypothesis

Resting plasma IL-6 is lower in sedentary, overweight and obese African American individuals who complete a high intensity exercise program than a moderate intensity exercise program. In addition, decreases in IL-6 correlate to increases in insulin sensitivity.

Purpose

The purpose of this study was to measure resting plasma IL-6 levels in response to exercise intensity in sedentary, overweight and obese African American individuals.

Delimitations

1. All individuals had a Body Mass Index (BMI) of 25.0 – 45.0 kg/m^2.
2. All individuals were sedentary.
3. All individuals would commit 24 weeks of 3-4 supervised exercise sessions per week.

Limitations

1. Exercise intensity was limited to moderate and vigorous, which limits the generalizability of the results to other exercise intensities.
2. All exercise training was done on a treadmill, which limits the generalizability of the results to other modes of exercise training.
3. The present study only used sedentary, overweight and obese African Americans, which limits the generalizability of these results to other BMI classifications and ethnicities.
4. This study used only aerobic exercise, thus limiting the generalizability of the results to other forms of exercise.
5. Only African American individuals were recruited, which did not allow for the direct comparison of intervention variables across different races.

Chapter II : Review of the Literature

Introduction

The purpose of the current study was to examine differences of the myokine interleukin-6 in plasma in response to different exercise intensities. The following literature review will discuss: **1)** myokines and their functions in skeletal muscle, **2)** interleukin-6 and its response to exercise, **3)** insulin sensitivity changes with exercise and IL-6, and **4)** racial disparities in exercise responses.

Myokines in skeletal muscle

The secretory properties of skeletal muscle were discovered in the past two decades[1], and thus myokine research is fairly new and the full scope of its functions and effects is still being determined. Myokines have been suggested to be classified as cytokines and peptides that have either an autocrine, paracrine, or endocrine action[1]. Through mRNA sequencing, almost 250 genes for encoding myokines were discovered that are regulated with exercise[3]. They have also been found to be especially important in tissue cross talk, such as with the liver, adipose tissue, brain, and bone[16]. Tissue cross talk, or communication between different tissues with a subsequent change in whole body metabolic homeostasis, is particularly important between adipose tissue and skeletal muscle tissue. Adipose tissue releases adipokines which are secreted proteins that also act in an endocrine manner and facilitate this tissue-to-tissue cross talk that is connected to the delicate balance of metabolism[17,18]. Myokines in connection to metabolic disease and function are an important area of research as studies have shown myokines can have positive effects on glucose uptake[19–23], glucose tolerance[24], fat oxidation regulation[24,25], and satellite cell proliferation[26–28].

Myokines are secreted with muscle contraction; thus it can be said that physical activity plays a large role in the expression of these myokines[8]. In response to exercise, there exist differences in myokine response rates. Some myokines are fast-responders meaning that their concentration is quickly changed after a brief bout of physical activity, but others are slow-responders and require more long-term, consistent physical activity to show a change in concentration[3]. Myokines also show both beneficial and harmful effects on the body dependent on the amount released. For many, a low concentration will be beneficial, but both a lack and a high concentration can cause adverse effects[29]. By viewing skeletal muscle as a secretory organ and understanding that for some myokines their production and release is fully dependent on the contraction of skeletal muscle, it allows an insight into physical inactivity being linked even further with many chronic diseases and disorders, such as insulin resistance, type 2 diabetes, cardiovascular disease, and cancer[16].

Interleukin-6 and exercise

IL-6 is considered both a myokine and a cytokine[29], and is one of the first extensively researched myokines[30]. It is considered to be a prototypic exercise myokine since it is one of the first myokines to start circulating after muscular contraction and is a fast responder to physical activity and can even be released after a single, acute bout of exercise. IL-6 levels can increase up to 100-fold after exercise[2]. The amount produced and released is dependent on both the intensity and duration of physical activity[2]. Studies have mapped out increases in IL-6 with varying exercise durations[4], but as of yet the same has not been done for exercise intensities. Exercise intensity is harder to control than exercise duration, so many studies focus on detailing IL-6 increases with duration.

Physical activity activates transcription factors creating an increase in IL-6 that is then released from both skeletal muscle and adipose tissue[29]. Regulation of IL-6 is mainly done at the transcriptional level[31]. If released from skeletal muscle, the amount of IL-6 produced also depends on the glycogen supply of the muscle. Lower amounts of IL-6 are released when skeletal muscle has a higher glycogen content. Regular training and exercise increase the glycogen content of the muscle, so lower basal plasma levels of IL-6 are seen with regular physical activity. Many studies have noted that basal IL-6 is sensitive to regular physical activity[14–16,32]. When basal IL-6 was categorized by weekly physical activity minutes, those who performed 180 or more minutes were found to have the lowest basal concentration of IL-6. Those who performed 1-180 minutes had the second lowest basal IL-6, and those who did no exercise had the highest basal IL-6. A linear trend was found for decreases in basal IL-6 and increases in weekly exercise, so it is suggested that basal IL-6 is particularly sensitive to total amount of exercise[14]. Regular physical activity also increases the basal expression of IL-6 receptors, so although circulating IL-6 is lower, the muscle becomes increasingly more sensitive to the myokine[29].

Previously, elevated IL-6 concentrations were connected with increased creatine kinase levels and skeletal muscle damage from exercise[33] making it unclear if damaged muscle caused the increased production of myokines or if it was the increased myokines causing the muscle damage. When skeletal muscles of rats were electrically stimulated for both concentric and eccentric contractions, mRNA levels of IL-6 were increased significantly in the stimulated muscle only with both types of contraction[34]. This helps indicate that the increased expression of IL-6 is through skeletal muscle locally producing the myokine as a response to damage in the muscle or connective tissue. Further, plasma IL-6 has been shown to be greater with eccentric

rather than concentric exercise although concentric exercise also increases plasma IL-6[33,35]. With all exercise, however, an increase in skeletal muscle IL-6 is accompanied by an increase in plasma IL-6. Many studies have shown a correlation between plasma and skeletal muscle IL-6 but few have shown the increase in plasma IL-6 to be the cause of increased skeletal muscle production of the myokine[11,16,30,36–38]. Steensberg et al.[30] looked at arterial IL-6 concentrations compared to skeletal muscle IL-6 production and found that IL-6 in skeletal muscle was about 17 times higher than in plasma. This shows that during muscular exercise, IL-6 has a high turnover rate in skeletal muscle, helping to explain the high variance seen between plasma and skeletal muscle IL-6. Further, it may be that IL-6 itself has a high clearance rate in general rather than specific to skeletal muscle tissue. When recombinant human IL-6 (rhIL-6) was injected in rats to measure biological activity, it was found that the peak concentration in plasma only accounted for half of the initially injected rhIL-6[31]. Together, this shows that IL-6 clears quickly in circulation when both produced in skeletal muscle and when injected intravenously. Castell et al.[31] also reported a half-life of 5-11 minutes for IL-6, and that IL-6 clearance occurs in a biphasic manner. The clearance of IL-6 from plasma occurs through two exponential components of which the initial component is rapid and the latter slow. The initial has an average of about 3 minutes, and the latter has an average of about 55 minutes. Morettini et al.[39] confirmed these findings through the use of a mathematical model and subsequent analysis of data sets from four experimental studies implementing exercise protocols with sampled plasma IL-6. A half-life of 13 minutes was found for IL-6 through this method. To the knowledge of the author, there is currently no evidence that the IL-6 half-life differs across different races.

Insulin sensitivity with exercise and IL-6

Physical activity is known to be beneficial for both non-insulin resistant and insulin resistant populations. One of the most important effects of physical activity is an increase in sensitivity to insulin. There are distinctive benefits from both a single acute bout of physical activity and general training adaptations. Benefits from an acute bout of physical activity last for at least 16 hours post-activity and include improved glucose uptake in skeletal muscle and an increase in plasma membrane associated glucose transporter type 4 (GLUT4)[40]. General training adaptations include a continued increase in glucose uptake, an increase in GLUT4 translocation to the plasma membrane upon insulin stimulation, an increase in insulin responsiveness and sensitivity compared to untrained individuals, upregulation of muscle GLUT4 protein, increased enzyme capabilities, and muscle capillarization[40]. To maximize benefits, an emphasis on total work performed should be placed rather than exercise duration or intensity[41].

The role of IL-6 in insulin sensitivity and the induction of insulin resistance has largely been found to be inconclusive. IL-6 has been shown to be capable of impairing insulin signaling and action through its effects on gene transcription, reduction in expression of insulin receptor substrate 1 (IRS-1), GLUT4, peroxisome proliferator-activated receptor (PPARγ), and decrease in insulin-stimulated glucose transport[29,42–45]. IL-6 has also been shown to increase GLUT4 translocation[46,47], increase glucose uptake[42,47], increase glycogen synthesis[46,48], increase fatty acid oxidization[46–48], correlate to lower blood glucose and body weight[49] and increase beta-cell proliferation in the pancreas[50]. Further, there exists more contradictory evidence whether IL-6 is beneficial or harmful and whether this occurs at high or at low concentrations of the myokine. Nieto-Vazquez et al.[42] reported the dual action of IL-6 when exposing myotubes and mice to the myokine. With short-term exposure, glucose uptake and systemic insulin sensitivity were

increased. However, with chronic exposure, insulin resistance was seen through the impairment of GLUT4 translocation to the plasma membrane and defects in IRS-1. Bruunsgaard[51] also notes the role of IL-6 as a "double edged sword". Large but short-lived increases of IL-6, as seen with exercise, result in systemic benefits. Chronic, low-grade increases in IL-6 that are commonly seen with metabolic disease, however, will cause detrimental systemic effects. The rapid rate at which IL-6 is cleared from circulation also suggests that chronic increases in IL-6 are not desirable. In summary, further research is needed to determine the precise role of IL-6 in relation to insulin sensitivity.

Racial disparities in exercise responses

Compared to Caucasians, African American individuals are disproportionately affected by type 2 diabetes (T2D) and obesity. In the United States, African Americans have a prevalence rate of 14.7% compared to 7.5% for Caucasians for diabetes[6]. In addition, African American women have a higher prevalence of T2D compared to African American men. Although the exact reason behind this disparity in cardiometabolic disease is unknown, it has been posed that differences in insulin sensitivity are partly to blame. An increase in insulin resistance is a precursor to the development of T2D[52–54] and physical activity is inversely related to both insulin resistance and the development of diabetes[55–57]. In the United States, the majority of African American individuals do not get the recommended amount of physical activity[6,58]. Supporting this, it is estimated that 30% of the racial disparity for T2D can be attributed to modifiable and non-modifiable risk factors, like physical inactivity, environment, and other lifestyle factors[59]. Because physical activity falls within these risk factors, it becomes increasingly important to understand the relationship between physical activity and its effects with this at-risk population.

Further, because IL-6 is still not entirely understood in its connection to insulin sensitivity, a deeper look into IL-6 and African American individuals needs to be done.

When examining muscle fiber composition, differences between African American and Caucasian populations have been noted. Tanner et al. compared the muscle fiber types of African American and Caucasian women in muscle samples collected from the rectus abdominus muscle[60]. The African American individuals were found to have more type IIb and fewer type I fibers than the Caucasian individuals. This difference in muscle fiber types is notable because studies have shown that different muscle fibers release IL-6 in different magnitudes. Liang et al.[61] found that IL-6 release is muscle fiber type specific, with IL-6 only being released from slow oxidative fibers but not fast glycolytic fibers with contraction. Another study found a similar result looking at gene transcription. IL-6 mRNA was higher in oxidative muscle fibers than glycolytic fibers[62]. From these results, it should follow that African American individuals have a lower production and release of IL-6 when compared to Caucasian individuals. However, Starzak et al. directly compared plasma IL-6 concentrations of African American and Caucasian individuals after a bout of exercise. Both groups performed the exercise for the same amount of time and at the same intensity. Cytokine concentrations were measured 3, 6, 9. 12, and 24 hours and 1, 2, and 3 weeks after the bout of exercise. They found that the African American individuals had significantly higher plasma IL-6 than the Caucasian individuals beginning at 6 hours post-exercise and continuing to 2 weeks post-exercise[63]. These results are contradictory to what is predicted from muscle fiber type research. Thus, it is not clear if IL-6 is produced at a higher or lower magnitude in African American individuals compared to Caucasian individuals.

Conclusion

Not only is IL-6 a relatively new research topic, but myokines in general are still not thoroughly understood in their pathways and peripheral effects on the body. There seem to be inconsistent results on the magnitude of IL-6 expression with physical activity. Concentrations of plasma IL-6 can vary significantly from mRNA expression of IL-6 in an individual[64]. Unfortunately, many papers do not specifically state the blood sampling method used for IL-6 making it difficult to compare results between studies. Even when looking at only plasma concentrations of IL-6, levels can vary dependent on if the sample was taken from a venous catheter or a venous puncture [65,66], which reflects a lack of a standardized sampling method of IL-6. There is also little research that has looked at plasma IL-6 with solely exercise intensity and rather focuses on mapping the effects of exercise duration. Since exercise intensity is frequently associated with a shorter duration, the relationship between the myokine and intensity may be more pronounced and needs further research. Finally, there exists contradictory research on IL-6 production in African Americans. It is suggested that African American women have more type IIb and fewer type I muscle fibers when compared to their Caucasian counterparts. Type I and type IIa muscle fibers have been shown to produce more IL-6, yet African Americans have shown a significantly higher IL-6 plasma concentration than Caucasians after an identical bout of exercise. In all, a better understanding of IL-6 may help to in turn explain its roles in crosstalk with other tissues and potentially the role it plays in various chronic diseases.

Chapter III: Methods

The purpose of the present study was to measure the changes in basal, plasma IL-6 and insulin sensitivity in sedentary, overweight and obese African American individuals after completion of a moderate and high intensity aerobic exercise training program. To help test this, data from the High Intensity exercise to Promote Accelerated improvements in Cardiorespiratory fitness (HI-PACE) study database has been used for the present study. The methodology of HI-PACE was approved by the East Carolina Institutional Review Board, and all participants signed a written informed consent prior to enrollment. Sedentary, overweight and obese African American participants were randomized to three different groups (two exercise and one non-exercise), performed an IVGTT pre-training and post-training, and resulting plasma samples were analyzed. This section provides a detailed summary of the study design, subjects, the exercise training protocol, IVGTT and sample analysis, and statistical analyses used to test the present study's hypothesis.

Study design

Two exercise intensities and a non-exercise control were examined: (1) control, (2) moderate intensity (45-55% VO_2 max), and (3) high intensity (70-80% VO_2 max). The rationale for examining these three groups was to provide a comparison of potential exercise effects in sedentary African American individuals. Participants were screened for exclusion criteria. A pretraining IVGTT was performed. Subjects then underwent 24 weeks of 3-4 days/week of supervised exercise training. A posttraining IVGTT was performed. Fasting baseline samples from the IVGTT were used to determine resting plasma IL-6 concentration and insulin sensitivity.

Participants

Recruitment of participants was done through e-mail, online advertisement, and flyer distribution. A sample size of 60 was used for total participant recruitment, but only those with a complete IVGTT were included in the present study. The main inclusion criteria for the study was age, sex, BMI, activity level, ethnicity, and informed consent. Individuals must be 35-65 years old. Both men and women were included but must have a BMI of 25.0-45.0 kg/m^2. Individuals must have been sedentary and not participating in exercise training at the time of enrollment (<20 minutes and ≤2 days per week for the past 3 months). Individuals must have self-identified as African American. Finally, individuals must show willingness and capability to provide their written consent and to understand the exclusion criteria. Individuals were excluded if they were either type 1 or type 2 diabetic or had a fasting plasma glucose of ≥126 mg/dL. No individuals could have cardiovascular disease or disorders including diagnosed congestive heart failure, serious arrhythmias, peripheral vascular disease with intermittent claudication, previous stroke, or myocardial infarction. Resting blood pressure could not include an excessively high resting systolic (>180 mmHg) or diastolic (>100 mmHg) blood pressure. Individuals that were on blood pressure medication at the time of recruitment were however permitted to enroll. Total cholesterol could not have been ≥240 mg/dL, low-density lipoprotein cholesterol ≥160 mg/dL, or triglycerides ≥300 mg/dL. Other exclusionary medical conditions included chronic or reoccurring neuromuscular, respiratory, gastrointestinal, neurological, HIV, or psychiatric conditions, musculoskeletal conditions affecting exercise, current treatment for medical illness or hospitalization from mental illness from previous 5 years, autoimmune or collagen vascular diseases, and other medical conditions that were considered life-threatening or that can be provoked from exercise training. Finally, other exclusion criteria include pregnancy or plans to

become pregnant, current engagement or plans to engage in a weight loss or dieting program, previous bariatric surgery or weight loss medications, and plans to leave Pitt County in North Carolina for more than 2 weeks in the 6 months following recruitment. Participants were screened by study staff for inclusion and exclusion criteria via phone interview. Next, the participants attended a screening session to review study design and associated benefits and risks of participating in the study. Finally, baseline measurements and values were recorded, which included a fasted blood draw that was sent to a clinical laboratory for analyses. Medical history and screening measurements were reviewed by study staff.

Anthropometry and body composition

After enrollment, baseline measurements were recorded including weight, height, body mass index (BMI), waist circumference (WC), and a dual energy x-ray absorptiometry (DXA) scan. A Digi Tol calibrated scale (Mettler Toledo, Columbus, OH) was used to record body weight in the fasted state. Height was recorded using a stadiometer. BMI was calculated from these measurements in kg/m^2. WC was measured using a tape measure at the natural waist. DEXA (GE Lunar Prodigy Advance, Fairfield, CT, USA) was used to measure body composition (fat mass and fat-free mass).

Exercise training

Upon completion of all baseline visits and approval received by the study physician, participants were randomized to the non-exercise control, moderate intensity, or high intensity group. All exercise sessions were supervised by study staff and performed on a treadmill. Participants randomized to the moderate-intensity exercise group performed exercise at a target

heart rate associated with 45-55% of their VO_2 max. Participants randomized to the high-intensity exercise group performed exercise at a target heart rate associated with 70-80% of their VO_2 max. The heart rate range was determined for each participant from a maximal exercise test at baseline and mid-intervention. A total of 600 MET-minutes per week was performed by both exercise groups, which is consistent with current public health guidelines[67]. Because all participants were sedentary at the start of the study, a ramp protocol was used to build up to 600-MET minutes per week. Individuals started at 50-MET minutes per week and increased by an additional 50-MET minutes per week until the exercise volume reached 600-MET minutes (week 9). Participants were instructed to do a 5-minute warm-up prior to each exercise session and a 5-minute cool-down at the end of each session. Heart rate was continuously monitored and recorded every 5 minutes during exercise to ensure maintenance of the target exercise intensity through the use of Zephyr Bioharness 3 Monitors (Annapolis, MD).

IVGTT and insulin sensitivity

Participants completed an intravenous glucose tolerance test (IVGTT) prior to beginning exercise training and once more after completing training. The IVGTT was done 18-24 hours following the last exercise session for the exercise groups. The procedure used for the IVGTT is as described by Bergman et al.[7]. Subjects reported to the laboratory in the morning after a 12-hour fast and completed a blood draw at the East Carolina Heart Institute. Three baseline samples were collected in addition to vials of archive plasma, serum, and red blood cells. Then, an intravenous injection of glucose (1.7 mmol/kg) was administered at time 0 and insulin (150 pmol/kg) was administered at minute 20. Including the baseline samples, a total of 31 samples

were collected between -15 and 180 minutes. Blood samples were then centrifuged and stored at -80° until sample analysis was performed.

Blood analysis

IVGTT samples were analyzed using a high sensitivity chemiluminescent immune assay for insulin and IL-6 using the Beckman Coulter Access II clinical analyzer. Samples were analyzed for lactate and for glucose using a hexokinase reaction on the Beckman Coulter DxC600 clinical chemistry analyzer (Beckman Coulter AU5800; Brea, CA). Bergman's Minimal Model[7] was be used to assess insulin and glucose kinetics of the IVGTT. The minimal model provides estimates of insulin secretion (acute insulin response to glucose, AIRG), insulin sensitivity (SI), and disposition index (DI). The SI is a measure that includes both the effect of insulin on glucose disappearance and reducing hepatic glucose production. The DI is a measure of pancreatic beta-cell function. HOMA-IR was calculated from baseline values and used as a measure of insulin resistance.

Statistical analysis

A repeated measures ANOVA was used to compare participants pre- and post-training, as well as between different randomization groups. To measure the change in IL-6, an ANOVA was performed with an alpha level of ≤ 0.05 for all statistical analyses. A one-way ANOVA was used to compare baseline values to ensure no significant differences exist between groups initially. To analyze the correlation between baseline variables and variable fold-change, a Spearman correlation was done. Change scores were calculated to measure change in IL-6 across other baseline variables.

Chapter IV: Results

The baseline characteristics for each treatment group are reported in Table 1. In the control group, there were 11 participants. In the moderate intensity exercise group, there were 6 participants. In the high intensity exercise group, there were 7 participants. No significant differences were found between randomization groups for age, gender, weight, BMI, waist circumference (WC), body fat percentage, glucose, insulin, and IL-6 ($p>0.05$). Baseline BMI was significantly associated with baseline HOMA-IR ($r=0.786$, $p<0.001$) (Figure 7) and baseline insulin ($r=0.784$, $p<0.001$) (Figure 8). Table 2 reports relative fold changes and standard error of the mean (SEM) of intervention statistics for each treatment group.

There was no significant difference in IL-6 with exercise ($p=0.1211$) (Figure 1). There was no significant change in IL-6 with the moderate intensity or the high intensity group when compared to the control group ($p=0.8364$) (Figure 2). Relative fold changes for IL-6 were 1.04, 1.43, and 1.03 for the control, moderate intensity, and high intensity group, respectively (Figure 3). In addition, there was no significant change in SI ($p=0.233$), DI ($p=0.422$), or HOMA-IR ($p=0.653$). Correlation analyses showed no significant association between IL-6 and SI ($r=-0.062$, $p=0.772$) (Figure 4), DI ($r=-0.130$,$p=0.545$) (Figure 5), or HOMA-IR ($r=-0.293$, $p=0.165$) (Figure 6).

Chapter V: Discussion

The primary finding of the present study was that neither moderate nor high intensity exercise training reduced interleukin-6 in overweight and obese African American individuals. Further, no significant changes in insulin sensitivity were found. These results are in contrast to previous studies in the literature, but it is important to note that other benefits from aerobic exercise training still exist [68,69]. This is the first study to the author's knowledge that has evaluated the effect of differing exercise intensities on IL-6 levels through an exercise training program, specifically in overweight and obese, sedentary African American individuals.

The purpose of this study was to examine the effect of moderate and high intensity training on basal, plasma IL-6 of overweight and obese, sedentary African American individuals, as well as further investigate the relationship between IL-6 and insulin sensitivity. Although other studies have suggested a relationship between exercise intensity and IL-6 as well as improvements in insulin sensitivity with exercise training, the present study did not observe any significant changes in basal, plasma IL-6 or any improvements in insulin sensitivity following 24 weeks of supervised aerobic exercise training. The present study found mean values of 4.15±3.74, 3.10±0.74, and 5.01±4.8 pg/mL for plasma IL-6 in our control, moderate, and high intensity groups, respectively. Although plasma IL-6 varies across populations and is influenced by geography, etiology, and health status, a reference range of 0.015-10.01 pg/mL was reported by Ridker et al.[70]. While our plasma IL-6 values fall within this reference range, our mean values differ largely from other studies. In populations of similar age, sex, and BMI, mean plasma IL-6 values of 1.89[71], 1.66[72], and 2.8[73] pg/mL have been measured. Gudmundsson et al.[65] reported that concentrations of plasma IL-6 may increase when sampled through an indwelling venous catheter. Although the use of an indwelling venous catheter for experimental models such as an

IVGTT is the accepted choice, their results indicated that the frequent sampling may result in a cytokine cascade that results in increased IL-6 production due to the continuous disruption of the endothelial layer. Endothelin is produced by epithelial cells, has been shown to be an effective stimulator of IL-6[74], and is released into circulation with indwelling venous catheters. It is suggested that the higher IL-6 concentration seen with this sampling method is reflective of local production rather than the circulating concentration. This data taken into account with the disproportionate difference of IL-6 production in skeletal muscle and IL-6 circulation in plasma helps to provide a potential explanation on the present study's results. In addition, the authors of the present study recognize that an IVGTT is not a stress-free environment for study participants. This is important to note due to the relationship between stress and IL-6. In previous sections, it has been noted that IL-6 acts as an indicator of systemic stress, such as with type 2 diabetes, obesity, and inflammation. Further, IL-6 is suggested to act as an indicator of all stress signals and be a fundamental component of the organism's stress sensor[75]. Thus, it is plausible that increased plasma IL-6 concentrations at the time of the IVGTT could be indicative of an acute stress response. To mitigate this problem, we suggest that plasma IL-6, mRNA for IL-6 in skeletal muscle, receptors for IL-6 in skeletal muscle, and adipocyte IL-6 be studied. Although skeletal muscle accounts for a large proportion of IL-6 production, other tissues should not be ignored. Adipose tissue has been shown to release significant amounts of IL-6 and there exists a strong, positive correlation between arterial IL-6 concentration and BMI[66]. In the present study, there was no significant change in BMI in either the moderate intensity or the high intensity exercise group when compared to the non-exercise control group. Due to this, it is also plausible that there needs to be a significant amount of weight loss to see a significant decrease in IL-6. In

addition, further research could allow enrollment for both Caucasian and African American individuals to allow a direct comparison of intervention variables between races.

Despite the results, the present study had many strengths. All exercise training was supervised, and participants were continuously monitored to ensure appropriate exercise intensity. This allowed for a high adherence rate for both exercise intensity groups. In addition, a particular strength of this study was the equivalent total exercise volume performed by the moderate and high intensity group, which allowed for mediation of the effects of exercise duration on IL-6. A weakness of the study was the majority of participants were female. Also, these results will not be able to be generalized to a diabetic population.

In conclusion, the present study did not find that moderate exercise intensity training nor high intensity exercise training decreased basal, plasma IL-6. In addition, insulin sensitivity was not improved in either group. We suggest a broader look at IL-6 for future studies as well as a larger sample size. Studying IL-6 with a multi-tissue approach would allow for a better understanding of IL-6 at a systemic level and could potentially provide more insight into the role IL-6 has in muscle-adipose tissue crosstalk. In addition, we suggest dietary interventions be combined with exercise to potentially lower BMI and compound into a decrease in basal, plasma IL-6.

Tables and Figures

Table 1: Baseline Participant Characteristics

Variable	Control (n=11)	Moderate Intensity (n=6)	High Intensity (n=7)
Age (yrs.)	49 ± 6	51 ± 8	46 ± 9
Sex (%)	1.73 ± 0.5	1.67 ± 0.5	1.71 ± 0.5
Weight (kg)	92.01 ± 17.9	95.78 ± 12.8	99.87 ± 19
BMI (kg/m^2)	32.5 ± 6.1	32.73 ± 2.9	35.9 ± 5.5
WC (cm)	94.05 ± 12.6	95.44 ± 10.5	102.73 ± 13.9
Fat Mass (kg)	39.55 ± 8.7	40.1 ± 8.1	43.03 ± 6.7
VO_2 (ml/kg/min)	21.76 ± 5.3	20.88 ± 5.4	18.53 ± 4.3
Glucose (mg/dL)	85.77 ± 8.2	92.76 ± 4.5	88.61 ± 4.7
Insulin (uU/mL)	8.92 ± 7.3	8.76 ± 3.4	12.43 ± 9.5
SI [min^{-1}/(μU/mL)]	4.95 ± 4.9	3.06 ± 1.1	2.25 ± 1.6
DI	6420 ± 10291	2067 ± 1441	1943 ± 1432
Lactate (mMol)	1.61 ± 0.2	1.67 ± 0.2	1.66 ± 0.3
HOMA-IR	1.96 ± 1.7	2.02 ± 0.8	2.71 ± 2.0
IL-6 (pg/mL)	4.15 ± 3.74	3.10 ± 0.74	5.01 ± 4.8

Table 2: Relative (post-training/pre-training) Changes in Intervention Statistics; Mean ± SEM

Variable	Control (n=11)	Mod Intensity (n=6)	High Intensity (n=7)
Δ Weight (kg)	1.00 ± 0.01	1.02 ± 0.01	0.99 ± 0.02
Δ BMI (kg/m^2)	1.00 ± 0.01	1.02 ± 0.01	0.99 ± 0.02
Δ WC (cm)	1.00 ± 0.01	1.04 ± 0.02	1.00 ± 0.01
Δ Fat Mass (kg)	0.99 ± 0.01	1.00 ± 0.03	0.97 ± 0.02
Δ VO_2 (ml/kg/min)	0.99 ± 0.04	1.05 ± 0.06	1.18 ± 0.04
Δ Glucose (mg/dL)	1.05 ± 0.03	1.00 ± 0.06	0.99 ± 0.04
Δ Insulin (mL)	1.05 ± 0.11	0.76 ± 0.10	1.04 ± 0.09
Δ SI	1.06 ± 0.24	1.33 ± 0.39	2.80 ± 1.69
Δ DI	1.25 ± 0.31	1.34 ± 0.54	2.66 ± 1.66
Δ Lactate	1.05 ± 0.03	0.92 ± 0.05	1.07 ± 0.07
Δ HOMA-IR	1.11 ± 0.12	0.77 ± 0.11	1.04 ± 0.10
Δ IL-6	1.04 ± 0.11	1.43 ± 0.28	1.03 ± 0.08

Figure 1. Basal plasma IL-6 before and after 24 weeks of aerobic training

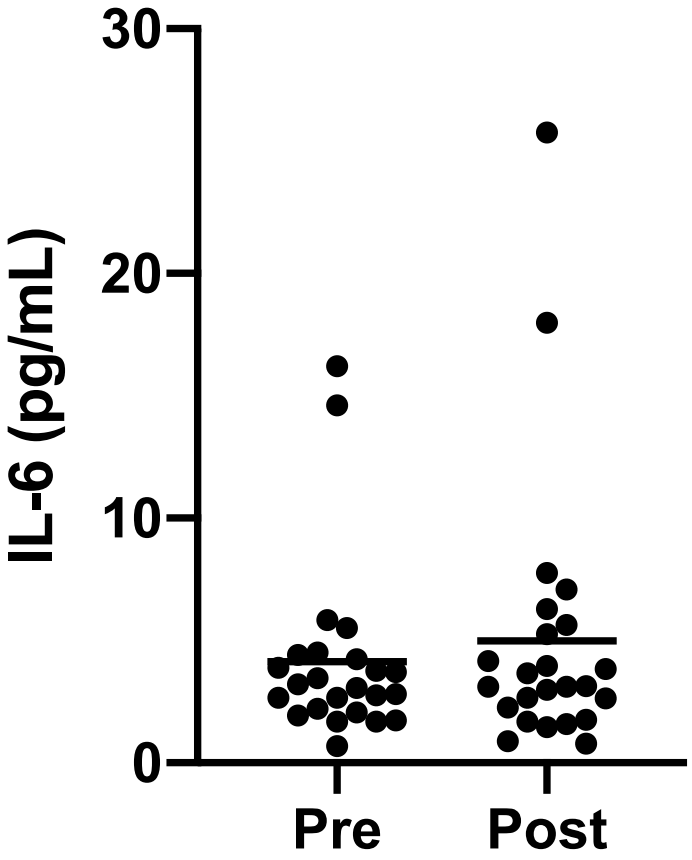


Figure 2. Mean basal plasma IL-6 before and after 24 weeks of aerobic exercise training separated by study group

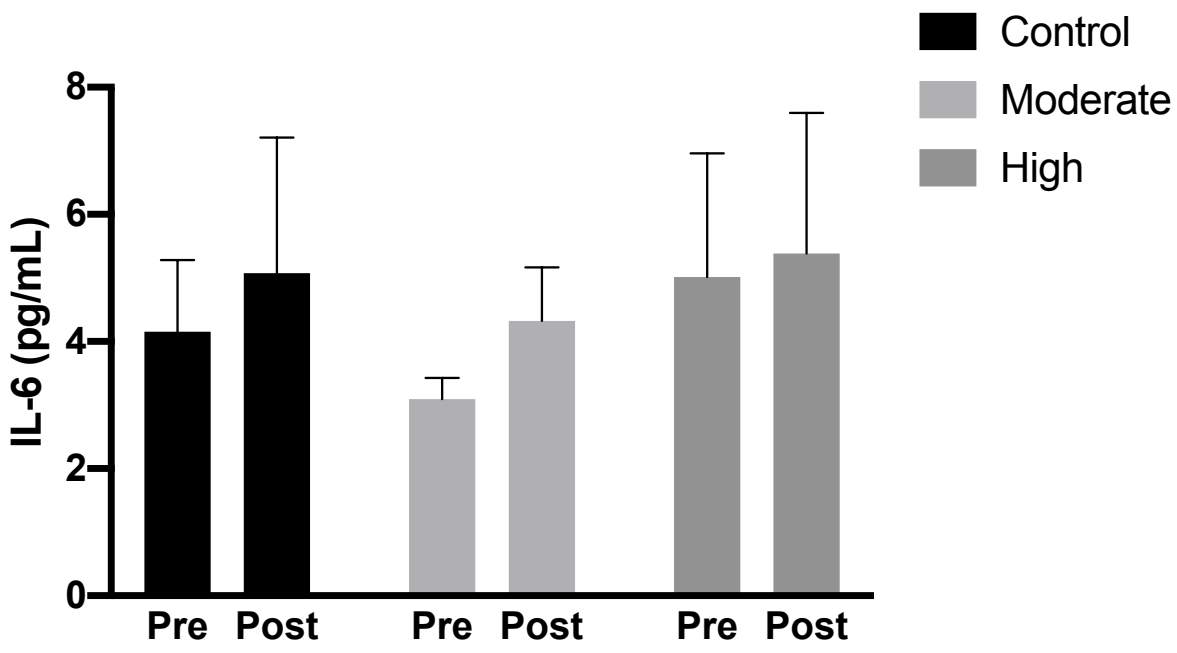


Figure 3. Relative basal plasma IL-6 fold change separated by study group

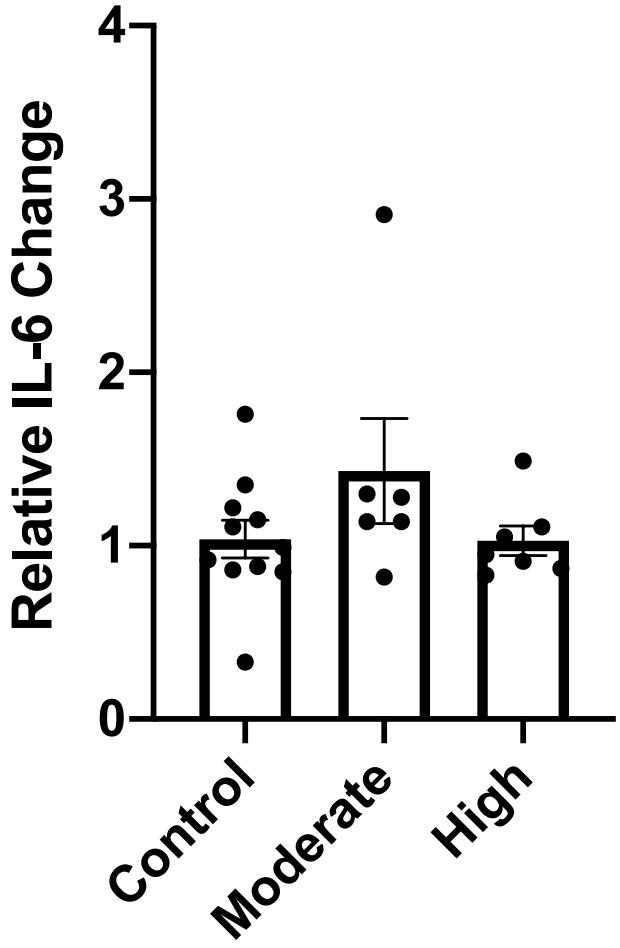


Figure 4. Relative fold change of IL-6 vs SI after 24 weeks of aerobic exercise training

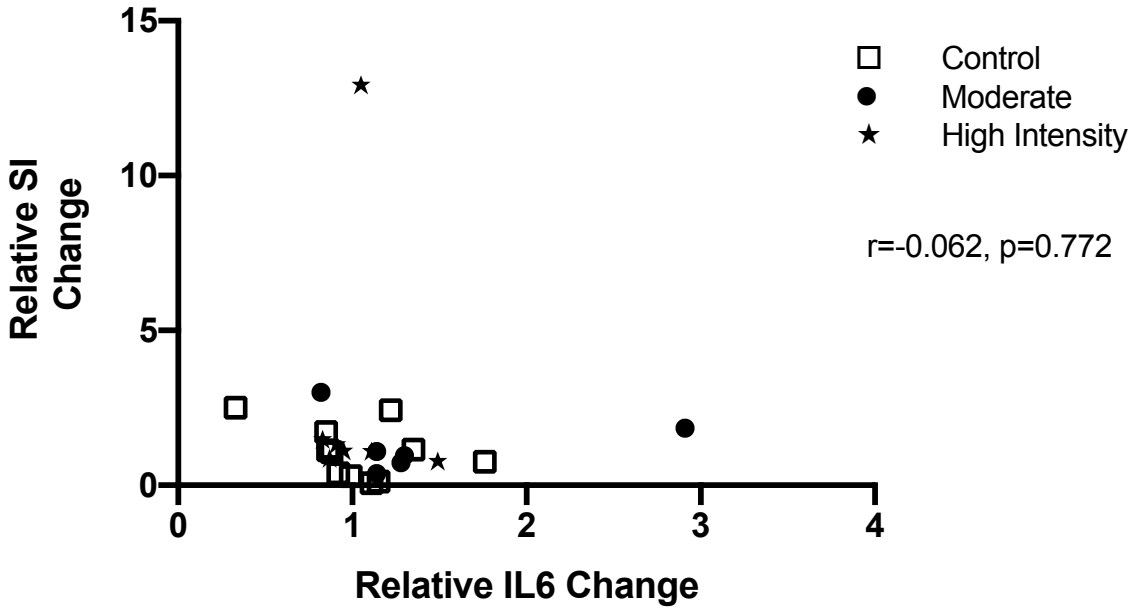


Figure 5. Relative fold change of IL-6 vs DI after 24 weeks of aerobic exercise training

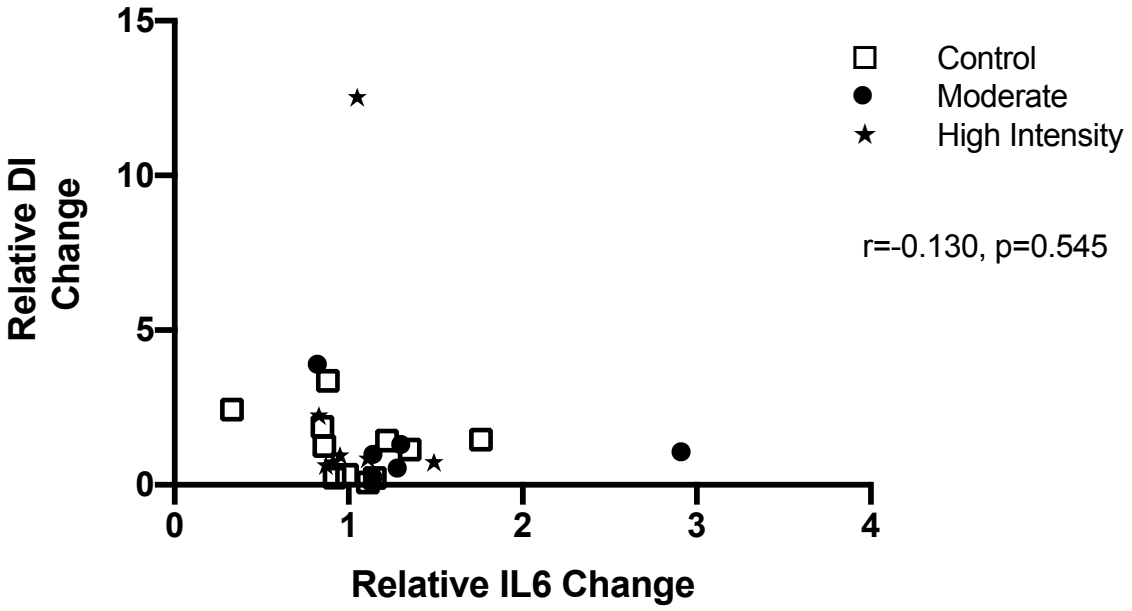


Figure 6. Relative fold change of IL-6 vs HOMA-IR after 24 weeks of aerobic exercise training

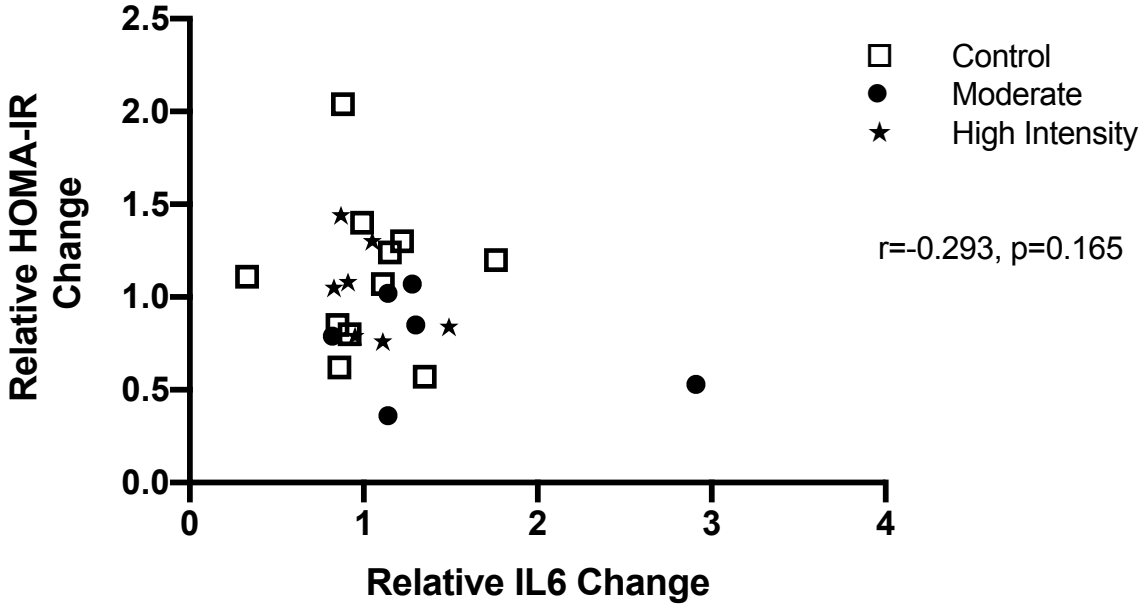


Figure 7. Baseline HOMA-IR vs baseline BMI

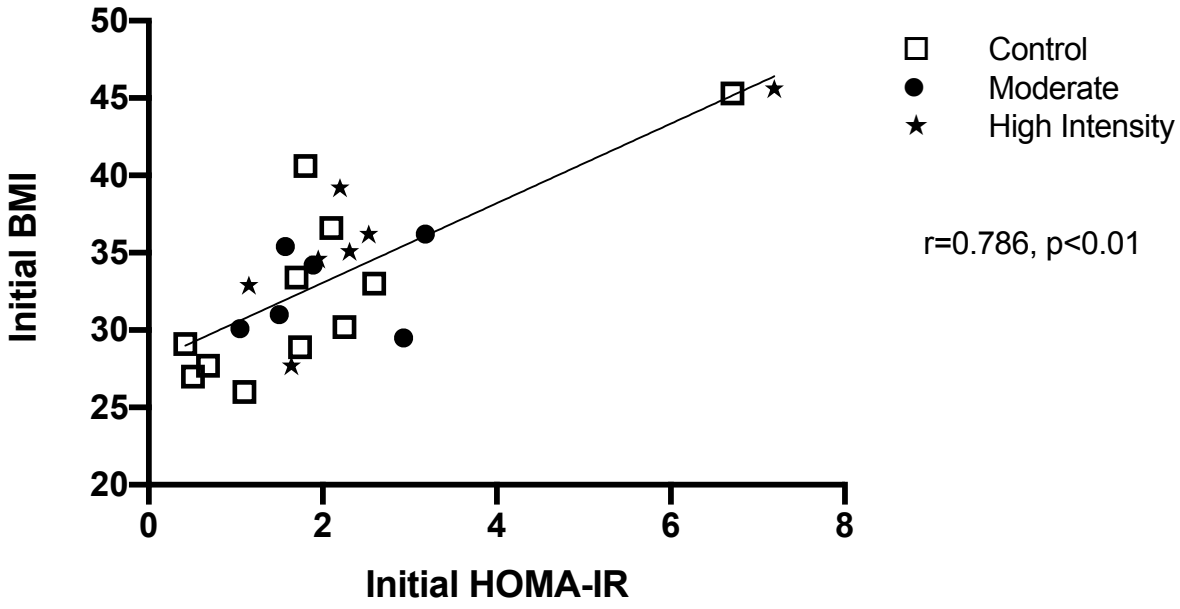
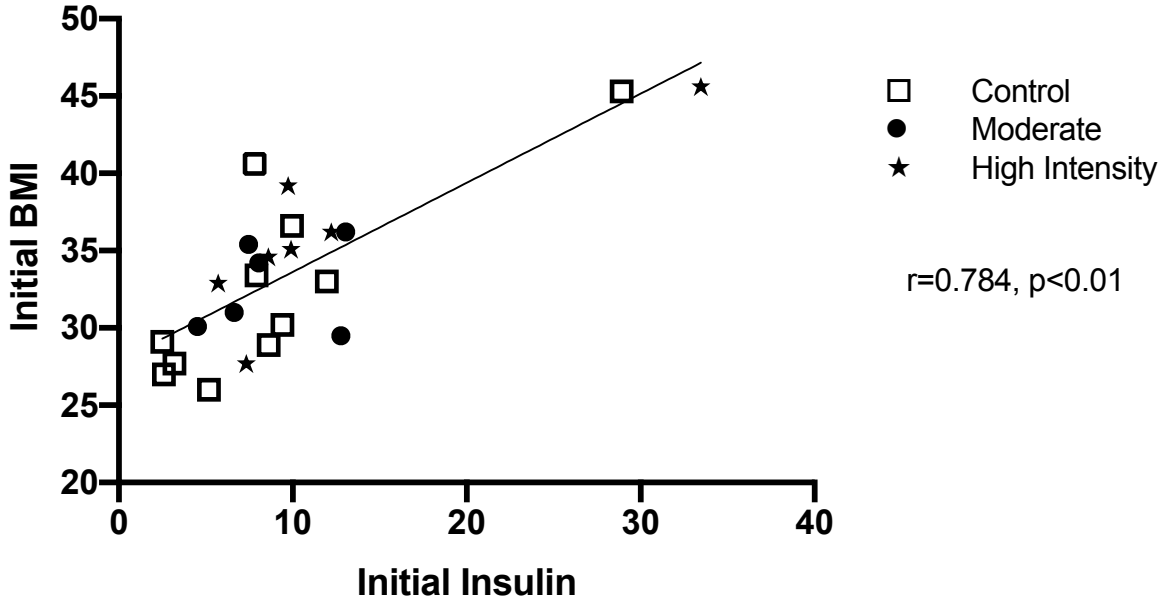


Figure 8. Baseline BMI vs baseline insulin



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Appendix

EAST CAROLINA UNIVERSITY
University & Medical Center Institutional Review Board
4N-64 Brody Medical Sciences Building· Mail Stop 682
600 Moye Boulevard · Greenville, NC 27834
Office **252-744-2914** · Fax **252-744-2284** ·
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Notification of Continuing Review Approval

From: Biomedical IRB
To: Damon Swift
CC:

Patricia Brophy
Date: 12/12/2019
Re: CR00008209
UMCIRB 14-001737
Effects of Exercise Training Intensity on Fitness and Insulin Sensitivity in African Americans (HI-PACE)

I am pleased to inform you that at the convened meeting on 12/11/2019 12:15 PM of the Biomedical IRB, this research study underwent a continuing review and the committee voted to approve the study. Approval of the study and the consent form(s) is for the period of 12/11/2019 to 12/10/2020.

The Biomedical IRB deemed this study Greater 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
Advertisment(0.04)	Recruitment Documents/Scripts
FFQ(0.01)	Surveys and Questionnaires
HD- HiPAce Flyer(0.01)	Recruitment Documents/Scripts
HiPace consent-CLEAN(0.03)	Consent Forms
HI-PACE flyer(0.04)	Recruitment Documents/Scripts
HiPace Muscle Biopsy Consent(0.01)	Consent Forms
HI-PACE R03-Main Application (FINAL).pdf(0.01)	Study Protocol or Grant Application
Mailer(0.01)	Recruitment Documents/Scripts
MTA agreement(0.01)	Additional Items
Radio Script(0.01)	Recruitment Documents/Scripts
REB for blood samples going to Univ of New Brunswick(0.01)	Additional Items
Short form-36 (Quality of Life Assessment)(0.01)	Surveys and Questionnaires

For research studies where a waiver of HIPAA Authorization has been approved, each of the waiver criteria in 45 CFR 164.512(i)(2)(ii) has been met. Additionally, the elements of PHI to be collected as described in items 1 and 2 of the Application for Waiver of Authorization have been determined to be the minimal necessary for the specified research.

The following UMCIRB members were recused for reasons of potential for Conflict of Interest on this research study:

P. Vos

The following UMCIRB members with a potential Conflict of Interest did not attend this IRB meeting: None

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