EFFECTS OF SEX AND RACE ON THE CHANGES IN INTRAHEPATIC LIPID, TOTAL AND VISCERAL ADIPOSE TISSUE IN RESPONSE TO EXERCISE TRAINING IN OBESE ADOLESCENTS

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BACKGROUD: Non-alcoholic fatty liver is the most frequent liver abnormality observed in obese children and adolescents. It is has yet to be determined whether sex and race plays a role in the effect of regular exercise without calorie restriction on intrahepatic lipid and regional adiposity in obese adolescent males and females.

OBJECTIVE: To examine the effect of sex and race after a 3-month regular exercise regimen alone without calorie restriction on intrahepatic lipid (IHL) and regional adiposity in overweight adolescent males and females. More specifically, we examined the influences of sex and race on the changes in total (TAT) and visceral fat (VAT) and IHL in response to aerobic (AE) versus resistance (RE) exercise in obese adolescents using data published previously.

STUDY DESIGN & METHODS: Thirty-one adolescent boys and twenty-eight overweight adolescent females (BMI $\geq 95^{th}$ percentile, 12-18 years, Tanner stage III-V) were randomly assigned to either: AE (n = 29, 60 min/session, 3 days/week) or RE (n = 30, 60 min/session, 3 days/week). Outcome measurements included IHL by proton magnetic resonance spectroscopy and TAT and VAT assessed by MRI. Cardiorespiratory fitness (CRF) and muscular strength was also assessed.

RESULTS: No significant sex differences were seen between obese adolescent males and females for IHL, TAT and VAT after 3 months of exercise regardless of modality. There were no

significant race differences between obese black and white adolescents for TAT. White adolescents (Δ -1.46 ± 0.2%) lost significantly more IHL than black adolescents (Δ -0.22 ± 0.1%) after 3 months of exercise regardless of modality. Improvement in CRF was not significantly different in the AE group compared to the RE group. Muscle strength index score significantly increased in the RE (Δ 0.33 ± 0.02) group compared to the AE group (Δ 0.04 ± 0.02).

CONCLUSIONS: Our results demonstrate that 3 months of AE versus RE exercise will improve body composition and fitness measurements consistently, with no influence of sex, between obese black and white adolescent males and females. Our observations suggest that regular exercise alone is an effective treatment strategy for the treatment of obesity in overweight black and white adolescents.

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ABBREVIATIONS

AE	Aerobic exercise
ALT	Alanine aminotransferase
ASAT	Abdominal subcutaneous adipose tissue
AST	Aspartate aminotransferase
AT	Adipose tissue
BMI	Body Mass Index
CRF	Cardiorespiratory fitness
CT	Computed Tomography
CVD	Cardiovascular disease
DEXA	Dual Energy X-ray Absorptiometry
FFA	Free fatty acid
FM	Fat mass
H-MRS	Proton magnetic resonance spectroscopy
HDL	High-density lipoprotein
HOMA-IR	Homeostasis Model of Assessment - Insulin Resistance
HR	Heart Rate
IHL	Intrahepatic lipid

IMCL	Intramyocellular Lipid
MRI	Magnetic resonance spectroscopy
NAFLD	Non-alcoholic fatty liver disease
NASH	Non-alcoholic steatohepatitis
PA	Physical Activity
RE	Resistance exercise
RM	Repetition Maximum
T2DM	Type 2 diabetes mellitus
TAT	Total adipose tissue
TG	Triglyceride
VAT	Visceral adipose tissue

1.0 CHAPTER ONE

1.1 INTRODUCTION

Currently, non-alcoholic fatty liver disease (NAFLD) is the most frequent liver abnormality observed in obese children and adolescents [1-7]. A strong body of evidence suggests that increased intrahepatic lipid content is significantly associated with visceral adiposity, metabolic syndrome and insulin resistance in obese children and adolescents [3, 8-12]. Diet and exercise are generally recommended to treat obese youth with NAFLD as they do not carry side effects and confer multiple cardiometabolic benefits.

Studies in adult populations report a beneficial effect of regular physical activity on reducing fatty infiltration of the liver [13, 14]. In children and adolescents, available data show that weight loss induced by increasing physical activity and calorie restriction is beneficial to reduce intrahepatic lipid content and associated health risk factors such as insulin resistance and dyslipidemia [8, 10, 15-18]. Currently, evidence regarding the independent effects of regular exercise alone (e.g, without calorie restriction) on NAFLD is unclear. Additionally, there are no data regarding the optimal exercise regimen (e.g., type, dose, intensity) that should be prescribed for reducing non-alcoholic fatty liver in children and adolescents.

The influence of gender and race on the prevalence of NAFLD in children remains unclear. Research of children with NAFLD consistently demonstrates a predominance of boys

versus girls [5, 19-21]. Recent research has concluded that Mexican-American children tend to have a greater prevalence of NAFLD compared to Caucasian and African-American children [22, 23]. Although African-American children are known to have high rates of risk factors for NAFLD, such as obesity and insulin resistance [24, 25], few children of African-American race are included in clinical NAFLD research [23]. Whether African-American children have lower rates of NAFLD or, alternatively, have NAFLD that remains undiagnosed are unknown [26]. A better understanding of the influence of gender, race, and ethnicity may provide additional insight into the pathophysiology of NAFLD. The purpose of this study was to examine the influences of sex and race on the changes in total and visceral fat and intrahepatic lipid in response to aerobic versus resistance exercise in obese adolescents using data published previously.

1.2 RATIONALE AND SIGNIFICANCE

Non-alcoholic fatty liver disease (NAFLD) is the most frequent liver abnormality in children and adolescents [21, 27]. NAFLD spans a spectrum of conditions ranging from simple hepatic steatosis (fat accumulation in hepatocyte) to non-alcoholic steatohepatitis (NASH, fat accumulation with inflammation) to advanced fibrosis and cirrhosis [21, 27] (**Table 1**). Since alcohol use and hepatic viral infections are not common in the pediatric population, most of the cases of fatty liver in children and adolescents are attributable to NAFLD [3, 28].

In clinical practice, the diagnosis of NAFLD is usually suspected upon the finding of elevated aminotransferases and/or evidence of hepatic steatosis on radiographic studies [1]. The gold standard for diagnosis of intrahepatic lipid is to perform a liver biopsy [29]. Although the

procedure is effective as a prognostic indicator, the invasive nature of liver biopsy limits its use in research settings, particularly in children and adolescents. Therefore, imaging techniques such as ultrasound, localized proton magnetic resonance spectroscopy (^{1}H-MRS), and hepatic fat fraction by magnetic resonance imaging (MRI) have been used in assessing hepatic steatosis in pediatric research.

Although the pathogenesis of pediatric NAFLD remains unclear, this condition is observed with increased frequency in obese children and adolescents. According to the SCALE (Study of Child and Adolescent Liver Epidemiology) study, hepatic steatosis was present in 5% of normal-weight (BMI <85th percentile) children compared with 16% in overweight (BMI $\geq$85th and <95th percentile) and 38% in obese (BMI $\geq$95th percentile) children based on liver histology [21]. Other studies report that the prevalence of hepatic steatosis is between 10 - 30% in overweight/obese adolescents based on alanine aminotransferase (ALT) level [30, 31], ultrasound [32] and ^{1}H-MRS [33, 34] and is substantially increased (83%) among morbidly obese (BMI >40 kg/m^2) adolescents based on liver histology [35].

A strong body of evidence shows that increased intrahepatic lipid content measured by MRI and ^{1}H-MRS is associated with visceral adiposity [8, 36], metabolic syndrome [36] [37], glucose intolerance [36] and insulin resistance [9, 38-40] in obese children and adolescents. Although the underlying mechanisms responsible for the development of NAFLD and metabolic complications in obese youth is not clear, Fabbrini et al. [41] reported that adipose tissue lipolytic activity and the rate of free fatty acid (FFA) release into the circulation are greater in obese adolescents with NAFLD (intrahepatic triglyceride content > 10% by ^{1}H-MRS) than in those without NAFLD (intrahepatic triglyceride content $\leq$ 5.5 by ^{1}H-MRS) during both postabsorptive and hyperinsulinemic conditions. These finding suggest that the failure of insulin

to adequately suppress lipolysis and the subsequent increased delivery of FFA to liver and muscle is involved in the development of intracellular fat accumulation in the liver and impairment in insulin action in obese youth with NAFLD [41].

Lifestyle intervention is considered the first line of approach to treat obese youth with NAFLD as it does not carry side effects and confer multiple cardiometabolic benefits [42-44]. Indeed, it has been shown that the prevalence of hepatic steatosis is significantly lower in physically active adult men who exercise regularly ($\geq$ 2-3 days per week) compared with sedentary men [45]. A significant inverse association was also noted between habitual physical activity level and intrahepatic lipid content in both men and women independent of age and BMI [46]. To date, very few studies have examined the influence of sex and race on intrahepatic lipid as well as regional adiposity in obese adolescents after a 3-month training intervention. Therefore, the present study examined the effects of sex and race on intrahepatic, total and visceral adipose tissue in overweight adolescents following a 3-month training intervention.

1.3 SPECIFIC AIMS

1.3.1 Primary specific aim

To examine the effects of sex on the change in intrahepatic lipid (IHL) and total (TAT) and visceral (VAT) adipose tissue in response to a 3-month aerobic (AE) versus resistance (RE) training program without calorie restriction.

Hypothesis 1)

It was hypothesized that the change in IHL, TAT and VAT will be significantly different between adolescent males and females in response to a 3-month AE versus RE training program without calorie restriction.

1.3.2 Secondary specific aim

To examine the effect of a 3-month AE vs. RE training program without calorie restriction on cardiorespiratory fitness (CRF) and muscular strength in overweight adolescent males and females.

Hypothesis 1)

It was hypothesized that the AE group will have significantly greater improvement in CRF compared with the RE group.

Hypothesis 2)

It was hypothesized that the RE groups will have significantly greater improvement in muscular strength compared with the AE group.

1.3.3 Exploratory Aim

To examine the effects of race on the change in IHL, TAT and VAT in response to a 3-month AE versus RE training program without calorie restriction.

Hypothesis 1)

It was hypothesized that white adolescents will have significantly greater reductions in IHL, TAT and VAT compared with black adolescents in response to a 3-month AE versus RE training program without calorie restriction.

2.0 CHAPTER TWO

2.1 LITERATURE REVIEW

2.1.1 Background

According to the National Health and Nutrition Examination Survey (NHANES), the prevalence of childhood obesity still remains high, currently affecting one in three US children and adolescents [47]. As with the increases in childhood obesity, pediatric NAFLD has become the most common form of liver disease that is strongly associated with obesity and insulin resistance in the pediatric population [3, 42]. Fatty liver disease is now recognized as a complication of obesity, and as a component of the cluster of obesity-related features that indicate a risk of cardiovascular diseases, known collectively as the metabolic syndrome.[48] The effects of obesity-related metabolic derangements in the liver are most commonly manifested by the accumulation of excess free fatty acids and triglycerides, which is referred to as hepatic steatosis. NAFLD is an umbrella term that refers to a broad spectrum of lesions that range from steatosis without necroinflammatory injury to active lesions of hepatocyte injury, cell death and inflammation.[49]

Limited evidence from the short-term and long-term lifestyle intervention studies suggest that weight loss induced by increasing physical activity and calorie restriction is beneficial to

reduce NAFLD and associated health risks such as insulin resistance and dyslipidemia in obese children and adolescents. At present, evidence regarding the role of regular exercise alone without calorie restriction or without weight loss as a treatment strategy for pediatric NAFLD is unclear and requires further investigation. Although the findings have been inconclusive, a few short-term efficacy trials [8, 10, 16] have sought to delineate the effect of regular exercise from that of calorie restriction. Given the adverse health outcomes (e.g., weight re-gain, binge eating, liver injury) of energy restrictive diets on long-term weight control in children and adolescents [50, 51], adoption of a physically active lifestyle and gradual weight loss should be considered when developing intervention strategies to reduce NAFLD in youth.

2.1.2 Effect of sex and race on non-alcoholic fatty liver

While most adolescents with NAFLD tend to be overweight or obese, only a subset of obese children or adolescents will develop NAFLD [26]. The prevalence of obesity among male children and adolescent's aged 2 through 19 years (18.6%) was significantly higher than among female children and adolescents (15.0%) between the years of 1999-2010 [47]. The impact that gender plays on the prevalence of NAFLD remains unknown. Early studies in adults with NASH present mixed results when examining the impact of gender. Lee et al. [52] performed a review of 543 liver biopsies diagnosed as alcoholic hepatitis that yielded 49 cases of NASH. The patients were commonly middle-aged women who were obese and often diabetic. Furthermore, women continue to outnumber men in clinical reports of NAFLD. However, some clinical series of NASH and epidemiologic studies of NAFLD suggest that men are at least as likely as women to have NAFLD. Data analyzed from the third NHANES revealed a prevalence of aminotransferase elevation in the United States was 7.9%. Aminotransferase elevation was more

common in men compared to women (9.3% vs. 6.6%, $p = 0.002$), in Mexican Americans (14.9%) and non-Hispanic blacks (8.1%) compared to non-Hispanic whites (7.1%, $p < 0.001$) [53].

Clinical series of children and adolescents with NAFLD consistently exhibit a predominance of males versus females. In a prospective series of 36 children with NASH, Rashid et al. [5] found heterogeneous results. Boys were more common than girls in this series of children. Obesity was the most common clinical denominator. The average patient had a body weight approximately 50% higher than the ideal for height. Tominaga et al. [4] determined that the overall prevalence of fatty liver in 810 Japanese children ages 4-12 years was 2.6%. Fatty liver prevalence, determined via ultrasound, was higher for boys (3.4%) than for girls (1.8%), although the difference was not statistically significant. Similarly, Schwimmer et al. [23] performed a retrospective analysis of 43 children with biopsy-proven NAFLD and identified significant predictors of liver steatosis, inflammation, and fibrosis. The researchers determined that 30 subjects (70%) were male. In a retrospective review of 82 patients with biopsy-proven hepatic steatosis, Baldridge et al. [54] determined that there is an association of obesity, hyperlipidemia, male gender, and possibly the hormonal changes associated with puberty that contributes to changes in the liver. In a similar retrospective study reviewing adolescent liver biopsies over a 27-year period, Australian researchers found that approximately 65% of subjects with biopsy-proven liver steatosis were male [20]. In a group of 127 obese adolescents in whom 27% had unexplained ALT elevation, Schwimmer et al. [26] concluded that there were a significantly higher percentage of males (44%) with increased ALT than females (7%). Whether the gender distribution of pediatric patients with NAFLD accurately reflects the predominance of NAFLD in boys over girls or represents a selection bias is unknown [26].

Early clinical series of pediatric NAFLD have included predominantly children of white or Asian race, possibly reflecting the community demographics of reporting centers. More recent research limited to the southwestern United States raise the possibility that Mexican American children have higher rates of NAFLD than non-Hispanic children. A retrospective review of 43 children with NAFLD resulted in a racial and ethnic distribution 53% Hispanic, 25% white non-Hispanic, 5% black non-Hispanic, and 17% other and did not differ by sex. The researchers diagnosed NAFLD more commonly in Mexican-American children than in children of other ethnic groups [23]. One motive for this may be that body fat distribution effects hepatic lipid accumulation and insulin sensitivity and varies by race and ethnicity. In a study population of 297 adolescent males, DEXA body composition analysis results showed that Hispanic males had higher body fat values than the white group, whereas the black males generally had lower values than the white group. When adjusted for body size, the Hispanic males continued to have significantly higher body fat and percentage fat than the white or black males [55].

In a more recent study, significant differences in obesity prevalence by race/ethnicity were found. In 2009-2010, 21.2% of Hispanic children and adolescents and 24.3% of non-Hispanic black children and adolescents were obese compared with 14.0% of non-Hispanic white children and adolescents [47]. This greater total body adiposity may lead to a greater degree of steatosis [23]. Flores-Calderon et al. [22] measured increased aminotransferases and associated metabolic anomalies among overweight and obese children in an elementary school in Mexico City. The researchers determined that 42% of obese and overweight children had increased ALT levels.

Although black children are known to have high rates of risk factors for NAFLD very few studies have measured NAFLD in African-American youth. In a study comparing interethnic

differences of muscle, liver and abdominal fat in obese adolescents researchers found that African-Americans had lower IMCL and liver fat content than Hispanics. In contrast, the Hispanic adolescents had significantly higher IMCL levels compared to their Caucasian and African-American peers, and had a significantly greater lipid content in the liver than the African Americans. The ethnic differences in IMCL were independent of age, gender and overall adiposity. Moreover, the liver of these obese Hispanic adolescents like that of the Caucasians had a two-fold increase in hepatic fat fraction than the African-American group [56]. Similar results were observed in obese adolescents by Schwimmer et al. [26], where the prevalence of abnormal ALT differed significantly by race and ethnicity (Hispanic: 36%; white: 22%; black: 14%). African-American children, relative to Caucasian children, may be more likely to develop T2DM due to obesity-independent hyperinsulinemia and insulin resistance, but appear less predisposed to the obesity-related clustering of risk factors associated with the metabolic syndrome. Mexican-American children may be more likely than Caucasian children to develop the metabolic syndrome due to greater obesity-related hyperinsulinemia and dyslipidemia [24]. Whether black children have lower rates of NAFLD or, alternatively, have NAFLD that remains undiagnosed are unknown. A better understanding of the influence of gender, race, and ethnicity may provide additional insight into the pathophysiology of NAFLD.

2.1.3 Measurements of intrahepatic adiposity

2.1.3.1 Liver Biopsy

Liver biopsy is usually the most specific test to assess the nature and severity of liver diseases [57] and has been acknowledged as the “gold standard” for diagnosis and assessment of extent of injury in NASH [58]. Whereas laboratory test abnormalities and radiographic findings may be

suggestive of NAFLD, histological evaluation remains the only means of accurately assessing the degree of steatosis, the distinct necroinflammatory lesions and fibrosis of NASH, and distinguishing NASH from "simple" steatosis, or steatosis with inflammation [59]. Liver biopsy not only provides important prognostic information but also remains crucial in the development of therapeutic protocols for certain forms of NAFLD [60].

However, this procedure has many drawbacks, even when performed under ultrasound guidance, including discomfort due to its invasive nature, risk of infection, hematoma formation or more significant internal bleeding, and potentially biliary leakage [61]. Furthermore, biopsies are subject to sampling error, providing quantitative information on a rather small volume of liver. The size of the biopsy specimen, which varies between 1 and 3 cm in length and between 1.2 and 2 mm in diameter, represents 1⁄50,000 of the total mass of the liver [57]. Another limitation of the method is the fact that a pathologist normally performs the estimation of fat semiquantitatively. All of these potential pitfalls might be corrected by multiple sampling and standardization in processing biopsies. However, another possible bias, which might definitively question the value of histology in assessing fat in the liver, is the degree of consistency among pathologists in interpreting the biopsies [62]. A study performed by El-Badry and colleagues [62] found inconsistent histologic assessment of hepatic steatosis among pathologists with the highest level of expertise from well-recognized European and American institutions. The need for an accurate noninvasive method is especially important since steatosis is becoming widespread in the child population, in which invasive methods must be avoided. [63]

2.1.3.2 Enzymes

Mildly to moderate elevated serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), or both are the most common and often the only laboratory abnormality

found in patients with NALFD [2]. Liver disease is the most important cause of increased ALT activity and a common cause of increased AST activity [64]. It has been shown that an AST/ALT ratio >1 significantly correlates with the presence of advance liver fibrosis possibly due to impaired AST clearance by the sinusoidal liver cells [65-67]. Sorbi et al. [67] determined that patients with biopsy-proven mild fibrosis had a mean AST/ALT ratio 0.9 ± 0.1 and patients with cirrhosis had a mean ratio of 1.4 ± 0.2. In an earlier study performed by Nanji et al. [68], a significant correlation between the AST/ALT ratio and degree of fatty infiltration of the liver was found. The authors hypothesized that damage mainly to the plasma membrane allowed for the loss of cytoplasmic enzymes rather than the loss of mitochondrial enzymes. This ratio can be of help when trying to identify liver disease progression in a NASH patient who does not initially present fibrosis in a biopsy. The American Academy of Pediatrics recommended that serum ALT and AST be performed in all overweight children starting at age 10 years if their BMI is ≥95th percentile or between the 85^{th} and 94^{th} percentile with risk factors. ALT and AST are to be checked in addition to fasting glucose and the lipid profile [69]. However, recent research by Browning et al. [7] provides evidence that normal serum ALT levels were prevalent in four fifths of subjects with biopsy proven hepatic steatosis. These results demonstrate that ALT levels may appear to be an insensitive marker for both hepatic steatosis and NASH in both children and adults [7, 69].

2.1.3.3 Ultrasound

Fatty liver is an entity histologically defined by the degree of fatty change and until 1976 liver biopsy was the only method of determining this change [70]. Taylor et al. [71] used ultrasonic images to determine that fatty infiltration of the liver gives rise to high-level echoes distributed in a well-defined pattern. Ultrasound is now the most common imaging modality used to initially

evaluate and diagnose hepatic steatosis because of its low cost, noninvasiveness, and widespread availability [72]. More recently, ultrasound has been correlated very closely with the severity of steatosis in large cohorts of children with biopsy-proven NAFLD [69]. Similar results have been seen in a recent study performed by Chiloiro et al. [73]. Researchers determined that ultrasound assessment of fatty liver was positively associated to anthropometric measurements and insulin resistance without a significant increase in liver enzymes. These results indicate that the measurement of liver enzymes alone are unreliable in fatty liver screening and that ultrasound may be an excellent screening tool for NAFLD in children due to its non-invasiveness and its accuracy in detecting fat infiltration in the liver.

Despite ultrasounds accuracy at detecting intrahepatic lipid content it has some undeniable flaws. The operator dependency of ultrasound, its inability to precisely quantify hepatic fat content, and its inability to detect small changes in liver fat with time, all potentially limit its use in longitudinal clinical studies [74]. In a very large and in-depth study performed by Saadeh et al. [75] radiological imaging modalities including ultrasound failed to distinguish between NASH and other forms of NAFLD and was also unable to detect individual pathologic features that are essential in the diagnosis of NASH [60, 76].

2.1.3.4 Computed Tomography

Some of the limitations of ultrasonography can be diminished with the use of a computed tomography (CT) scan. Hepatic steatosis produces a decrease of hepatic parenchymal attenuation in comparison to the surrounding blood vessels as well as the spleen and the kidneys [77]. A CT scan is capable of depicting fatty infiltration of the liver as a decrease in attenuation where the degree of fatty infiltration of the liver correlates with the degree of decreased attenuation [78]. After multiple areas in liver and spleen are scanned, the average of these measurements is

calculated as average density (in Hounsfield units) in the region of interest [79]. Typically, the attenuation of the liver is 50 to 75 Hounsfield units in a non-contrast CT scan and decreases by 1.6 Hounsfield units for every milligram of triglyceride deposited per gram of liver tissue [80]. The liver-to-spleen ratio has been reported to correlate well with the degree of fatty liver infiltration as measured by calibrated CT [81]. The spleen is chosen as the organ to relate to the liver because the spleen is a large organ that can easily be ascribed a CT number; the spleen is at approximately the same transverse level as the liver and beam-hardening artifacts would affect both organs similarly; and the spleen is relatively inactive metabolically, and large fluctuations in chemical content do not occur [82]. The spleen remains an accurate internal constant when the liver stores lipid, glycogen or iron due to the fact that the spleen does not participate in these storage processes. It has been determined that the optimal liver-to-spleen ratio to predict less than 30% of hepatic steatosis would be a ratio of 1.1, with a sensitivity and specificity of 0.833 and .815 respectively [83].

Obtaining a CT image that contains both liver and spleen presents a challenge; variations exist not only in the vertical positioning of the spleen relative to the liver but also in positioning of both organs within the abdominal cavity [84]. While CT has a high sensitivity and specificity for detecting moderate to severe levels of fatty infiltration in the liver, sensitivity is poor at lower levels [85]. In addition, CT scanning has the drawback of exposing subjects to ionizing radiation making a multi-image approach not feasible. The above two factors limit its potential use in longitudinal studies and in children [74].

2.1.3.5 Magnetic Resonance Spectroscopy

For medical diagnosis or biochemical analysis accurate and efficient quantification of magnetic resonance spectroscopy (MRS) signals is of utmost importance [86]. MRS provides a means of

accurately measuring intrahepatic adiposity with a non-invasive technique. Of all the MR techniques, chemical shift gradient-echo technique is the most sensitive to detect fat and has been used to evaluate hepatic steatosis [87]. Chemical shift magnetic resonance imaging techniques are easy to perform, widely available, and provide spatial coverage of the entire liver [88]. This method also enables the identification of tissues that contain a significant portion of intracellular lipid [74]. The Dixon technique utilizes the phase shift between water and fat resonances due to their different Larmor frequencies [89]. Adding and subtracting the in-phase and opposed-phase images, even in body regions with inhomogeneities of the static magnetic field, can obtain water- and fat-selective images. The Dixon techniques require acquisition and post-processing of several data sets with different echo times for calculation of the fat content [90].

Localized ^{1}H-MRS is an alternative, noninvasive method to measure intrahepatic lipid content. Because values given by ^{1}H-MRS correlate with liver biopsy results [91], it is widely considered to be the optimal noninvasive method to assess and diagnose hepatic steatosis [92]. Longo et al. [91] studied a population of subjects with NAFLD and found the percentage of intrahepatic lipid measurements using ^{1}H-MRS correlated well with those obtained by CT biopsy. ^{1}H-MRS involves the use of a strong magnet and nonionizing radio frequency waves to acquire data and provides quantitative information on the biochemical profile within tissues. The primary scientific principle of MRS is based on the influence of the cellular chemical environment on the local magnetic field experienced by nuclear protons. Protons in different chemical environments oscillate at different frequencies, known as the resonance frequency. Therefore, chemically different atoms, such as the ^{1}H in water and fat, and morphologically different atoms, such as the ^{1}H in intramyocellular and extramyocellular fat, can be distinguished

by their resonance frequencies. The change in resonance frequency is called the chemical shift and is used to distinguish between different metabolites [93]. ^{1}H-MRS relies on a single measurement from a predetermined region of interest where a voxel is positioned at the right hepatic lobe, avoiding inclusion of the diaphragm, edges of the liver and vascular and biliary structures [79]. Although intrahepatic lipid content has been found to be homogenous throughout the right lobe of the liver [61], it has become common practice to average 3 separate voxels to help correct for any potential regional differences in lipid content [93]. Research performed by Szczepaniak et al. [94] demonstrated the use of ^{1}H-MRS, which is primarily used in small-scale protocols, could be a reliable method in a large clinical trial to assess intrahepatic lipid. This study as well as others provided much needed information to help define the normal amount of intrahepatic fat in healthy subjects [94, 95]. The 95th percentile for intrahepatic lipid content was 5.6% [94] and 3% [95] in these 2 studies, supporting the notion that an intrahepatic fat content of approximately 5% is an appropriate upper limit.

2.1.4 Intrahepatic adiposity and health risks

With the increasing prevalence of obesity and diabetes mellitus in the pediatric population, NAFLD has become the most common form of pediatric liver disease [3, 7]. NAFLD represents a spectrum of conditions characterized by macrovesicular hepatic steatosis and little or no exposure to ethanol. The liver pathology encompasses a range from isolated fatty liver to steatohepatitis, advanced fibrosis, and cirrhosis [23]. Under normal conditions the liver is a non-adipose, highly cellular, solid organ tissue with a limited capacity for lipid storage [90]. Current evidence on intrahepatic lipid points to a disarrayed interaction between adipocytes and muscle cells in an inflammatory background resulting from excessive macrophage infiltration and

inflammatory mediator production. The end result is increased lipid delivery to non-adipose tissues such as the liver, which overpowers their clearance capacity and produces ectopic fat deposition [96].

Hepatic glucose output is increased among subjects with a high liver fat content. This phenomenon contributes to glucose intolerance and largely explains the hyperglycemic state of patients with type 2 diabetes mellitus, because their hepatic glucose production also becomes resistant to the inhibitory effect of insulin [97]. The most widely held view is that central obesity is the initial event in the development of insulin resistance, because it leads to increased free fatty acid (FFA) levels in the portal and peripheral circulations [98]. Failure of insulin to inhibit triglyceride lipolysis in insulin-resistant states leads to the oversupply of FFA to the liver, excess hepatic triglyceride synthesis, and intracellular accumulation of toxic lipid products that impair insulin signaling and activate inflammatory pathways [99]. FFAs have been shown to enhance hepatic gluconeogenesis, to impair insulin metabolism with resultant hyperinsulinemia and to stimulate triglyceride-rich VLDL synthesis in the liver [100].

Fabbrini et al. [101] determined that increased intrahepatic lipid is an independent indicator of multi-organ insulin resistance and increased hepatic secretion of very low-density lipoprotein-triglyceride (VLDL-TG). In addition, fatty acids released from lipolysis of intrahepatic lipid might stimulate hepatic VLDL-TG production, demonstrating that intrahepatic lipid in itself could be directly involved in the pathogenesis of dyslipidemia associated with NAFLD [101]. NAFLD is a marker of pathological ectopic fat accumulation combined with a low-grade chronic inflammatory state affecting adipose tissue and characterized almost universally by insulin resistance. There is convincing evidence that worsening grades of NAFLD contribute to progressive cardiometabolic risk, such that NASH represents a marker as well as a

mediator of increased cardiovascular risk more than simple steatosis [102]. Recent research by Schwimmer et al. [37] showed that children with fatty liver disease had significantly higher fasting glucose, insulin, total cholesterol, and blood pressure than children of a similar weight and age without NAFLD. Children with fatty liver disease also had significantly lower levels of HDL "good" cholesterol than the others [37]. Intrahepatic fat content is frequently referred to as an additional feature of the metabolic syndrome. Research by Kotronen et al [103], revealed that liver fat was fivefold higher in participants with than in those without the metabolic syndrome, a finding that was independent of obesity.

2.1.5 Physical activity and non-alcoholic fatty liver

2.1.5.1 Associations between leisure-time physical activity, cardiorespiratory fitness and non-alcoholic fatty liver

To date five cross-sectional studies have examined the association between leisure-time physical activity and pediatric non-alcoholic fatty liver in obese youth (Table 1). The majority of studies used questionnaires to evaluate physical activity levels and reported that participation in leisure-time physical activities are significantly lower and time spent in sedentary activities are higher in obese youth with NAFLD compared with their lean or obese counterparts without NAFLD. Lee et al. [104] have shown that in Chinese and Malay children (11.1 ± 3.0 yrs), elevated ALT and aspartate aminotransferase (AST) levels are significantly associated with reduced recreational physical activities after accounting for age, gender and ethnicity. Using an Armband, which provides a better proxy of leisure-time physical activity levels than questionnaires, Fintini et al. [105] reported that youth with biopsy-proven NAFLD had significantly lower total energy expenditure, spent less time in physical activity [>3 metabolic equivalent of task (METs)] and

more hours in sedentary activities (<3 METs) compared with their normal-weight healthy controls. Similarly, a recent study in Canada reported that youth with hepatic steatosis evaluated by sonography spent more than 65% of leisure time during the day in sedentary activities such as watching television or playing computer/video games [106].

Given that self-reported physical activity using questionnaires are prone to error and misclassification [107] and that CRF is more strongly associated with metabolic risk factors than leisure-time activity in children and adolescents [108], several studies employed CRF tests as a surrogate measure of physical activity (Table 2). Martins et al. [109] reported that obese fit children (10-11 yrs) have 48% less risk of being classified as having the metabolic syndrome compared with their obese unfit counterparts. In a large sample of normal-weight and obese adolescents (n = 100, 15.6 ± 1.4 yrs.), Kelishadi et al. [110] have shown that the prevalence of hepatic steatosis evaluated by ultrasound was higher in obese healthy youth (12.4%) and obese youth with metabolic syndrome (21.1%) than in normal-weight youth with (5.6%) and without (3.2%) metabolic risk factors. In that study, CRF had the strongest inverse correlation with ALT level and homeostatic model assessment of insulin resistance (HOMA-IR), independent of obesity and metabolic abnormality status.

To our knowledge, we are aware of only two studies wherein the influence of CRF on intrahepatic lipid content was measured by ^{1}H-MRS in children and adolescents. Currently, ^{1}H-MRS is considered the gold-standard non-invasive imaging technique for quantifying liver fat since intrahepatic lipid content measured by ^{1}H-MRS correlates well with histological analysis of liver biopsy samples [91, 111]. Using this technique, Wittmeier et al. [40] have shown that peak oxygen uptake during a maximal graded cycle ergometer test was 10% lower and intrahepatic lipid content was 2.6 times higher in youth with type 2 diabetes mellitus (T2DM) compared to

age, BMI and total fat matched overweight controls. Further, collapsed across groups, CRF was inversely associated with intrahepatic lipid content (r = -0.22) and positively associated with insulin sensitivity (r = 0.29) after accounting for confounding variables (sex, age, ethnicity and visceral to subcutaneous fat ratio). However, the same research group recently reported no significant associations between CRF and intrahepatic lipid in healthy overweight and obese adolescents without impaired glucose tolerance and T2DM [34]. As the methodologies used (^{1}H-MRS and cycle ergometer) were identical in both studies [34, 40], these disparate findings may be attributed to differences in metabolic status.

To date limited data are available regarding the influence of CRF on non-alcoholic fatty liver in children and adolescents. Given the strong associations between intrahepatic lipid, visceral fat and metabolic risk factors, it is unclear whether the effect of CRF on hepatic steatosis is mediated by the degree of adiposity and/or insulin resistance or if there is an independent relationship between CRF and hepatic steatosis. Nonetheless, given that low CRF is a strong risk factor of cardiovascular disease (CVD), insulin resistance [112-114] and visceral adiposity [115], effective intervention strategies are needed to improve CRF and related health risk factors.

2.1.5.2 Effects of short-term exercise with and without calorie restriction on non-alcoholic fatty liver

Currently, no specific lifestyle intervention guidelines are available for the prevention and management of NAFLD both in adults and in children. This is perhaps due to the small number of children enrolled, short-duration of the follow-up period, and the lack of histological confirmation of NAFLD in the current literature. This results in insufficient knowledge of the pathogenesis of the disease, thereby focusing on the treatment of the morbidities associated with NAFLD, rather than to the direct treatment of NAFLD per se [44, 116]. Diet and exercise are

generally recommended as they do not carry side effects and confer multiple cardiometabolic benefits and small changes in weight are associated with significant reductions in intrahepatic lipid content in obese adolescents [8].

To date, we are aware of six short-term (<6 month) experimental studies, wherein the effect of exercise alone or in combination with calorie restriction on the prevalence of NAFLD or intrahepatic lipid content was examined in children and adolescents (Table 3). van der Heijden et al. [10] performed a non-randomized controlled study to examine the effect of 12-weeks aerobic exercise alone (e.g, without calorie restriction) performed on treadmill, elliptical or a bike (120 min/week, target heart rate >140 beats/min) in normal-weight and obese Hispanic adolescents. Despite no changes in body weight and calorie intake in the study, significant reductions in intrahepatic lipid content (-3.3%) by ^{1}H-MRS, visceral fat (-5 cm^{2}) and HOMA-IR was observed in obese adolescents, but not in normal-weight adolescents. Subsequently, the same group [16] performed a non-randomized study to examine the effects of resistance training (supervised, 2 times/week, 60 min/session), independent of calorie intake, on intrahepatic lipid and associated metabolic risk factors in a small number of obese Hispanic adolescents (n = 12). Although significant increases in strength, lean body mass, and hepatic insulin sensitivity were noted after 12 weeks, no improvements in intrahepatic lipid, visceral fat and peripheral insulin sensitivity were found in the study.

At present, there has only been one study that examined the influence of aerobic versus resistance exercise alone on intrahepatic lipid content and associated health risk factors in youth. Using a randomized controlled design, Lee et al. [8] have recently demonstrated that 3 months of supervised aerobic and resistance training significantly reduces intrahepatic lipid content (aerobic: -1.9% vs. resistance: -2.0%) and visceral (aerobic: -0.1 kg vs. resistance: -0.2 kg) and

total fat (aerobic: -3.0 kg vs. resistance: -2.5 kg) in previously sedentary, obese adolescent boys. Furthermore, our observation that visceral fat is associated with intrahepatic lipid content (r = 0.55) at baseline and that changes in visceral fat are significantly associated with corresponding changes in intrahepatic lipid content (r = 0.72) suggest a potential link between the two fat depots. Further our observation [8] is similar to a previous finding by Tock et al. [117] who reported significant reductions in NAFLD prevalence by ultrasonography (from 52% to 29% on the right side and from 48% to 29% on the left side) and visceral adiposity in obese adolescents after a 12-week exercise program with calorie restriction.

Currently, very little is known about the effect of exercise alone as a treatment strategy to reduce NAFLD in children and adolescents. Additionally, there is sparse data regarding the optimal exercise regimen (e.g., type, dose, intensity) that should be prescribed for the treatment of youth with NAFLD. It appears that in studies reporting significant reductions in intrahepatic lipid content in response to exercise with and without calorie restriction, there were concurrent reductions in visceral fat and insulin resistance, which suggest that exercise strategies to target abdominal obesity reduction and insulin resistance may also be an effective treatment for youth with NAFLD.

2.1.5.3 Effects of long-term lifestyle intervention on non-alcoholic fatty liver

Currently, we are aware of five studies [17, 118-121] that have studied the long-term ($\geq$6 months) effect of lifestyle interventions on NAFLD in children and adolescents. In majority of these studies, lifestyle intervention strategies consisted of both increasing physical activity in conjunction with reducing caloric intake tailored on the individual basis. In 53 children and adolescents with biopsy proven NAFLD (5.7-18.8 yrs), Nobili et al. [119] demonstrated that a 2-

year lifestyle intervention consisting of monthly dietary counseling and physical activity (45 min/d aerobic exercise) was associated with a weight loss (~ -5 kg), resulting in significant improvements in liver histology and insulin resistance and reductions in dyslipidemia and liver enzyme levels (ALT and AST). Similarly, Reinehr et al. [120] reported that unlike obese control subjects without lifestyle intervention, obese children with NAFLD who participated in a 2-year lifestyle intervention consisting of physical activity, nutrition education and behavior therapy resulted in a significant weight loss and decreases in liver enzymes and the prevalence of hepatic steatosis as evaluated by ultrasound. In this study, the greater obesity reduction was associated with greater decreases in liver enzymes and prevalence of NAFLD [120].

To our knowledge, we are aware of only one long-term study that examined the effect of increasing physical activity alone on intrahepatic lipid content in obese youth. Pozzato et al. [121] examined the effect of a 1-year lifestyle intervention composed of increasing physical activity (30-45 min/day, aerobic exercise) and maintaining a normocaloric balanced diet (55-60% carbohydrate, 25-30% fat, 12-15% protein) in 26 obese children (6-14 yrs). After the intervention, the average reduction of hepatic fat fraction was 8% as evaluated by a chemical-shift MRI and the prevalence of liver steatosis (defined by hepatic fat fraction $\geq$ 9%) decreased significantly from 34.6% to 7.7%. In addition, significant reductions in triglycerides, total cholesterol, apolipoprotein A1 and apolipoprotein B were also observed in addition to decreases in BMI z score (change: -0.26) and waist circumferences (change: -1.5 cm).

Although there is evidence to suggest that weight loss accomplished by long-term lifestyle intervention is beneficial to NAFLD as evaluated by liver enzymes and imaging modalities (e.g., MRI and ultrasound) in children and adolescents, no specific dietary and physical activity guidelines are available to achieve optimal weight loss that is associated with

reductions in metabolic complications related to NAFLD. This is perhaps due to the small number of long-term studies and lack of a liver biopsy data at follow-up in the current pediatric literature [43]. It has been suggested that sudden or quick weight loss achieved through dietary modification may accelerate the progression from simple hepatic steatosis to non-alcoholic steatohepatitis, therefore gradual weight loss is recommend [51]. Further, long-term lifestyle intervention strategies to treat youth with non-alcoholic fatty liver should focus on increasing physical activity, which is shown to be effective in reducing visceral adiposity [8, 10] and insulin resistance [8, 15, 122] in obese children and adolescents.

Table 1. Associations between leisure-time physical activity and NAFLD in children and adolescents (cross-sectional studies)

References	N	Age (y)	BMI or BMI % tile	Assessments		Main Findings
				PA	Fatty Liver	
Lee et al. [104]	201Obese (Chinese + Malay)	11.1 ± 3.0	31.9 ± 5.5 kg/m^2	Questionnaire	Liver function test (ALT, AST, GGT)	Elevated liver transaminases are significantly associated with lower physical activity level (OR =2.4, 95% CI; 1.1-5.1).
Tsuruta et al. [123]	288 (Japanese)	NR (high school students)	19.2 ± 2.7 kg/m^2	Questionnaire	Liver function test (ALT, GGT) Ultrasound	Participation in extracurricular sports activities is significantly lower in students with NAFLD compared with students without NAFLD.
Mager et al. [106]	38 Overweight and obese with NAFLD	14.1 ± 3.2	30.2 ± 5.3 kg/m^2	Questionnaire	Liver function test (ALT, GGT) Ultrasound	In youth with NAFLD, more than 65% of leisure time during waking hours was spent in sedentary activities (e.g., TV watching or playing computer/video games).
Hattar et al. [124]	17 Lean 20 Obese 20 NASH	12.4 ± 2.1 11.9 ± 2.0 12.2 ± 2.0	48.9 ± 21.8% tile 98.3 ± 1.4% tile 98.1 ± 1.7% tile	Questionnaire	Biopsy proven NASH	Average physical activity score was lowest in the NASH group (NASH =1.3 vs. obese=2.3 vs. lean=2.4).
Fintini et al. [105]	41 Lean 30 Obese 40 NAFLD	12.4 ± 2.7 12.9 ± 2.9 12.8 ± 2.3	19.7 ± 2.7 kg/m^2 27.6 ± 2.5 kg/m^2 26.2 ± 3.2 kg/m^2	Questionnaire Armband (5-7 consecutive days)	Biopsy proven NAFLD	Armband measured sedentary activity was higher in youth with NAFLD compared with lean and obese youth without NAFLD. Obese youth with and without NAFLD spent less time in physical activity compared with lean youth (NAFLD=1.6 h/day vs. obese=1.7 h/day vs. lean=2.3 h/day).

BMI, body mass index; BMI % tile, BMI percentile; PA, physical activity; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyl transpeptidase; OR, odd ratio; CI, confidence interval; NR, not reported; NASH, nonalcoholic steatohepatitis; NAFLD, non-alcoholic fatty liver disease.

Table 2. Associations between cardiorespiratory fitness and NAFLD in children and adolescents (cross-sectional studies)

References	N	Age (y)	BMI or Bodyweight	Assessments		Main Findings
				CRF	Fatty Liver	
Kelishadi et al. [110]	23 NWMN 24 NWMA 24 POMN 24 POMA	12-18	20.7 ± 0.5 kg/m^2 20.2 ± 0.7 kg/m^2 26.4 ± 0.5 kg/m^2 26.2 ± 0.6 kg/m^2	Cycle	Ultrasound, ALT	Independent of obesity and metabolic abnormality status, CRF had the highest inverse association with ALT level.
Manco et al. [125]	11 Lean 20 Obese 20 NAFLD	12.7 ± 1.6 12.1 ± 2.4 12.7 ± 2.6	18.7 ± 1.8 kg/m^2 26.3 ± 3.1 kg/m^2 26.3 ± 4.0 kg/m^2	Cycle	Biopsy proven NAFLD	During a cycle ergometer max test, the highest changes in cardiac output and total vascular peripheral resistance were observed in the NAFLD group compared to the lean and obese groups. 10 children in NAFLD group (50%) showed hypertensive response to exercise.
Wittmeier et al. [40]	13 Lean 97 Overweight 27 T2DM	16 ± 1.8 15 ± 1.7 15 ± 1.5	60.0 ± 10.8 kg 87.9 ± 18.4 kg 96.0 ± 22.1 kg	Cycle	^{1}H-MRS	CRF was ~10% lower and hepatic triglyceride content was 2.6 times higher in overweight youth with T2DM compared with overweight controls. Collapsed across groups, CRF was inversely associated with hepatic triglyceride content ($r = -0.22$) and this remained significant after adjusting for age, gender, ethnicity and visceral to SAT ratio.
Wicklow et al. [34]	11 Lean 68 Overweight 30 Overweight with NAFLD	16 (13-17) 15 (13-18) 15 (13-17)	body fat=23.4 % body fat=37.1 % body fat=38.7 %	Cycle	^{1}H-MRS	CRF was not significantly different between youth with (26.0 mg/kg/min) and without NAFLD (27.2 mg/kg/min).
Martins et al. [109]	79 Obese	11-13	NR	20-m shuttle run	ALT	Fit obese children with high ALT values were less likely (OR=0.52) to be classified as having the metabolic syndrome than their unfit peers.

CRF, cardiorespiratory fitness; NWMN, normal-weight metabolically normal; NWMA, normal-weight metabolically abnormal; POMN, phenotypically obese metabolically normal; POMA, phenotypically obese metabolically abnormal; CRF, cardiorespiratory fitness; ALT, alanine aminotransferase; NAFLD, non-alcoholic fatty liver disease; T2DM, type 2 diabetes mellitus; NR, not reported; OR, odd ratio.

Table 3. Effects of short-term (<6 months) exercise with and without calorie restriction on NAFLD

References	Subjects	Treatment	Age (y)	BMI	Exercise Prescription	Duration	Liver fat measure	Δ Body weight or BMI	Δ VAT	Δ Liver fat
Exercise without calorie reduction										
van der Heijden et al. [10]	14 Lean 15 Obese (Hispanic)	AE AE	15.1 ± 0.3 15.6 ± 0.4	20.6 ± 0.8 kg/m^2 33.7 ± 1.1 kg/m^2	Supervised, 4 d/wk, 30 min/d, treadmill, elliptical or bicycle, HR at ~70% VO_{2peak}.	12 weeks	^{1}H-MRS	NS NS	NS -5 cm^2	NS -3.3%
van der Heijden et al. [16]	12 Obese (Hispanic)	RE	15.5 ± 0.5	35.3 ± 0.8 kg/m^2	Supervised, 2 d/wk, 60 min/d, 10 exercises (2-3 sets, 8-20 reps, ~80-85% 3-rep max)	12 weeks	^{1}H-MRS	+ 2.6 kg	NS	NS
Lee et al. [8]	45 Obese boys	Control	14.8 ± 1.4	33.9 ± 4.2 kg/m^2	Remain sedentary	13 weeks	^{1}H-MRS	+2.6 kg	+0.2 kg	+0.9 %
		AE	15.2 ± 1.9	36.6 ± 5.9 kg/m^2	Supervised, 3d/wk, 60 min/d, ~60-75% VO_{2peak} (treadmill, elliptical)			-0.04 kg	-0.1 kg	-1.9 %
		RE	14.6 ± 1.5	34.5 ± 2.4 kg/m^2	Supervised, 3d/wk, 60 min/d, 10 exercises, (2 sets, 8-12 reps to fatigue)			-0.6 kg	-0.2 kg	-2.0 %
Exercise with calorie reduction										
Tock et al. [117]	73 Obese	Exercise + calorie restriction	17.0 ± 1.6	36.5 ± 2.9 kg/m^2	2d/wk, 60 min/d (team sports, aerobic activities, no specific intensity) + reduce daily calorie intake (no specific target)	12 weeks	Ultrasound	~ -4.6 kg	~ -16%	Reduction in NAFLD prevalence (from 52% to 29% on the right side and from 48% to 29% on the left side).

de Piano et al. [32]	30 Obese	AE + calorie restriction + psychological intervention for both groups	15-19 yrs	33.5 ± 2.3 kg/m^2	3d/wk, 60 min/d, ~50-70% VO_{2peak} aerobic exercise + reduce daily calorie intake (no specific target)	12 weeks	Ultrasound	-2.9 kg	~ -8%	Reduction in NAFLD prevalence from 30.2% to 18.6%.
	13 Obese +NAFLD			35.8 ± 3.4 kg/m^2				-7.1 kg	~ -28%	
Wang et al. [126]	76 Obese	Control	14.0 ± 1.8	29.8 ± 2.4 kg/m^2	None	4 weeks	ALT, AST Ultrasound	+0.02 kg/m^2 -2.43 kg/m^2	NA	Greater reductions in BMI and liver enzyme in lifestyle vs. vitamin E group. No changes in ultrasound data.
		Lifestyle	13.4 ± 2.5	29.6 ± 1.5 kg/m^2	Aerobic exercise (3h/d, 7d/w, ~50-60% Max HR) + Calorie reduction (~250 kcal)			-1.45 kg/m^2		
		Vitamin E	13.4 ± 1.6	29.4 ± 3.1 kg/m^2	Vitamin E 100 mg/d					

AE, aerobic exercise; RE, resistance exercise; HR, heart rate; NS, changes are not significant; VAT, visceral adipose tissue; ^{1}H-MRS, proton magnetic resonance spectroscopy. NA, not available

3.0 CHAPTER THREE

3.1 METHODS

3.1.1 Subjects

Adolescent males and females with a BMI ≥ 95th percentile for age and gender were the target for recruitment in this 3-month exercise intervention. Posters were placed around the University of Pittsburgh campus and in the Weight Management and Wellness Clinic at Children's Hospital of Pittsburgh (CHP). Flyers were also posted in city public bus transportation and advertisements were placed in newspapers in the greater Pittsburgh area. All inclusion and exclusion criteria are summarized in Table 4. The inclusion criteria for participating subjects was as follows: 12-18 years of age, Tanner stages III-V, non-diabetic, non-smokers and sedentary (no structured physical activity, except school physical education classes, for the past 3 months). Exclusion criteria included: chronic diseases (e.g., asthma, syndromic obesity, psychiatric disorder, or diabetes), medications that may result in a change in glucose metabolism or body composition, significant weight change (BMI > 2-3 kg/m^2) over the past 3 months, and participation in structured physical activity. The participants were identified as either White or Black. A nurse practitioner assessed pubertal development based on Tanner criteria (genital development and pubic hair). All subjects were given a thorough physical examination and routine hematologic

and biochemical tests at the Pediatric Clinical and Translational Research Center (PCTRC) at CHP.

Table 4. Inclusion and Exclusion Criteria

Inclusion Criteria	Exclusion Criteria
• 12-18 years of age in Tanner stages III-V • BMI $\geq 95^{th}$ percentile for age and gender • Non-smokers • Non-diabetic • Sedentary • Black or White	• Chronic diseases - Asthma - Diabetes - Psychiatric disorders - Syndromic obesity • Medications • Significant weight change (BMI > 2-3 kg/m^2)

3.1.2 Informed consent and screening procedures

Subject eligibility was determined by phone screening. If subjects were determined to be eligible they were requested to visit the PCTRC at CHP for an informed consent and physical exam to evaluate their eligibility that lasted approximately 40-45 minutes. During this screening visit, subjects and their parents/guardians were informed about the nature of the research, risks and potential benefits of participation in this study, and their rights as a subject in this study. Once agreement was reached for participation and both, parent and child, signed the consent form, a thorough medical and physical exam was performed by the certified nurse practitioner. The physical exam included medical history, physical activity participation, Tanner stage, and measurements of height and weight.

3.1.3 Randomization

All subjects were admitted to the PCTRC for baseline evaluations including anthropometric measurements (body weight, height, BMI and WC), DEXA, abdominal MRI, CRF (VO_{2peak}), and muscular strength (1-repetition maximal, 1-RM) before and after the intervention.

After finishing baseline evaluations (e.g., body composition and metabolic health evaluations) subjects were randomly assigned to one of three groups: aerobic exercise, resistance exercise or non-exercise control group using a completely randomized design with cell sizes of 16. A staff member in the Weight management and Wellness Department who is not affiliated with this study randomly selected a piece of paper from a container with group assignments. In this study, thirty-two obese adolescent boys and thirty-two obese adolescent girls were randomized to AE (n = 32) or RE (n = 32). Of the total group 59 subjects completed the three-month intervention (Figure 1, Figure 2).

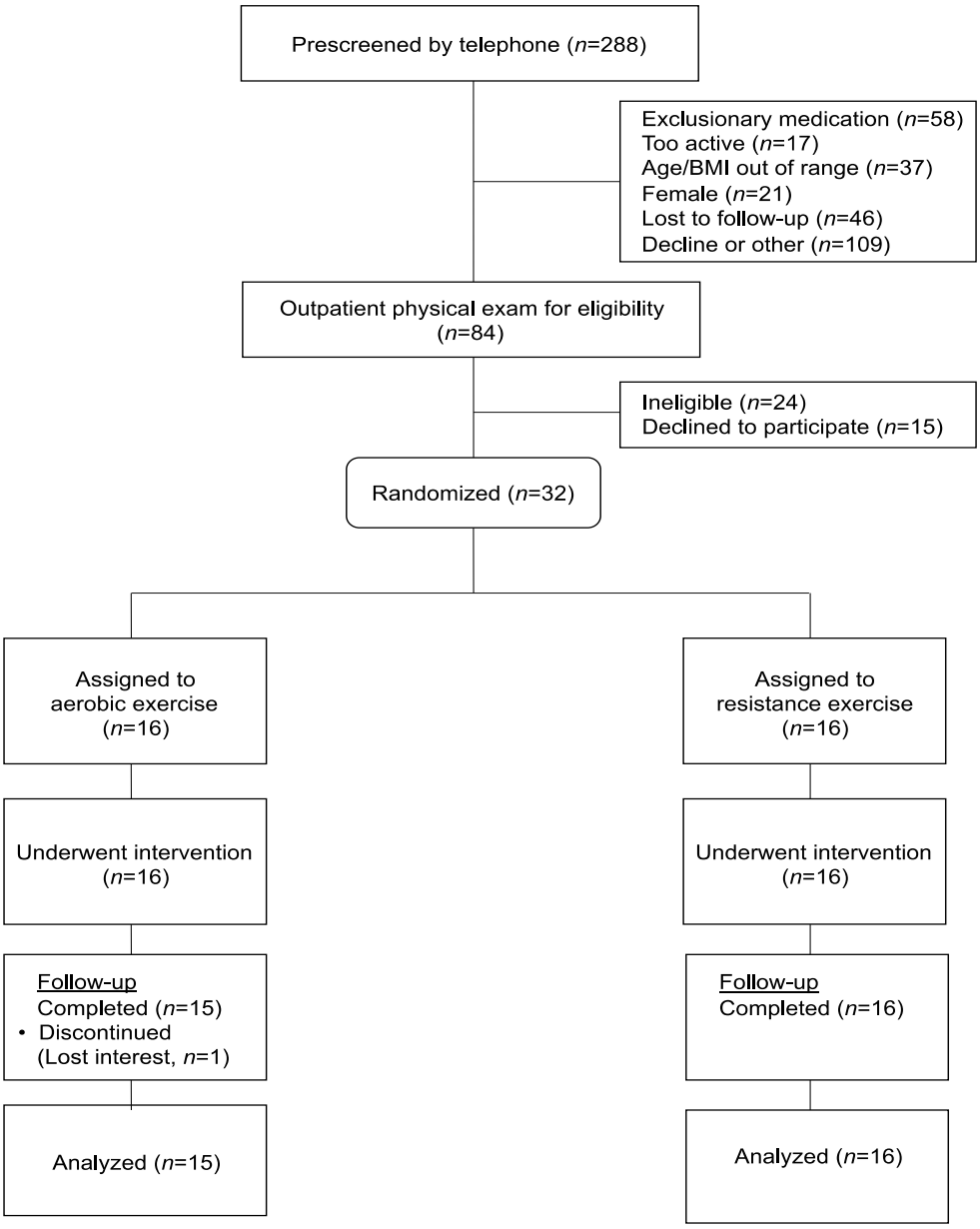


Figure 1. Flowchart (Boys)

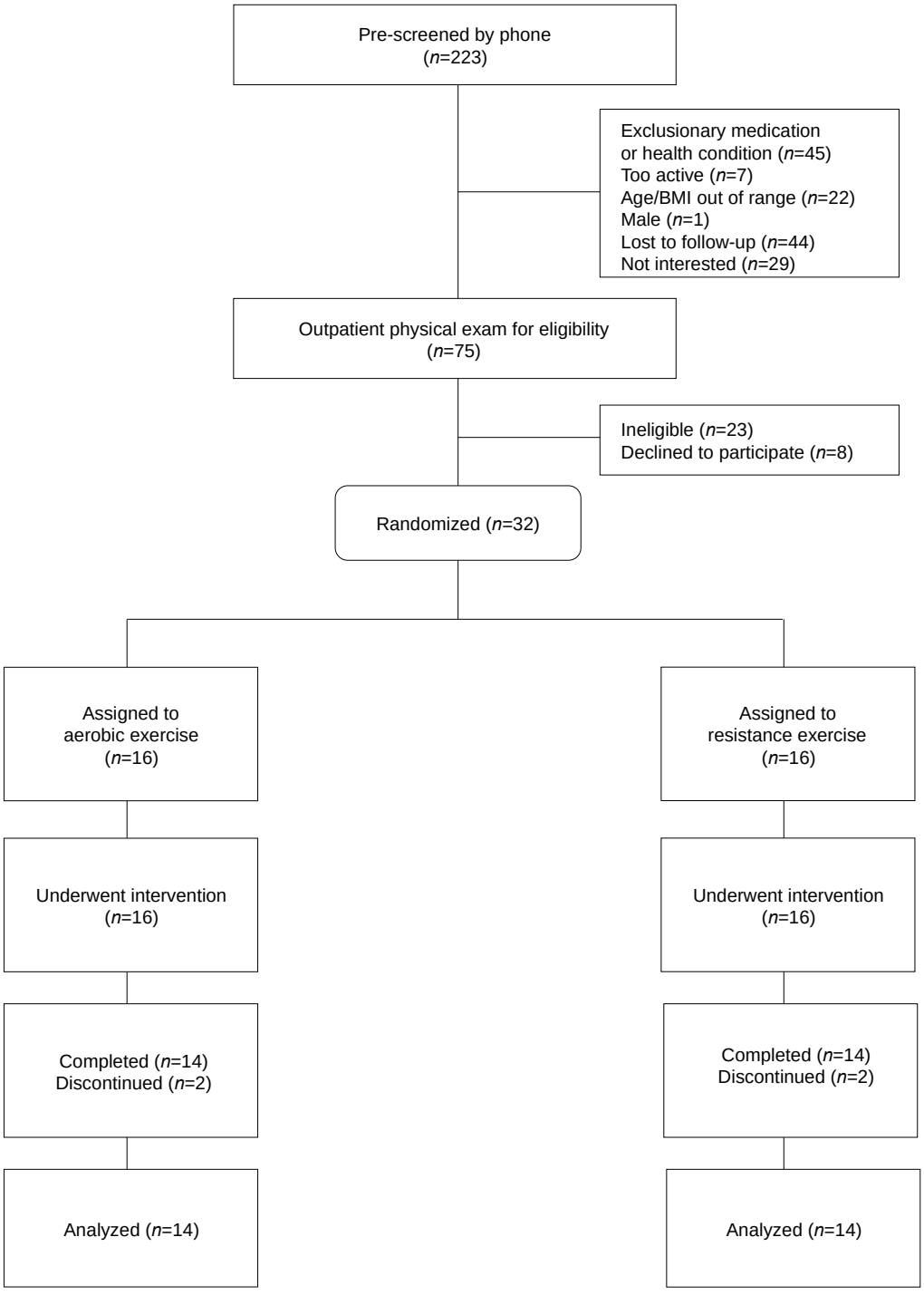


Figure 2. Flowchart (Girls)

3.2 ASSESSMENTS

3.2.1 Anthropometric measurements

Height was measured to the nearest 0.1 cm using a wall-mounted stadiometer (Heightronic Digital Stadiometer, QuickMedical, Issaquah, WA) with the subject standing with out shoes on. Body weight was measured to the nearest 0.1 kg using a calibrated medical balance-beam scale (MediChoice, BEFOUR INC, Saukville, WI). Subjects were required to wear either light clothing or a hospital gown during measurements.

Three sites were used for measuring waist circumference using a tension-regulated measuring tape. The sites included were: level of last rib, iliac crest, and umbilicus using a tension-regulated tape. Two measurements were taken at each site and the average value was used for the analysis. External landmarks were used to identify last rib and iliac crest on both sides of the body to ensure a horizontal plane of the measuring tape. The subjects were then asked to stand with legs apart (shoulder width) with both feet parallel and to fold their arms across their chest. Waist circumference was then taken at the end of a normal expiration and assessed to the nearest 0.1 cm.

3.2.2 Whole-Body Magnetic Resonance Imaging (MRI) protocol

TAT, VAT and ASAT were measured using a 3.0 Tesla MR scanner (Siemens, Magnetom TIM Trio, Germany) at the University of Pittsburgh Magnetic Resonance Research Center (MRRC). Axial images were obtained using a T1-weighted, spin-echo sequence with a 700-ms repetition time, a 5.5-ms echo time, a 48 cm x 36 cm field of view, and a 320 x 240 matrix during a 20-sec

breath hold. Subjects were instructed to lie in the magnet in a prone position with their arms stretched straight overhead. To define the point of origin, a sagittal and transverse image of the abdomen region was taken to identify the intervertebral space between the lumbar fourth (L_4) and fifth (L_5) vertebrae. Cross-sectional images (10 mm image thickness) were then acquired every 40 mm, beginning at the L_4-L_5, space and continue toward the feet. The subjects were repositioned for the upper body scan. The L_4-L_5 was identified in the same manner, and equidistant images (10 mm thickness with 40 mm interslice space) were obtained from L_4-L_5 to the hands. Approximately 40 ~ 45 images were acquired depending on the height of the subject. MRI data acquisition took approximately 30 minutes to acquire MRI images of the total body.

3.2.2.1 Segmentation of MRI Images

TAT, VAT and ASAT area (cm^2) were segmented using commercially developed imaging software (Slice-O-Matic, Version 4.3, Tomovision, Montreal, Canada). As described previously [127-129], the surface area of TAT, VAT and ASAT was traced using different color codes. During this procedure, interactive editing tools and transparency modes that allow correcting and verifying the segmented result were used to review the color tag image or to remove unnecessarily segmented areas (e.g., bone structures or organs). The surface area (cm^2) of VAT and ASAT in each image was calculated by multiplying the total number of pixels of each AT by the surface area of individual pixel. The volume (cm^3) of VAT and ASAT in each image was calculated by multiplying the segmented area (cm^2) of each AT by the image thickness (10 mm). The estimated volume of VAT and ASAT between images was calculated using the truncated pyramid method [128]. Volume units (cm^3) were converted to mass units (kg) by multiplying the volumes by the assumed constant density for adipose tissue (0.92 kg/L) [130].

3.2.3 Intrahepatic lipid by Proton Magnetic Resonance Spectroscopy (^{1}H-MRS)

For intrahepatic liver contents, ^{1}H-MRS spectra was obtained with a 3.0 Tesla MR system (Siemens, Tim Trio, Erlangen, Germany) using a body matrix coil and a spine matrix (Siemens, Erlangen, Germany) as shown previously [8]. The subject was closely monitored on localizer images during repositioning and voxel and coil placement to ensure that the same location was used on the repeated scan. A voxel (30 x 30 x 20 mm^3) was placed within the posterior part of segment 7 of the liver, avoiding blood vessels and intrahepatic bile ducts, using the following parameters (TR = 4000ms, TE = 30ms). Eight acquisitions were recorded in a measuring time of 32 sec without water suppression and the average of eight spectra were used for liver triglyceride calculation as shown in Figure 3. Attempt to minimize movement artifact was performed by requesting the subject to breathe within the repetition time (TR) interval and to expire during each data acquisition. Liver spectra was fitted using the AMARES algorithm in the Java-based magnetic resonance user interface (jMRUI) software package [131].

$$\text{Intrahepatic lipid (\%)} = \frac{\text{Lipid Peak}}{\text{Water Peak + Lipid Peak}} \text{ X } 100$$

Figure 3.

3.2.4 Muscular Strength

Pre and post 1-repetition maximal (1-RM) tests were performed for lower and upper body muscular strength for all intervention groups. Lower-body muscular strength was measured using leg extension and leg press. Upper-body muscular strength was measured using latissimus pull down and chest press. Proper lifting techniques and testing procedures, based on the American

College of Sports Medicine's (ACSM) guidelines, were given to subjects prior to the beginning of the test [132]. The test began with a perceived maximum weight from the subject following a warm-up of 5 to 10 repetitions at 40-60% of the perceived maximum weight (light to moderate exertion) and a subsequent test of 4 to 5 repetitions at 60-80% of their perceived maximum weight (moderate to heavy exertion). Finally, weight (5-10 lb.) was added, and a 1-RM lift was attempted. If the lift was successful, the subject rested for ~3 minutes and then attempted to lift a heavier weight (additional 5~10 lb.). If the lift was not successful, 5 lbs. were removed and another 1-RM was attempted. The subjects were given a maximum of four attempts to reach their 1-RM.

3.2.5 Cardiovascular Fitness

Cardiorespiratory fitness (VO_{2peak}) was measured at baseline and at the end of the 3-month intervention in all subjects. In the AE group, VO_{2peak} was measured at the 4^{th} and 8^{th} week of the intervention to re-evaluate target heart rate and energy expenditure, which may have been altered in response to aerobic exercise training. A graded maximal treadmill test utilizing standard open-circuit spirometry techniques (MOXUS Metabolic System, AEI Technology, Pittsburgh, PA) was used to measure VO_{2peak}. Before each test begins, there was a five-minute warm-up period so that subjects could become comfortable walking on a treadmill and allow the researcher to identify the subject's testing treadmill speed. During the test, speed was held constant between 2.5 ~ 4.0 mph while the grade began at 0% for the initial three minutes and then increased by 2% for the third minute, and by 1% every minute thereafter. Heart rate (Polar Electro Oy, Kempele, Finland) was recorded every 20 seconds throughout the testing procedure. VO_{2peak} was determined as the peak level recorded at the point when the subjects reached volitional fatigue

(the point at which subjects can no longer maintain the required walking speed or continue the test), and subjects met at least one of the following criteria: 1) no increase in VO_2 despite a further increase in treadmill grade, 2) the heart rate ≥ 85% of age-predicted maximal heart rate (220-age), or 3) a respiratory exchange ratio (RER) ≥ 1.1.

3.3 DIETARY AND EXERCISE REGIMENS

3.3.1 Dietary Regimen

The primary aim of this study was to assess the effects of exercise alone, without caloric restriction, on hepatic fat and body composition. It was requested of the subjects to not restrict their calorie intake but to maintain a healthy isocaloric diet determined at baseline containing a ratio of 55-60% carbohydrate, 25-30% fat, and 15-20% protein. Adherence to the dietary program was determined by examination of body weight prior to each exercise session. If weight deviated significantly more than 4% of initial weight on two consecutive weeks, nutrition counseling was provided to identify the deviation.

3.3.2 Aerobic Exercise Training (AE) Regimen

Subjects that were selected to participate in the AE group underwent a supervised aerobic exercise program for three months. Subjects were required to perform 60 minutes of continuous aerobic exercise using the treadmill, elliptical, and/or bike on three non-consecutive days per week. The exercise intervention spanned 13 weeks with week 1 used as an orientation week.

CRF testing sessions were also included into total exercise sessions attended. All exercise sessions were performed at the Downtown YMCA of Pittsburgh. The aerobic exercise program was individually prescribed based on the subject's baseline VO_{2peak} levels. During the first two weeks of aerobic exercise, the subjects were familiarized with the exercise equipment and the exercise sessions lasted for 30-40 minutes at a heart rate equivalent to approximately 50-60% VO_{2peak}. Beginning on week 3, the subjects exercised for 60 minutes at a heart rate equivalent to 60-75% of VO_{2peak}. All aerobic exercise subjects were required to wear a heart rate monitor (Polar Electro Oy, Kempele, Finland) to verify appropriate levels of exercise intensity were being maintained. Heart rate was recorded every five minutes to estimate energy expenditure during each exercise session. The target heart rate (50~75% of VO_{2peak}) during exercise was determined using the baseline VO_{2peak} test and was re-evaluated at the 4^{th} and 8^{th} weeks using additional VO_{2peak} tests. Each exercise session included 5 minutes of warm-up and 5 minutes of cool-down in addition to ~50 minutes of the main exercise phase.

3.3.3 Resistance Exercise Training (RE) Regimen

Subjects that were selected to participate in the RE group underwent a 3-month supervised resistance exercise program, 3 non-consecutive days per week for 60 minutes per session. The exercise intervention spanned 13 weeks with week 1 used as an orientation week. CRF testing sessions were also included into total exercise sessions attended. All exercise sessions were performed at the Downtown YMCA of Pittsburgh. All resistance exercise sessions included the following exercises: 1) chest press, 2) lateral pull down, 3) seated row, 4) bicep extension, 5) triceps extension, 6) leg press, 7) leg extension, 8) leg flexion, 9) sit-ups, and 10) push-ups. The exercise protocols for the AE and RE groups are summarized in Table 5. This resistance exercise

program has been individually prescribed based on baseline 1-RM test and designed to improve muscular strength. The main emphasis of the first two weeks of the resistance exercise program was to make sure the subjects are familiarized with the exercise equipment as well as learn proper lifting techniques to minimize the risk of injury. During these first two weeks, the subjects performed 1 set of 12 repetitions of each exercise with 50-60% of their baseline 1-RM. At the beginning of the third week, the subjects performed 2 sets of 12 repetitions at greater than 60% of baseline 1-RM. Once a subject completely performed 2 sets of 12 repetitions of each exercise using proper technique and form, then the resistance gradually increased (5-10lb) to maintain adequate loads within 12 repetitions and to stimulate further strength gains. Similar to the aerobic exercise group, each exercise session included 5 minutes of warm-up and 5 minutes of cool-down in addition to 50 minutes of the main exercise phase.

Table 5. Aerobic and Resistance Exercise Protocols

	AE	**RE**
	5 min of warm-up and cool down Treadmill, elliptical, bike CRF (VO_{2peak}) test at 4^{th} and 8^{th} week	5 min of warn-up and cool down Stretching, chest press, lateral pull down, seated row, biceps, triceps, leg press, leg extension, leg flexion, sit-ups and push-ups.
1^{st} ~ 2^{nd} week	30-40 min/session 50-60% of VO_{2peak} 3-5min of break at 20min	30-40 min/session, 1set of 12 reps 50-60% of baseline 1-RM 1-2 min of stretching between exercises
3^{rd} ~ 12^{th} week	~ 60 min/session 60-75% of VO_{2peak} 3-5min of break at 30min	~ 60 min/session, 2 set of 12 reps > 60% of baseline 1-RM 1-2 min of stretching between exercises

3.4 STATISTICAL ANALYSIS

3.4.1 Power Analysis

This study is the secondary data analysis and that the primary outcome variables in the study were already published and currently under review. The primary endpoint of the parent study [8] is the visceral fat at 3 months post-intervention. The power calculation is based on a paired *t*-test of the decrement of visceral fat at 3 month (baseline level measured at pre-intervention) using a power analysis equation as described previously [133]. Previously, the PI [134] reported that a 3-month aerobic exercise without weight loss was associated with MRI measured visceral fat reduction of 0.56 kg (17% loss) with a standard deviation of 0.29 kg. Assuming that our overweight/obese adolescent subjects would have similar reductions in visceral fat in response to a 3-month training, we calculated the sample size required to detect reductions in visceral fat at 3 levels (10%, 15% and 20%) (Table 6).

Table 6. Power Calculations

		Reductions in Visceral Fat Mass		
Power	Sample Size	10%	15%	20%
80%	*N*	7	3	2
90%	*N*	9	4	3
95%	*N*	11	5	3

As with any intervention, it was expected that some participants might drop out of the study during the intervention period. We expected that the dropout rate would be ~20%. Thus, a sample size of 14 subjects per group, similar proportion of Black and White youth was needed to detect a reduction of 10% of visceral fat with a 95% power using a two-sided test and with a significance level (alpha) of 0.05 (14 subjects x 3 groups x 2 gender = total 84 subjects).

3.4.2 Statistical Analysis

A 2 × 2 × 2 mixed-design analysis of covariance was performed on each outcome (TAT, VAT, IHL, CRF and muscular strength) separately as a function of time, exercise training group, and sex after adjusting for age and height. The within-subjects independent variable was time with two levels (pre and post). The between-subjects independent variables were group with two levels (aerobic and resistance) and sex with two levels (male and female). Age and height were considered as a covariate due to the large age range (12-18 yrs). If any assumptions were violated a bootstrap ANCOVA will be performed. When the interaction terms were significant ($P< 0.05$), interaction contrasts were performed to locate the differences. All statistical procedures will be performed using SPSS 21 (SPSS, Inc., Chicago, IL). *F* and *P* values for all main effects and interaction effects are displayed in Table 8.

A 2 × 2 × 2 mixed-design analysis of covariance was performed on each outcome (TAT, VAT, IHL, CRF and muscular strength) separately as a function of time, exercise training group, and race after adjusting for age and height. The between-subjects independent variables were group with two levels (aerobic and resistance) and race with two levels (black and white). Age and height were considered as a covariate due to the large age range (12-18 yrs). If any assumptions were violated a bootstrap ANCOVA will be performed. When the interaction terms were significant ($P< 0.05$), interaction contrasts were performed to locate the differences. All statistical procedures will be performed using SPSS 21 (SPSS, Inc., Chicago, IL). *F* and *P* values for all main effects and interaction effects are displayed in Table 9. Absolute changes in anthropometrics, body composition and fitness are listed in Table 10.

4.0 CHAPTER FOUR

4.1 RESULTS

4.1.1 Baseline subject characteristics

Subject baseline characteristics are shown in Table 7. In respect to anthropometrics, there were no significant differences ($P < 0.05$) in age and weight. Males (169.16 ± 7.9 cm) were significantly taller than females (163.57 ± 7.8 cm). Males (36.2 ± 1.1) in the AE group had a significantly higher BMI than females (33.2 ± 1.1) in the AE group. Males (99.08 ± 0.7%) also were at a significantly higher BMI percentile than females (98.24 ± 1.2%).

In regards to body composition measurements, there were no significant differences ($P < 0.05$) in IHL, TAT, ASAT, and fat mass. Males (102.5 ± 2.4) had significantly greater waist circumference than females (96.9 ± 2.6). Females (51.22 ± 13.1%) had significantly higher total percent body fat than males (43.39 ± 5.5%). White males (1.74 ± 0.5 kg) had significantly greater visceral adipose tissue than black males (1.10 ± 0.4 kg). White females (1.38 ± 0.5 kg) had significantly greater VAT than black females (0.91 ± 0.4 kg). Subsequently, white adolescent subjects (1.61 ± 0.5 kg) had significantly more VAT than black adolescents (0.99 ± 0.4 kg) and males (1.42 ± 0.5 kg) had significantly more VAT than females (1.06 ± 0.5 kg). At

baseline males had significantly greater lean body mass (56.26 ± 8.4 kg) and fat free mass (59.06 ± 8.8 kg) than females (48.21 ± 7.0 kg; 50.90 ± 7.3 kg respectively).

Males (29.97 ± 4.7 ml/kg/min^{-1}) had significantly greater CRF than females (26.11 ± 4.6 ml/kg/min^{-1}). Males (1.15 ± 0.2) were also significantly stronger based on muscular strength index score than females (1.03 ± 0.2). Muscular strength index score was calculated by adding 1-RM chest press (kg) and 1-RM leg press (kg) then dividing the total by body weight in (kg). There were no race differences at baseline.

Table 7. Subject characteristics at baseline

	Male		**Female**		**Main Effects**		**Interaction Effects**
	AE	RE	AE	RE	Sex	Group	Sex × Group
Race (B/W)	(8/7)	(7/9)	(9/5)	(10/4)	*P* value		*P* value
Anthropometry							
Age (yr)	14.9 ± 0.5	14.6 ± 0.4	14.8 ± 0.5	14.8 ± 0.5	0.971	0.689	0.643
Ht (cm)	170.3 ± 2.1	168.1 ± 1.2	164.5 ± 2.1	162.7 ± 2.1	0.009	0.330	0.925
Wt (kg)	105.2 ± 4.1	97.7 ± 4.0	90.6 ± 4.3	96.2 ± 4.3	0.059	0.817	0.122
BMI (kg/m^2)	36.2 ± 1.1	34.5 ± 1.1	33.2 ± 1.1	36.1 ± 1.1	0.545	0.585	0.043
BMI (%)	99.1 ± 0.2	99.1 ± 0.2	97.8 ± 0.3	98.7 ± 0.3	0.001	0.098	0.084
Body Composition							
Total Body fat (%)	40.0 ± 1.1	41.5 ± 1.1	43.4 ± 1.2	46.3 ± 1.2	0.001	0.064	0.537
WC (cm)	103.8 ± 2.5	101.1 ± 2.4	93.3 ± 2.6	100.6 ± 2.6	0.031	0.357	0.052
Liver Fat (%)	4.8 ± 1.0	2.9 ± 1.0	2.4 ± 0.8	1.9 ± 0.8	0.073	0.197	0.456
Total Fat (kg)	45.7 ± 2.9	43.4 ± 3.0	44.2 ± 3.1	49.9 ± 3.0	0.420	0.573	0.198
ASAT (kg)	7.7 ± 0.5	6.8 ± 0.5	6.9 ± 0.6	8.0 ± 0.6	0.707	0.886	0.086
VAT (kg)	1.5 ± 0.1	1.4 ± 0.1	0.88 ± 0.1	1.2 ± 0.1	0.011	0.398	0.098
Fat Mass (kg)	42.2 ± 2.5	39.9 ± 2.4	39.4 ± 2.6	44.5 ± 2.6	0.711	0.564	0.142
LBM (kg)	59.3 ± 1.9	53.4 ± 1.9	47.8 ± 2.0	48.6 ± 2.0	0.000	0.206	0.097
FFM (kg)	62.2 ± 2.0	56.1 ± 2.0	50.5 ± 2.1	51.3 ± 2.1	0.000	0.209	0.100
Fitness and Strength							
CRF (ml/kg/min^{-1})	29.5 ± 1.2	30.4 ± 1.2	28.1 ± 1.2	24.1 ± 1.2	0.002	0.179	0.041
MSI	1.2 ± 0.1	1.2 ± 0.1	1.0 ± 0.1	1.0 ± 0.1	0.011	0.696	0.987

Values are Mean ± Standard Error of the mean

4.1.2 Exercise adherence

A summary of exercise training sessions is shown in Table 8. Average (Mean ± SD) attendance at the exercise sessions was 97.6% ± 2.6% in the AE and 98.0% ± 2.5% in the RE groups and average exercise duration was similar between the AE (56.9 ± 1.2 minutes/session) and RE (58.2 ± 2.2 minutes/session) group. Average attendance for females was 95.8% ± 4.3% in the AE and 97.0% ± 2.8% in the RE groups. For females the average exercise duration between the AE (56.0 ± 1.1 minutes/session) and RE (57.0 ± 0.7 minutes/session) group were also similar. Average attendance for males was 99.7% ± 0.8% in the AE and 99.0% ± 2.1% in the RE groups. The average exercise duration for males was similar between the AE (57.7 ± 1.2 minutes/session) and RE (59.4 ± 3.6 minutes /session) group.

Table 8. Exercise Summary

	Males		**Females**	
	AE (n = 15)	RE (n = 16)	AE (n = 14)	RE (n = 14)
Total Exercise Sessions (Attendance Rate)	40.7 ± 0.6 (99.7%)	40.1 ± 0.7 (99%)	38.1 ± 0.7 (96%)	37.9 ± 0.3 (97%)
Duration per session (min)	57.7 ± 1.2	59.4 ± 3.6	56.0 ± 1.1	57.0 ± 0.7
Exercise Intensity (Mean HR, beats/min)	155 ± 3	NA	153 ± 7	NA
Energy Expenditure (Kcal/Session)	740.6 ± 57.8	NA	536.6 ± 72.9	NA

Values are Mean ± Standard Error of mean; NA, Not available

4.2 EXERCISE INTERVENTION

4.2.1 Effect of a 3-month aerobic versus resistance training program on anthropometrics in obese adolescent youth

4.2.1.1 Time by group by sex

Age and height did not significantly predict BMI or body fat percent. There was a significant positive prediction of body weight by height, F (1, 49) = 33.967, p < .000, η^2_p = .409. Age did not significantly predict body weight. After adjusting for age and height, the main effects of time, group, and sex were not found to be significant for body weight, BMI or body fat percent. All interaction effects were not significant implying that there were no significant changes in body weight after the 3-month exercise intervention between group and sex.

4.2.1.2 Time by group by race

After adjusting for age and height, the main effect of race was not statistically significant for body weight, BMI or body fat percent. All interaction effects were not significant implying that there were no significant changes in body weight after the 3-month exercise intervention between group and race.

4.2.2 Effect of sex and race on total adipose tissue in response to a 3-month aerobic versus resistance exercise training program in obese adolescent youth

4.2.2.1 Time by group by sex

There was a significant positive prediction of TAT by height, F (1, 49) = 11.37, p = .001, η^2_p = .188. Age did not significantly predict TAT. After adjusting for age and height, the main effects of time and group were not found to be significant. There was a significant main effect difference on TAT among sex averaged across groups and time, F (1, 49) = 5.036, p = .029, η^2_p = .093. Males (M = 41.581 kg, SE = 2.024) had significantly less TAT than females (M = 48.360 kg, SE = 2.064). The 3-way interaction effect of time by group by sex on TAT was not significantly different, F (1, 49) = 2.719, p = .106, η^2_p = .053. The interaction effects of time by group and group by sex were also not statistically significant.

4.2.2.2 Time by group by race

After adjusting for age and height, the main effect of race was not statistically significant. All interaction effects involving race were not significant.

4.2.3 Effect of sex and race on intrahepatic lipid in response to a 3-month aerobic versus resistance exercise training program in obese adolescent youth

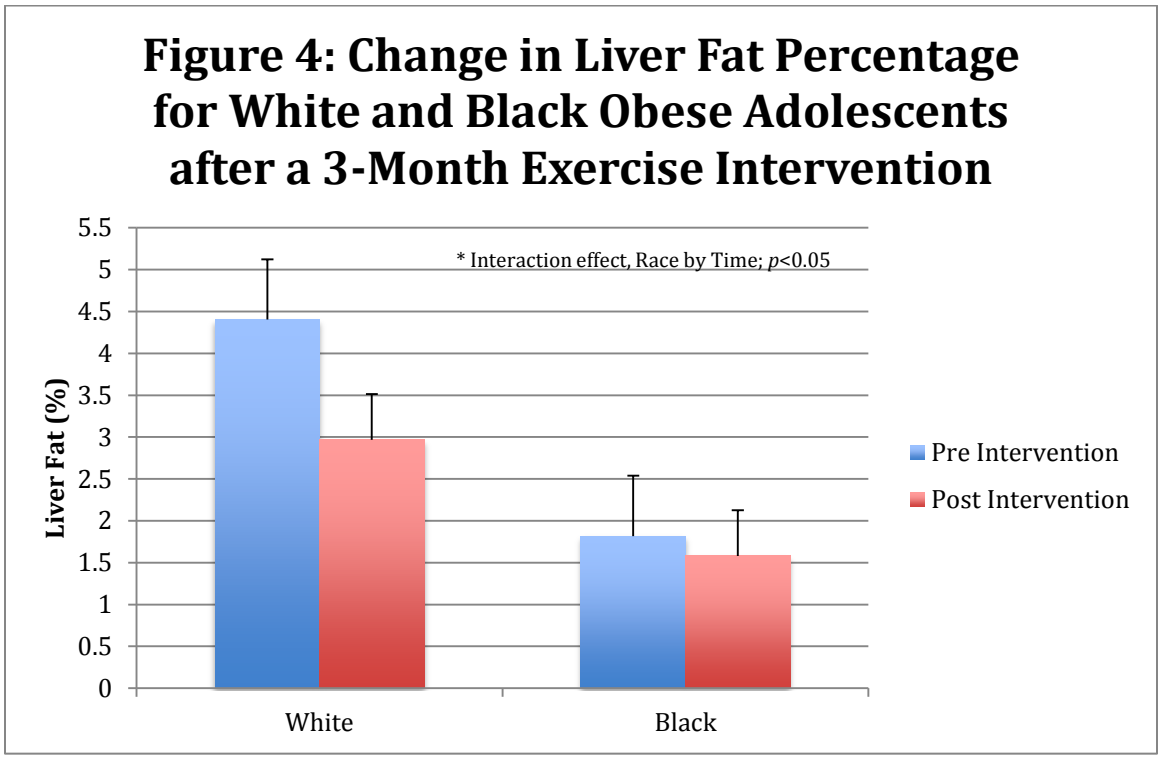
4.2.3.1 Time by group by sex

Age and height did not significantly predict IHL. After adjusting for age and height, there were no significant main effect differences on IHL among time, sex or group. The pattern of difference on IHL was not significantly different between groups for males and females from

pre-intervention to post-intervention, F (1, 38) = 1.374, p = .248, η^2_p = .035. All interaction effects were not significant. Only 18 out of 31 boys were analyzed for IHL content compared to 26 out of 28 females due upgrades in equipment.

4.2.3.2 Time by group by race

The main effect difference on IHL among race was significant, F (1, 34) = 5.007, p = .032, η^2_p = .128, after adjusting for age and height. White adolescents (M = 3.687 %, SE = .613) had significantly greater IHL than black adolescents (M = 1.579 %, SE = .546) averaged across group and time. There was a significant interaction on IHL between race and time. White obese adolescents (M = 2.969 %, SE = .543) lost significantly more IHL than black adolescents (M = 1.579 %, SE = .546) after a 3-month exercise program (Figure 4). All other interaction effects were not significant.



4.2.4 Effect of sex and race on visceral adipose tissue in response to a 3-month aerobic versus resistance exercise training program in obese adolescent youth

4.2.4.1 Time by group by sex

Age and height did not significantly predict VAT. After adjusting for age and height, there were no significant main effect differences on VAT among time, sex or group. The 3-way interaction effect of time by group by sex on VAT was not significantly different, F (1, 51) = 4.010, p = .051, η^2_p = .073. The interaction effects of time by group and group by sex were also not statistically significant.

4.2.4.2 Time by group by race

After adjusting for age and height, the main effect difference on VAT among race was found to be significant, F (1, 47) = 24.112, p < .000, η^2_p = .339. Black adolescents (M = .913 kg, SE = .073) had significantly less VAT than white adolescents (M = 1.481 kg, SE = .088) averaged across groups and time. All interaction effects involving race were not significant.

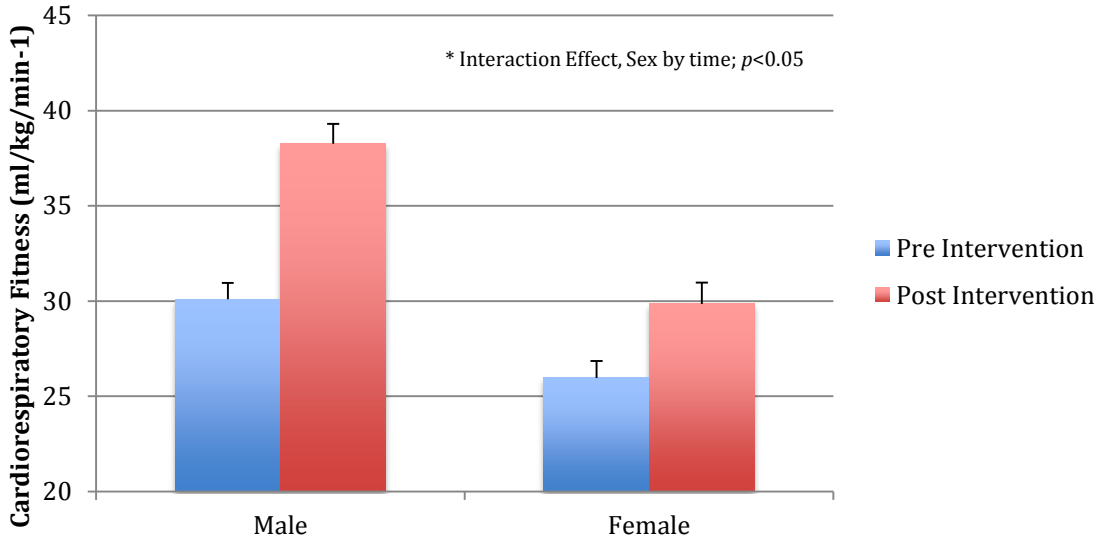
4.2.5 Effect of a 3-month aerobic versus resistance training program on cardiorespiratory fitness in obese adolescent youth

4.2.5.1 Time by group by sex

Age and height did not significantly predict CRF. After adjusting for age and height, there was a significant main effect difference on CRF for sex, F (1, 53) = 21.675, p < .000, η^2_p = .290 averaged across groups and time. Males (M = 34.180 ml/kg/min^{-1}, SE = .889) had significantly greater CRF than females (M = 27.906 ml/kg/min^{-1}, SE = .939). The interaction of time by sex

was found to be statistically significant, F (1, 53) = 15.569, p < .000, η^2_p = .227. Males (M = 38.259 ml/kg/min^{-1}, SE = 1.052) significantly improved their CRF after a 3-month exercise intervention compared to females (M = 29.853 ml/kg/min^{-1}, SE = 1.111) averaged across groups (Figure 5). The pattern of difference on CRF was significantly different between groups for males and females averaged across time, F (1, 53) = 4.075, p = .049, η^2_p = .071. Males in the aerobic (M = 34.122 ml/kg/min^{-1}, SE = 1.267) group had significantly greater CRF than females in the aerobic (M = 30.347 ml/kg/min^{-1}, SE = 1.284) group and males in the resistance (M = 34.237 ml/kg/min^{-1}, SE = 1.202) group had significantly greater CRF than females in the resistance (M = 25.465 ml/kg/min^{-1}, SE = 1.314) group averaged across time. No other interaction effects were found to be significant.

Figure 5: Change in Cardiorespiratory Fitness Between Obese Adolescent Males and Females After a 3-Month Exercise Intervention



4.2.5.2 Time by group by race

After adjusting for age and height, the main effect of race was not statistically significant. All interaction effects involving race were not significant.

4.2.6 Effect of a 3-month aerobic versus resistance training program on muscular strength in obese adolescent youth

4.2.6.1 Time by group by sex

There was a significant positive prediction of muscular strength by height, F (1, 53) = 26.416, p < .000, η^2_p = .333 and age, F (1, 53) = 14.912, p < .000, η^2_p = .220. After adjusting for age and height, there was a significant main effect difference on muscular strength between males and females averaged across groups and time, F (1, 53) = 37.237, p < .000, η^2_p = .413. Males (M = 1.374, SE = .039) had significantly greater muscular strength compared to females (M = 1.015, SE = .041). There was a significant interaction between sex and time, F (1, 53) = 9.777, p = .003, η^2_p = .156. Males (M = 1.515, SE = .053) significantly increased their muscular strength after the 3-month exercise intervention compared to females (M = 1.053, SE = .056) averaged across groups (Figure 6). There was also a significant interaction between group and time, F (1, 53) = 20.370, p < .000, η^2_p = .278. The resistance group (M = 1.399, SE = .052) significantly increased their muscular strength compared to the aerobic group (M = 1.168, SE = .052) after the 3-month exercise intervention averaged across sex (Figure 7). No other interaction effects were found to be significant.

Figure 6: Change in Muscular Strength Between Obese Adolescent Males and Females After a 3-Month Exercise Intervention

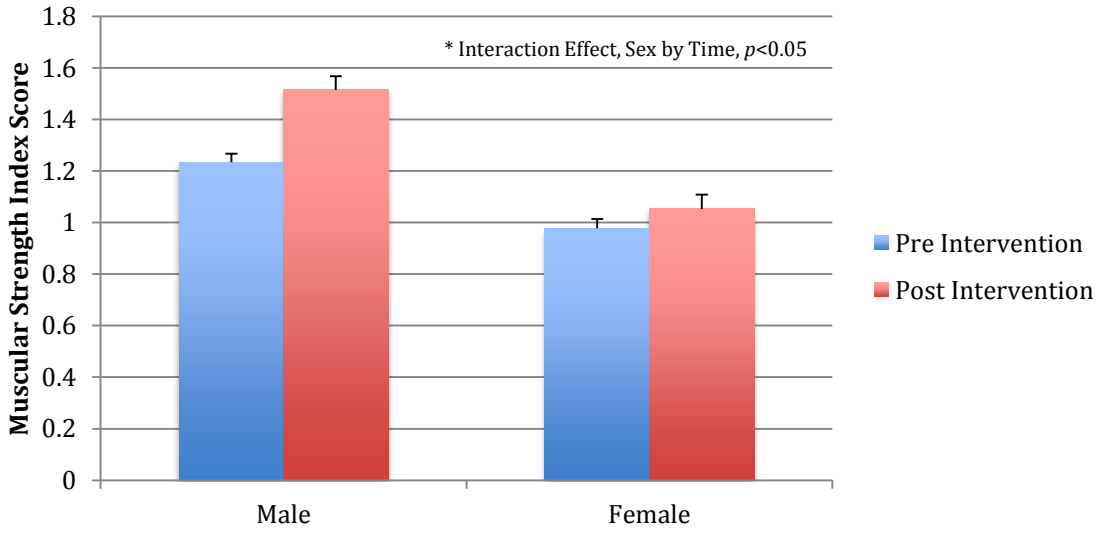
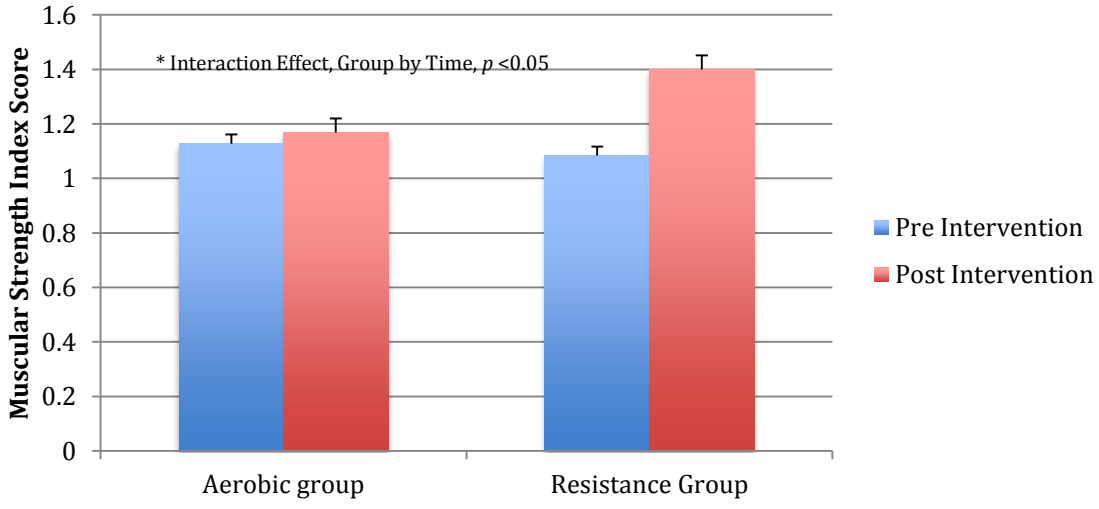


Figure 7: Change in Muscular Strength Between the Aerobic and Resistance Groups After a 3-Month Exercise Intervention



4.2.6.2 Time by group by race

After adjusting for age and height, the main effect of race was not statistically significant. All interaction effects involving race were not significant.

Table 9. *F* and *P* value table for all main and interaction effects

	Total Adipose Tissue			Visceral Adipose Tissue			Liver Fat		
	F Value	*P* Value	Effect Size	*F* Value	*P* Value	Effect Size	*F* Value	*P* Value	Effect Size
Main Effects									
Time	0.805	0.374	0.016	0.434	0.513	0.008	0.330	0.569	0.009
Sex	5.036	0.029	0.093	3.872	0.055	0.071	1.104	0.300	0.028
Group	0.972	0.329	0.019	1.083	0.303	0.021	1.278	0.265	0.033
Race	0.033	0.857	0.001	24.112	0.000	0.339	5.007	0.032	0.128
Interaction Effects									
Time × Sex	1.609	0.211	0.032	0.240	0.626	0.005	2.761	0.105	0.068
Time × Group	0.447	0.507	0.009	0.035	0.853	0.001	1.949	0.171	0.049
Time × Race	0.538	0.467	0.012	0.003	0.959	0.000	5.998	0.020	0.150
Sex × Group	2.719	0.106	0.053	4.010	0.051	0.073	1.374	0.248	0.035
Sex × Race	0.106	0.747	0.002	0.091	0.764	0.002	0.006	0.937	0.000
Group × Race	0.274	0.603	0.006	2.150	0.149	0.044	0.163	0.689	0.005
Sex × Group × Race	0.002	0.965	0.000	0.342	0.561	0.007	0.312	0.580	0.009
Time × Sex × Group	0.088	0.768	0.002	0.526	0.472	0.010	1.303	0.261	0.033
Time × Sex × Race	0.028	0.868	0.001	0.162	0.689	0.003	1.588	0.216	0.045
Time × Group × Race	0.381	0.540	0.008	1.913	0.173	0.039	3.144	0.085	0.085
Covariates									
Age	0.047	0.828	0.001	2.498	0.120	0.047	0.002	0.966	0.000
Height	11.37	0.001	0.188	0.625	0.433	0.012	0.630	0.432	0.016

Table 9. *F* and *P* value table for all main and interaction effects (continued)

	Cardiorespiratory Fitness			Muscular Strength		
	F Value	*P* Value	Effect Size	*F* Value	*P* Value	Effect Size
Main Effects						
Time	0.043	0.836	0.001	0.788	0.370	0.015
Sex	21.675	0.000	0.290	37.237	0.000	0.413
Group	3.669	0.061	0.065	2.994	0.089	0.053
Race	1.937	0.170	0.038	0.958	0.332	0.019
Interaction Effects						
Time × Sex	15.569	0.000	0.227	9.777	0.003	0.156
Time × Group	2.295	0.136	0.042	20.370	0.000	0.278
Time × Race	0.013	0.911	0.000	1.442	0.236	0.029
Sex × Group	4.075	0.049	0.071	0.974	0.328	0.018
Sex × Race	0.338	0.564	0.007	0.109	0.743	0.002
Group × Race	0.230	0.634	0.005	1.466	0.232	0.029
Sex × Group × Race	0.050	0.824	0.001	0.583	0.449	0.012
Time × Sex × Group	0.000	0.998	0.000	2.407	0.127	0.043
Time × Sex × Race	0.414	0.523	0.008	0.680	0.414	0.014
Time × Group × Race	0.014	0.907	0.000	0.137	0.713	0.003
Covariates						
Age	0.188	0.666	0.004	14.912	0.000	0.220
Height	0.022	0.882	0.000	26.416	0.000	0.333

Table 10. Absolute changes in anthropometrics, body composition and fitness

	Male		Female	
	Black (n = 15)	White (n = 16)	Black (n = 18)	White (n = 10)
Anthropometry				
Wt (kg)	-0.05 ± 0.1	-0.51 ± 0.1	-0.40 ± 0.1	-1.40 ± 0.1
BMI (kg/m^2)	-0.38 ± 0.0	-0.58 ± 0.1	-0.19 ± 0.1	-0.79 ± 0.0
Body Composition				
Total Body fat (%)	-2.66 ± 0.1	-1.12 ± 0.1	-1.03 ± 0.1	-0.86 ± 0.1
WC (cm)	-2.42 ± 0.1	-1.54 ± 0.1	-1.26 ± 0.1	-3.40 ± 0.1
Liver Fat (%)	-0.43 ± 0.3	-2.21 ± 0.2	-0.07 ± 0.2	-0.65 ± 0.2
Total Fat (kg)	-2.70 ± 0.1	-3.19 ± 0.1	-1.53 ± 0.1	-2.33 ± 0.2
ASAT (kg)	-0.50 ± 0.01	-0.49 ± 0.01	-0.14 ± 0.01	-0.20 ± 0.02
VAT (kg)	-0.16 ± 0.01	-0.18 ± 0.01	-0.21 ± 0.01	-0.19 ± 0.01
Fat Mass (kg)	-2.39 ± 0.1	-1.28 ± 0.1	-0.81 ± 0.1	-1.28 ± 0.2
LBM (kg)	2.08 ± 0.02	1.17 ± 0.02	0.57 ± 0.01	0.28 ± 0.02
FFM (kg)	2.15 ± 0.02	1.25 ± 0.02	0.61 ± 0.02	0.22 ± 0.02
Fitness and Strength				
CRF ($ml/kg/min^{-1}$)	8.50 ± 0.3	7.96 ± 0.3	3.72 ± 0.3	4.57 ± 0.4
MSI	0.35 ± 0.03	0.22 ± 0.03	0.10 ± 0.03	0.07 ± 0.03

Values are Mean ± Standard Error of the mean

5.0 CHAPTER FIVE

5.1 DISCUSSIONS

The primary aim of this study was to examine the effect of sex on the change in IHL, TAT and VAT in response to a 3-month AE versus RE training program without caloric restriction in obese adolescent males and females. No significant differences were observed between males and females on the change in IHL, TAT and VAT in response to exercise training. We observed that males significantly improved their cardiorespiratory fitness and muscular strength compared to females averaged across groups and race. Despite the absence of weight loss, we also observed a significant reduction in liver fat percent for white adolescents compared to black adolescents averaged across groups and sex after a 3-month exercise intervention.

5.1.1 Effect of sex on the change in IHL, TAT and VAT in response to a 3-month AE versus RE without calorie restriction

Although we hypothesized that there would be a significant change in IHL between obese adolescent males and females in response to 3-months of AE versus RE, the changes remained comparable between sexes. There were no significant differences in IHL percent at baseline for males and females. NAFLD is defined as ≥ 5% of hepatocytes containing macrovesicular fat [58]. 6 boys and 3 girls were determined to have IHL content greater than 5% at baseline. The

data from this study support the contention that NAFLD is more common in boys than in girls. In case reports of children with NAFLD, boys out number girls usually two to one [12, 135, 136].

To date, few studies have examined the effects of sex on IHL in response to exercise. Similar results were seen by Gronbaek et al. [17] after a 10-week weight loss camp in obese Danish children. Boys had more frequently increased liver fat echogenicity compared to girls at baseline; however there was no sex effect on liver fat changes during the study period. Devries et al. [137] found no sex differences before or after 3-months of endurance training in obese and lean adults. One possible explanation for our results could be that our study was not designed to induce weight loss, and exercise-induced weight reduction appears to be more strongly associated with improvement in liver condition. Research by Suzuki et al. [138] found 5% or greater weight reduction for one year was associated with ALT improvement and 3.6-fold increased odds of ALT normalization in adults. Another possible explanation might be the small sample size used for this study. Only 18 out of 31 boys were analyzed for IHL content compared to 26 out of 28 females due upgrades in equipment. This sample size resulted in sex only explaining 6.8% of the change in IHL after 3-months of exercise.

While the changes in our study were not statistically significant, obese adolescent boys decreased their IHL by 33.5% and females decreased their IHL by 16.2% after 3-months of exercise without calorie restriction. By breaking this down by exercise group, we can see that males in the AE group lost 27.9% of IHL content while females in the AE group lost 23.2%. Males in the RE group lost 42.8% IHL content compared to a 9.3% increase in IHL for females in the RE group. These findings extend the previous observation by Lee et al. [8] who demonstrated that after 13-weeks of aerobic or resistance exercise obese adolescent boys had significant reductions in IHL content independent of exercise modality compared to non-exercise

controls. In a more recent study, Lee et al. [139] found that obese adolescent females significantly reduced their IHL content after 13-weeks of aerobic exercise compared to non-exercise controls while the resistance group demonstrated a slight increase in IHL. In a similar study by van der Heijden et al. [10], obese males and females substantially decreased their hepatic fat (37%) after a 12-week aerobic exercise program without weight loss. No comparisons between males and females for hepatic fat loss were performed.

Our hypothesis that there would be a significant change in TAT between obese adolescent males and females in response to 3-months of AE versus RE was also not met. At baseline, there were no significant differences between males and females for TAT. After 3-months of AE, obese adolescent males lost 7.3% of TAT while females in the AE group lost 4.1%. Males in the RE group lost 5.9% of TAT and females in the RE group lost 3.0%. Again, these findings extend the previous observation by Lee et al. [8] who demonstrated that after 13-weeks of aerobic or resistance exercise obese adolescent boys had significant reductions in TAT independent of exercise modality compared to non-exercise controls. In a more recent study, Lee et al. [139] found that obese adolescent females significantly reduced their TAT after 13-weeks of aerobic and resistance exercise compared to non-exercise controls.

To date few studies have examined the effect of sex on TAT in response to exercise. In a similar study employed by Savoye et al. [140], overweight children (BMI > 95th percentile) were enrolled in a weight management program consisting of high intensity exercise (2 days/week, 65-80% age-adjusted maximal heart rate) combined with nutrition education for 12 months. Although mean body weight essentially remained unchanged from baseline to 12 months in the weight management group, BMI (-3.3), body fat (-9.2 kg) and percent body fat (-6.0%) was all significantly decreased compared to non-exercise controls. The authors [140] found no

differences in any outcome measures between sexes. In order to examine whether there were sex-differences in the response of energy balance to physical training, Andersson et al. [141] recruited slightly obese men and women to participate a 3-month physical training program with the same individual relative intensity. There were no significant differences in sex. The men became somewhat leaner losing 2.9 kg of body fat while the women showed a decrease of 2.6 kg of body fat. More recently, Donnelly et al. [142] reported a significant decrease in fat mass (4.9 ± 4.4 kg) in men compared to women (0.3 ± 2.7 kg) after 16 weeks of moderate-intensity aerobic exercise in young adults (17-35 yrs).

Irrespective of sex, Gutin et al. [143] demonstrated that an 8-month aerobic exercise training program (2-5 days/week, 55-80% of VO_{2peak}) combined with lifestyle education significantly reduced body fat (-3.6%) compared with a lifestyle education program in overweight adolescent males and females. In a different study [144], these researchers also reported significant reductions in percent body fat (-2.2%) after a 4-month moderate intensity aerobic exercise program in overweight adolescent males and females. Conversely, Eliakim et al. [112] reported no changes in TAT after a 5-week endurance-type exercise training (5 days/week, ~2h/day) in adolescent girls. In this study [112], the subjects were varied with respect to fatness and ethnicity (Asian, White, Hispanic), which may have confounded the findings. Furthermore, reductions in TAT would be less likely to occur in non-obese individuals. This is supported by a recent report by van der Heijden and colleagues [10] who demonstrated significant reductions in percent body fat (-1.0%) after 12 weeks of aerobic in overweight Latino adolescent boys and girls, but not in their lean peers.

Sexual dimorphism of body fat distribution had previously been reported to emerge during puberty, implying that factors regulating sex-specific total body composition are present

in prepubertal children but that those determining regional body composition appear during puberty [145]. Results in prepubertal children confirm that sex-specific patterns of greater relative extremity or gynoid fat deposition in girls start well before the appearance of the physical signs of puberty. Using the DEXA method of determining body composition, He et al. [146] determined that prepubertal girls had greater extremity fat deposits compared with boys. In adults, women are generally characterized by a greater body fat content compared to men. Furthermore, women also show a preferential accumulation of adipose tissue in the gluteofemoral region, whereas men are more prone to abdominal fat deposition, particularly in the abdominal cavity, a condition that has been described as visceral obesity [147, 148]. Our baseline characteristics for VAT revealed similar results in obese adolescents. Males (1.42 ± .05 kg) had a significantly greater amount of VAT compared to females (1.06 ± .05 kg).

Our hypothesis that there would be significant changes in VAT between obese adolescent males and females in response to 3-months of aerobic versus resistance exercise was also not met. Males in the aerobic group had a 10.1% decrease in VAT compared to a 29.7% decrease for females. Males in the resistance group had a 14.5% decrease in VAT compared to a 13.9% decrease for females. These findings extend the previous observation by Lee et al. [8] who demonstrated that after 13-weeks of aerobic or resistance exercise obese adolescent boys had significant reductions in VAT content independent of exercise modality compared to non-exercise controls. A follow up study, by the same authors [139], in obese adolescent females reported a significant reduction in VAT after 13-weeks of aerobic exercise but not resistance exercise compared to non-exercise controls. Our data is consistent with van der Heijden et al. [10], who reported that a 12-week aerobic exercise program without weight loss decreased VAT (9.3%) in mixed sample of obese adolescent males and females. Another study by Gutin et al.

[149] demonstrated that 4-months of aerobic exercise (40 minutes, 4 days/week) significantly decreased VAT compared to non-exercise controls in a mixed sample of obese adolescent males and females. When investigating the effects of exercise-induced weight loss in adults, Ross et al. [150] reported a resistance to VAT reduction in obese women, whereas exercise-induced weight loss is associated with significant reductions in VAT in obese men.

Our findings provide evidence that 3-months of physical exercise, without caloric restriction, can be beneficial to reduce IHL, TAT and VAT in obese adolescent males and females. Although the improvements were not significantly different between males and females, substantial decreases were seen in both IHL and VAT, which are strong predictors of health outcomes such as insulin resistance and NAFLD in adolescents [151-153]. Our results emphasize the importance of regular exercise as an effective strategy for the treatment of obesity and obesity-related health risks in obese adolescent boys and girls regardless of ethnicity.

5.1.2 Effect of race on the change in IHL, TAT and VAT in response to a 3-month AE versus RE without calorie restriction

An exploratory aim of this study was to examine the effect of race on the change in IHL, TAT and VAT in response to a 3-month exercise intervention without calorie restriction on obese adolescent males and females. At baseline, there were no significant differences in body weight, BMI, body fat percent, IHL and TAT for white and black adolescents As we hypothesized, regular exercise for 180 min/week, independent of exercise modality, is associated with significant reductions in IHL in the absence of changes in body weight in obese white adolescents (-32.5%) compared to obese black adolescents (-13.1%). It is important to note that

there was a significant main effect of race with obese black adolescents (1.69%) having significantly less IHL than obese white adolescents (3.69%).

These findings extend the previous histological findings by Schwimmer et al. [3] and Louthan et al. [154] that black children, even when obese, are much less likely to have a fatty liver. In a study by the same group [26], obese black adolescents (14%) had the lowest rate of elevated ALT, a surrogate for suspected fatty liver, compared to obese white (22%) and Hispanic adolescents (36%). The authors [26] speculate that blacks either have a protective factor, making fatty liver less common, or lack a vulnerability factor for the development of fatty liver. Similar results were seen by D'Adamo et al. [155], at similar concentrations of VAT, obese black youth had lower liver fat contents than did whites and Hispanics. Larson-Meyer et al. [156] performed a 6-month calorie restriction and aerobic exercise intervention (5 days/week) on black and white obese adult males and females. A significant treatment effect, with no influence of race, ($P < 0.01$) was found on IHL levels, which was reduced from baseline in the exercise group. It was determined that liver lipid stores determine by MRS, were higher in white than black individuals, particularly in white men, despite higher insulin sensitivity.

Recently published data [157] provide solid evidence that physical inactivity and low aerobic capacity leads to a greater susceptibility to IHL, NAFLD and the metabolic syndrome. By contrast, high aerobic fitness or physical activity protects against NAFLD and metabolic syndrome. Our findings provide evidence that 3-months of physical exercise, without caloric restriction, can be beneficial to reduce IHL in obese adolescent males and females, particularly white adolescents. More importantly, because exercise training and/or increased physical activity is the proven method to increase fitness, and because it positively improves the metabolic

function of multiple tissues, it should remain the cornerstone therapy for preventing and treating both IHL and NAFLD.

Our hypothesis that there would be a significant change in TAT between obese black and white adolescents in response to 3-months of AE versus RE without calorie restriction was not met. At baseline, there were no significant differences between blacks and whites for TAT. In a recent study by Lee et al. [158] using MRI to determine whole-body AT distribution, overweight black boys had significantly greater TAT and lower-body subcutaneous AT compared with overweight white boys with no difference in body weight. Similarly, compositional development in different ethnic groups of healthy female adolescents by Ellis et al. [159] indicated that healthy black adolescent females had significantly ($P < 0.002$) more total fat mass than white female adolescents.

After 3-months of physical exercise, we determined that obese black adolescent lost 4.6% of TAT while obese white adolescent whites lost 5.9%, independent of group and sex. Research is sparse when observing the effect of race on TAT in response to exercise in obese adolescent youth. A mixed sample of obese adolescents who participated in an afternoon lifestyle education plus physical training (5 day/week, 250 kcal/session) program where shown to improve in cardiovascular fitness and decline TAT, independent of race, to a greater degree than did a group who engaged in the lifestyle education alone [143]. The HERITAGE study, one of the largest, well-controlled studies of its kind, reported that after 20-weeks of aerobic exercise (3 days/week, 50 minutes, 75% of baseline VO_{2max}) there were no significant differences in total fat mass when analyzed by black and white adults [160]. Our results are consistent with a cross-sectional review of the HERITAGE study by Janssen et al. [161], who divided the adults into groups by CRF. Similar decreases (2.5—4.8%) in TAT in black and white adults with moderate levels of CRF

compared to subjects with low CRF after 20-weeks of aerobic exercise (3 days/week, 50 minutes, 75% of baseline VO_{2max}) training. The recognition of race differences in fat distribution is of clinical importance, especially because the metabolic implications of particular body composition parameters may vary among races. For example, the strength of association between specific fat depots and insulin sensitivity or HDL-cholesterol was found to be different in black and white children [162].

Our hypothesis that there would be a significant change in VAT between obese black and white adolescents in response to 3-months of AE versus RE without calorie restriction was not met. At baseline, white (1.61 ± 0.5 kg) adolescents had significantly more VAT than black (0.99 ± 0.4 kg) adolescents. Our observation that there is lower VAT in black compared to white adolescent boy and girls is consistent with the previous literature in children and adolescents [158, 163, 164]. After 3-months of physical exercise, we determined that obese black adolescent lost 18.1% of VAT while obese white adolescent whites lost 11.8%, independent of group and sex. In a mixed sample of obese black and white boys, Lee et al. [8] demonstrated that after 13-weeks of AE or RE obese adolescent boys had significant reductions in VAT independent of exercise modality and race compared to non-exercise controls. In a follow up study in a mixed sample of obese black and white girls Lee et al. [139] demonstrated that after 13-weeks of AE versus RE exercise obese adolescent girls had significant reductions in VAT independent of race in the AE group but not in the RE group. Similarly, Gutin et al. [143] identified that a mixed sample of obese adolescents who participated in an afternoon lifestyle education plus physical training (5 day/week, 250 kcal/session) program where shown to improve in cardiovascular fitness and decline VAT, independent of race, to a greater degree than did a group who engaged in the lifestyle education alone. In a cross-sectional study by Owens et al. [165] investigating the

relationship of VAT and CRF to markers of insulin resistance, researchers identified obese black adolescents to be significantly less fit than obese white adolescents but to have significantly less VAT than their white counterparts.

Although our results show that African Americans, compared with whites, may, in fact, have less VAT mass, the risk for chronic metabolic diseases associated with VAT is higher in African Americans compared with whites [147, 166]. Because African-American persons seem to have less VAT compared with white persons, one could infer that they are exposed to greater environmental risk factors for chronic disease, such as a diet high in fat or processed carbohydrates or low levels of physical activity [167]. Because of the health implications associated with increased abdominal adiposity and VAT, there is a need for intervention strategies, such as exercise training, to prevent the deposition of adipose tissue and the obesity-associated deterioration of metabolic status.

5.1.3 Effect of a 3-month AE versus RE without calorie restriction on CRF and muscular strength in overweight adolescent males and females

We hypothesized that CRF (VO_{2peak}) would be significantly increased after 3-months of AE compared to RE in obese adolescent males and females. Our results show that there was no statistically significant difference in CRF after the 3-month intervention when comparing the AE (23.5%) group to the RE (19.4%) averaged across sex. Our results reveal a significant improvement for males (27.1%) compared to females (15.0%) averaged across group after 3-months of exercise. Again, although not statistically significant, our results do demonstrate substantial improvements in response to regular exercise training in overweight adolescents. It has been well established that aerobic exercise is used as the primary modality to improve CRF

in youth [168, 169]. Our findings support the association that aerobic training (≥ 3 days/week, ≥ 80% HR max) leads to mean improvements of 5-10% in CRF of children or adolescents [170].

These findings extend the previous observation by Lee et al. [8] who demonstrated that after 13-weeks of aerobic or resistance exercise obese adolescent boys had significant improvements in CRF independent of exercise modality compared to non-exercise controls. In a more recent study, Lee et al. [139] found that obese adolescent females significantly improved their CRF after 13-weeks of aerobic exercise but not the resistance group compared to non-exercise controls. In a similar study by van der Heijden et al. [10], obese (13 ± 2%) and lean (16 ± 4%) males and females significantly increased CRF after a 12-week aerobic exercise program without weight loss. In an 8-month, high-intensity (≥ 75% of VO_{2peak}) plus lifestyle education intervention, Gutin et al. [143] reported that CRF was significantly improved in obese adolescent males and females compared to subjects in the lifestyle education only group. Contrary findings have recently been reported [171] in adolescent (13-14 yrs old) females. After a 20-week AE program (3 days/week, 20 min., 75-80% HR max) no improvement was observed in CRF compared to non-exercise controls. The researchers [171] concluded that their exercise program might not have been frequent enough, intense enough, or long enough to elicit changes in CRF. Our protocol consisting of individually prescribed exercise intensity (60-75% VO_{2peak}), with exercise duration of 60 minutes, three days per week for 3 months resulted in improvements in CRF for obese adolescent males and females regardless of modality or ethnicity.

Our observation of 19.4% improvement in CRF in response to resistance exercise in obese adolescents is contrary to previous findings [122]. The average baseline CRF level (28.03 $ml/kg/min^{-1}$) in our study was noticeably lower than in a study by Shaibi et al. [122] (34.4 $ml/kg/min^{-1}$) that saw no change in CRF after 16-weeks of resistance exercise. A sedentary

cohort with a low baseline CRF, such as ours, may have lead to the improvement in CRF. The mechanism by which resistance exercise improves CRF in adolescents remains unclear. Hickson et al. [172] suggested that improvements in endurance exercise capacity without improvements in CRF in adults was due to increases in muscular strength and power. It is conceivable that the improvements we encountered in CRF may be due to changes in fat free mass and muscular strength which might have allowed our subjects to perform a single bout of acute exercise, such as a maximal CRF test, at a greater intensity and duration.

As we hypothesized, a significant improvement in muscular strength was observed in response to RE compared to AE in obese adolescents. The increase in strength for males was significantly greater than the changes in strength for females, regardless of modality. Females increased strength in the RE group but not the AE group. These findings extend the previous observation by Lee et al. [8] who demonstrated that after 13-weeks of aerobic versus resistance exercise obese adolescent boys had significant improvements in muscular strength in the RE group but not in the AE group compared to non-exercise controls. In a more recent study, Lee et al. [139] found that obese adolescent females significantly improved their muscular strength after 13-weeks of RE but not AE compared to non-exercise controls. In a study by Benson et al. [173], overweight and obese adolescents (12.2 yrs) were recruited for an 8-week high-intensity resistance-training program that resulted in significant increases in upper and lower body strength compared to non-exercise controls. The training-induced gains in muscular strength during childhood have been attributed primarily to neuromuscular adaptations as opposed to hypertrophic factors [174, 175]. Although not assessed in this study, increases in motor unit activation, improvements in motor skill coordination, and perhaps some qualitative changes in the muscle have been suggested as possible mechanisms by which children increase their

muscular strength in response to resistance training. Further studies should address this.

CRF has emerged as an independent determinant of weight status in youth [176, 177]. More specifically, cross-sectional studies demonstrate that low CRF [115] and muscular strength [178] are characteristic features of overweight youth and independently associated with adiposity [179]. Additionally, average cardiorespiratory fitness levels in American youth have declined in parallel with the rising prevalence of childhood obesity [180]. Our observation that regular exercise training alone may increase CRF (VO_{2peak}) and muscular strength in overweight adolescents suggests the health benefits of regular physical activity in this population.

5.2 STRENGTHS AND LIMITATIONS

The strengths of this study warrant mention. Our study extends previous observations by employing a multiple-slice MRI technique to quantify total, visceral and abdominal subcutaneous adipose tissue in response to regular exercise. We also employed the gold standard non-invasive technique of using proton magnetic resonance spectroscopy to assess intrahepatic lipid. Further, high attendance rates in the AE (97.6%) and RE (98%) groups suggest that our exercise regimen is feasible and could be implemented in school settings. Furthermore, no subjects in the exercise groups were injured during the 3-month intervention, suggesting that our exercise prescription is acceptable and enjoyable for overweight adolescent boys and girls.

The limitations of this study warrant mention. The potential limitation in this study is the small sample size. Although the power and sample size were calculated to detect a reduction of 10% in VAT with 95% power at a significance level of 0.05, the calculation was based on previous findings in an adult study that was not designed to examine the effects of sex and race

on total and visceral adipose tissue and liver fat. Thus, the small sample size may have limited the ability to detect statistical significance on measurement variables within or between groups.

Secondly, our findings are limited to obese healthy black and white adolescent boys and girls. Further studies are required to explore whether our observations remain true in different pediatric populations such as: lean adolescent boys, lean adolescent girls, prepubertal girls and girls with oral or injectable contraceptives and other ethnicities. Since our findings are limited to obese healthy black and white adolescents our findings cannot be generalized to the general population of obese adolescent boys and girls. All subjects were offered compensation, free access to facilities, travel reimbursement and personal supervision from an exercise physiologist. These amenities almost certainly increased subject compliance. Due to all these factors our results cannot be generalized to the general population of obese adolescent boys and girls. Further, our study examined the effects of sex and race after a short-term exercise training intervention in overweight adolescent boys and girls. Evidence regarding the benefits of long-term exercise on obesity-related health outcomes in youth is unclear.

Another limitation is that a subsequent CRF test was assessed at 4^{th} and 8^{th} week to re-evaluate target HR and energy expenditure in the AE group only. These repeated test exposure may influence the changes in CRF in this group. Energy expenditure was not monitored in the RE group which would have allowed for comparison of total energy expended between groups, sex and race. Participants were asked to report daily energy intake three times per week. A significant portion of subjects did not track their food consumption and the subjects that did usually performed this task incorrectly. Leisure time physical activity was also not monitored.

5.3 FUTURE RECOMMENDATIONS

Although our findings did not reveal effects of sex and race on total and regional adipose tissue, substantial decreases were observed. One possible explanation might be the small sample size in this study. Future studies should include a larger sample size to detect the statistical significances with higher power levels. Our subjects were divided into two exercise groups, AE and RE. The combination of exercise modes (e.g., AE + RE) might result in even greater losses of IHL, TAT and VAT as well as increases in both fitness and strength. Another variable that might have an impacted our outcome measures was the length of the intervention. The effect of a long-term exercise intervention in obese adolescents has yet to be performed. One could hypothesize that greater improvements might be seen with an extended intervention. Further investigations might also equip subjects with pedometers or accelerometers to track daily physical activity and require strict adherence to the tracking of daily energy consumption.

5.4 CONCLUSION

In conclusion, 3 months of regular exercise training (60 min/day, 3 times/week) without calorie restriction reduced TAT and VAT in the absence of changes in weight that were not significantly different between males and females, independent of exercise modality and race. Males reduced IHL in the AE and RE groups while females reduced IHL in the AE group only. AE but not RE might be more effective in reducing IHL and improving metabolic health in obese adolescent girls. Significant improvements in CRF and muscular strength were seen in males compared to females after 3 months of regular exercise, independent of exercise modality and race. 3 months

of regular exercise training without calorie restriction resulted in reductions in TAT and VAT and improvements in CRF and muscular strength in the absence of changes in weight that were not significantly different between obese black and whites adolescents, independent of exercise modality and sex. Obese white adolescents significantly reduced IHL after 3 months of regular exercise compared to black adolescents, independent of exercise modality and sex. Our observations suggest that regular exercise alone is an effective treatment strategy for the treatment of obesity in overweight black and white adolescents.

Declines in physical activity levels over the last two decades have led to considerable increases in NAFLD and obesity in children and adolescents. Given that intrahepatic lipid content, abdominal obesity and physical inactivity are strongly associated with many health outcomes, our findings have clinical implications that could be implemented in public health settings to combat the current epidemic of childhood obesity and NAFLD in children and adolescents.

APPENDIX A

[EXERCISE MEASUREMENTS FORMS]

AEROBIC EXERCISE DATA FORM

RESISTANCE EXERCISE DATA FORM

CARDIORESPIRATORY FITNESS TEST

MUSCULAR STRENGTH TEST

WAIST CIRCUMFERENCE MEASUREMENT

AEROBIC EXERCISE DATA FORM

Week: _____

Name (ID): _____ Age: _____ Sex: male / female

Height (cm): _____ Max HR (bpm): _____

	Mon Tue Wed Thu Fri Sat				Mon Tue Wed Thu Fri Sat				Mon Tue Wed Thu Fri Sat				Mon Tue Wed Thu Fri Sat			
	Date: / /				Date: / /				Date: / /				Date: / /			
	Time (min)	Speed (mph)	Grade (%)	HR (bpm)	Time (min)	Speed (mph)	Grade (%)	HR (bpm)	Time (min)	Speed (mph)	Grade (%)	HR (bpm)	Time (min)	Speed (mph)	Grade (%)	HR (bpm)
Warm-Up	Resting	-	-		Resting	-	-		Resting	-	-		Resting	-	-	
	0~5				0~5				0~5				0~5			
	5~10				5~10				5~10				5~10			
Aerobic Exercise	10~15				10~15				10~15				10~15			
	15~20				15~20				15~20				15~20			
	20~25				20~25				20~25				20~25			
	25~30				25~30				25~30				25~30			
	30~35				30~35				30~35				30~35			
	35~40				35~40				35~40				35~40			
	40~45				40~45				40~45				40~45			
	45~50				45~50				45~50				45~50			
Cool-Down	50~55				50~55				50~55				50~55			
	55~60				55~60				55~60				55~60			
Comments:	Body Weight (kg):				Body Weight(kg):				Body Weight(kg):				Body Weight(kg):			
	Time: Min:				Time: Min:				Time: Min:				Time: Min:			

RESISTANCE EXERCISE DATA FORM

Week: _____

Name (ID): _____ Age: _____ Sex: male / female

Height (cm): _____

	1 RM (lb)	Mon Tue Wed Thu Fri Sat Date: / / Weight (lb) / Repetition (8~12)			Mon Tue Wed Thu Fri Sat Date: / / Weight (lb) / Repetition (8~12)			Mon Tue Wed Thu Fri Sat Date: / / Weight (lb) / Repetition (8~12)			Mon Tue Wed Thu Fri Sat Date: / / Weight (lb) / Repetition (8~12)		
Warm-Up		1st set	2nd set	3rd set	1st set	2nd set	3rd set	1st set	2nd set	3rd set	1st set	2nd set	3rd set
1. Leg Press													
2. Leg Extension													
3. Leg Flexion													
4. Chest Press													
5. Lat Pull-Down													
6. Seated Row													
7. Bicep Curl													
8. Triceps Extension													
9. Sit-Up													
10. Push-UP													
Comments:		Body Weight (kg):			Body Weight (kg):			Body Weight (kg):			Body Weight (kg):		
		Time: Min:			Time: Min:			Time: Min:			Time: Min:		

CARDIORESPIRATORY FITNESS TEST (VO_2peak Test)

Name: _____ Test Date: _____

ID: _____ Gender: male / female

Age: _____ Test: pre / post

Weight (kg): _____ Height (cm): _____

Age-adjusted Max HR (bpm): 220 – age = _____ Speed (mph): _____

Time (min)	Grade (%)	HR (bpm)	Time (min)	Grade (%)	HR (bpm)
00:00	**0**		10:20		
0:20			10:40		
0:40			11:00	**10**	
1:00			11:20		
1:20			11:40		
1:40			12:00	**11**	
2:00			12:20		
2:20			12:40		
2:40			13:00	**12**	
3:00	**2**		13:20		
3:20			13:40		
3:40			14:00	**13**	
4:00	**3**		14:20		
4:20			14:40		
4:40			15:00	**14**	
5:00	**4**		15:20		
5:20			15:40		
5:40			16:00	**15**	
6:00	**5**		16:20		
6:20			16:40		
6:40			17:00	**16**	
7:00	**6**		17:20		
7:20			17:40		
7:40			18:00	**17**	
8:00	**7**		18:20		
8:20			18:40		
8:40			19:00	**18**	
9:00	**8**		19:20		
9:20			19:40		
9:40			20:00	**19**	
10:00	**9**				

Cool-Down	1:00	2:00	3:00	4:00	5:00
HR (bpm)					

Max HR (bpm): _____

Max VO^2 (ml/kg/min): _____ Staff: _____

MUSCULAR STRENGTH TEST

Name: _____ Test Date: _____

ID: _____ Gender male / female

Age: _____ Test: pre / post

Weight (kg): _____ Height (cm): _____

	Warm-Up		Trial 1	Trial 2	Trial 3	1 RM
Upper Body	40-60%	60-80%	1 RM	1 RM	1 RM	
1. Chest Press						
2. Lat Pull-Down						

Lower Body	40-60%	60-80%	1 RM	1 RM	1 RM	
1. Leg Press						
2. Leg Extension						
Comments:						

Staff: _____

WAIST CIRCUMFRENT MEASUREMENT

Name: _____ Test Date: _____

ID: _____ Gender: male / female

Age: _____ Test: pre / post

Weight (kg): _____ Height (cm): _____

Waist Circumference (cm)	1st	2nd	Average
1. Last Rib			
2. Iliac Crest			
3. Umbilicus			

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