Effect of Exercise Training on Metabolic Syndrome z-score: the Association of C- reactive protein

by

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PURPOSE: Previous studies have shown that metabolic syndrome z-score (MetSynZ) is improved with exercise training. Metabolic syndrome is based upon insulin resistance, such that it would be expected for insulin resistance to improve with exercise training. Currently, it is unknown if improvements in MetSynZ from exercise training are associated with improvements in systemic inflammation. The purpose of the present study is to evaluate the effect of exercise training on MetSynZ in participants with elevated C- reactive protein (CRP), and determine if changes in MetSynZ with exercise training were associated with changes in CRP and HOMA-IR (Homeostatic Model Assessment- Insulin Resistance). METHODS: The study sample included 123 participants with elevated CRP levels from the Inflammation and Exercise (INFLAME) study. The participants were randomized into a control group and an exercise group. MetSynZ was defined as the sum of the z-scores from the NCEP-ATP III criteria. MetSynZ, CRP, and HOMA-IR were evaluated at baseline and follow up. The intervention consisted of aerobic exercise training for 4 months where total energy expenditure was approximately 16 kilocalories/week per kilogram of body weight. RESULTS: Baseline CRP showed small, non-significant, associations with MetSynZ. Analysis of covariance showed no significant change in MetSynZ between the exercise (-0.3, CI: -0.7 to 0.2) and control groups (0.2, CI: -0.2 to 0.6). Change in MetSynZ was not associated with change in CRP (r=-0.15, p=0.914), but was

associated with change in the homeostatic model assessment of insulin resistance (HOMA-IR) ($r=0.286$, $p=0.036$). DISCUSSION: Results from the present study suggest that 4 months of aerobic exercise training may not be a sufficient time to favorably change MetSynZ. Reductions in MetSynZ with exercise training were not associated with improvements in systemic inflammation, but were associated with significant improvement in insulin resistance.

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List of Abbreviations

National Cholesterol Education Program- Adult Treatment Panel III (NCEP-ATP III)

World Health Organization (WHO)

International Diabetes Federation (IDF)

Metabolic Syndrome (MetSyn)

C- reactive protein (CRP)

Homeostatic Model Assessment-Insulin Resistance (HOMA-IR)

Inflammation and Exercise study (INFLAME)

High – Density Lipoprotein (HDL)

Low- Density Lipoprotein (LDL)

Kilocalries per kilogram bodyweight per week (KKW)

Mean Arterial Pressure (MAP)

Analysis of Covariance (ANCOVA)

Chapter I: Introduction

The increased prevalence of insulin resistance, cardiovascular disease, and type 2 diabetes are all major public health concerns that are growing in the United States [1]. The National Cholesterol Education Program- Adult Treatment Panel III (NCEP-ATP III) has defined a clustering of 3 or more risk factors (elevated blood pressure, elevated waist circumference, impaired fasting glucose, and dyslipidemia) known as the metabolic syndrome (MetSyn)[1, 2]. MetSyn is centered around insulin resistance, such that it increases overall risk for cardiovascular disease and type 2 diabetes [2-6]. Current estimates suggest that 22.9% of adults in the United States have the MetSyn [7]. Increasing physical activity and exercise training are potential strategies to reduce the prevalence of MetSyn and the severity of the aforementioned risk factors. An inverse relationship has been observed between high physical activity levels and cardiorespiratory fitness with MetSyn prevalence. Several exercise training studies have observed a reduction in MetSyn prevalence following intervention [8-15].

Much of the current scientific literature has described the MetSyn using categorical MetSyn definitions (NCEP [2], WHO [16], IDF definitions [17]); however, a limitation of this approach is that it may not thoroughly account for continuous changes in individual MetSyn risk factors. For example, if systolic blood pressure decreases from 140 to 132 mmHg, this would still be identified as a MetSyn risk factor (based on categorical definitions) despite the fact that a clinically significant reduction in blood pressure was achieved. Thus, the categorical definition is dependent on the presence or absence of 3 MetSyn risk factors [18]. Recently, researchers have utilized MetSyn z-score which uses a continuous risk score assessment scale [18]. Researchers have proposed that the categorical definition of MetSyn may not recognize these improvements in individual risk factors following exercise interventions because the reduction is not below the

cut off values for a risk factor [9]. MetSyn z-score uses a continuous risk score which may better identify improvements in individual risk factors despite reductions below recommended values.

Similar to the presence of MetSyn risk factors, an elevated level of C- reactive protein (CRP) is associated with an increased risk of cardiovascular events and other disease processes as it is represented by chronic elevated systemic inflammation [19-21]. Several studies suggest that a positive association exists between CRP level and the prevalence of MetSyn [19, 21-23]. Current data indicates that aerobic exercise training has been established as an effective treatment to reduce MetSyn z-score [8-15]. Recent data have shown that reductions in CRP with exercise training are associated with weight reduction [24]. It is possible that a reduction in waist circumference, a MetSyn risk factor, may be related to a reduction in weight and fat loss, which is a potential mechanism that may link the association between improvements in MetSyn z-score and CRP reduction following exercise training. This relationship may be of importance because of the pro-inflammatory pathway, as represented by elevated CRP, which is associated with the progression of multiple disease processes including increased cardiovascular disease risk and type 2 diabetes risk. However, to our knowledge, the effect of exercise training on changes in MetSyn Z-score and CRP has not been previously assessed in the literature.

The purpose of the present study was to investigate the effect of aerobic exercise training on MetSyn z-score. Secondly, we evaluated whether reductions in MetSyn z-score were associated with reductions of CRP levels as a result of aerobic exercise training. The analysis of the study utilized data from the Inflammation and exercise (INFLAME) study which evaluated the effect of 4 months of aerobic exercise training on inflammatory markers in adults with elevated CRP at baseline. We hypothesize that aerobic exercise training will result in reduction

in MetSyn z-score, and that the reductions in MetSyn z-score following exercise training will be correlated with reductions in CRP levels.

Limitations

Limitations of this study design include not accounting for changes in medication throughout the intervention, the four month intervention duration, only using one exercise intensity during the exercise intervention, measuring the waist circumference at the umbilicus rather than the minimum waist, and z-scores were tabulated from the study population such that it can only be generalized for adults with elevated CRP levels.

Delimitations

Delimitations include not currently taking medication with confounding effects on inflammation, no current tobacco use in the past 6 months, randomized controlled design study, controlled and supervised exercise doses, and closely monitored energy expenditure.

Key Terms

Metabolic syndrome, metabolic syndrome z-score, C- reactive protein.

Metabolic syndrome (MetSyn): a clustering of risk factors (elevated blood pressure, elevated waist circumference, impaired fasting glucose, and dyslipidemia) that increases the overall risk for insulin resistance, cardiovascular disease, and type 2 diabetes [6]. NCEP-ATP III guidelines define MetSyn as having three of the five following risk factors: waist circumference > 102 cm in men and 88 cm in women; triglycerides ≥ 150 mg/dL; High Density Lipoprotein (HDL) cholesterol < 40 mg/dL in men and 50 mg/dL in women; blood pressure ≥ 130 mmHG for

systolic blood pressure and/or 85 mmHg for diastolic blood pressure; and impaired fasting glucose ≥ 110 mg/dL [1, 2].

Metabolic syndrome z-score: a statistically normalized z-score using means and standard deviations of the individual components of MetSyn that are then combined for a continuous risk score [9].

C- reactive protein (CRP): a biomarker of systemic inflammation measured in the blood [22]. The National Institute of Health classifies CRP as low, average, and high risk with values <1.0 mg/L, 1.0 to 3.0 mg/L, and >3.0 mg/L, respectively [25].

Significance

Although there are studies that have observed the relationship between MetSyn prevalence and CRP levels, limitations do exist. Few studies have evaluated the use of CRP levels as a predictor for having the MetSyn. It has been observed that exercise training has positive effects on both MetSyn z-score and CRP, independently. Despite this potential association between MetSyn and CRP, there are no studies to date that examine reductions in MetSyn z-score following an exercise intervention that are associated with reductions in inflammation. Determining a target pathway, such as inflammation, to improve cardiovascular risk as represented by MetSyn z-score could have important clinical relevance for developing exercise interventions. Since the relationship between MetSyn z-score and CRP has not been clearly defined, investigating the effects of exercise on this relationship may aid in the understanding of the potential mechanism of cardiovascular risk reduction.

Chapter II: Literature Review

Metabolic Syndrome

MetSyn is a constellation of risk factors (elevated blood pressure, elevated waist circumference, impaired fasting glucose, and dyslipidemia) that increases the overall risk for insulin resistance, cardiovascular disease, and type 2 diabetes [6]. Several health organizations (e.g. World Health Organization, International Diabetes Federation, and the National Cholesterol Education Program) have developed specific criteria and guidelines to identify individuals with the metabolic syndrome [2]. The National Cholesterol Education Program Adult Treatment Panel III (NCEP-ATP III) criteria are the most utilized among researchers and clinicians [1]. NCEP-ATP III guidelines define MetSyn as having three of the five following risk factors: waist circumference > 102 cm in men and 88 cm in women; triglycerides ≥ 150 mg/dL; HDL cholesterol < 40 mg/dL in men and 50 mg/dL in women; blood pressure ≥ 130 mmHG for systolic blood pressure and/or 85 mmHg for diastolic blood pressure; and impaired fasting glucose ≥ 110 mg/dL[1]. Present research approximates that 22.9% of U.S. adults have MetSyn [7]. Cheal et al. [3] observed that the odds of insulin resistance increased 10-fold (odds ratio: 10.7; 95% CI: 6.2,18.3) for those individuals who met the criteria for MetSyn compared to those without MetSyn. Adults with MetSyn have an increased significant relative risk for developing type 2 diabetes of 3.97 (95% CI:1.35,11.6) [5]. The relative risk of cardiovascular disease in presence of MetSyn is also increased at 2.96 (95% CI: 2.36,3.72) [26]. Thus, reducing the prevalence of MetSyn and its aforementioned risk factors has high clinical and public health importance.

Metabolic Syndrome z-score

Recently, researchers have questioned whether the categorical definition of MetSyn is the best indicator of quantifying the overall risk associated with the MetSyn risk factors. The categorical method of defining MetSyn is dependent on the presence or absence of each risk factor. It has been suggested that the categorical definition of MetSyn may limit the evidence of improvement of individual risk factors for MetSyn as compared to a MetSyn z-score [9, 10]. For example, if a participant's baseline blood pressure is 140/80 mmHg and reduces the value to 132/80 mmHg following an exercise intervention, the categorical definition of MetSyn would not recognize the improvement as the recommended blood pressure value is below 130/85 mmHg [1]. Consequently, the effects of exercise are attenuated as the components of MetSyn are viewed as a whole rather than individually [9].

To determine continuous risk score for MetSyn, each risk factor's individual z-score is computed from a standard deviation/sample mean of the sample. The z-scores for waist circumference, blood pressure, glucose, cholesterol, and triglycerides are then summed to represent the continuous risk for MetSyn [18]. Earnest et al. [9] proposes that defining MetSyn with the z-score accounts for intraindividual responses that differ within each component.

When utilizing the categorical approach to determine MetSyn risk, exercise training could yield a reduction in certain risk factors which may not be below recommended values, such that the improvement is not recognized. MetSyn z-score recognizes improvement despite reductions below the threshold of recommended values. This in turn can benefit healthcare providers in defining the severity of an individual's metabolic syndrome and risk for disease. Many studies have evaluated MetSyn through the categorical approach, but determining the

severity of the MetSyn in subjects through z-scores may translate more appropriately into the healthcare field when providers are creating a treatment plan.

Effect of Aerobic Fitness Levels on Metabolic Syndrome

It has been suggested that fitness level plays a role in MetSyn prevalence and MetSyn z-score. Recent literature has observed that high cardiorespiratory fitness levels are inversely associated with MetSyn and MetSyn z-score [8, 9]. Farrell et al. [8] (N=7104) divided women into cardiorespiratory fitness quintiles according to age following a maximal treadmill test. MetSyn was identified by ATP III guidelines. Women in the highest fitness quintile had a significantly lower prevalence of MetSyn (2.7%) compared to those in the lowest fitness quintile (20.2%) [8]. Earnest et al. [9] also reported an inverse relationship between fitness level and MetSyn z-score for both sexes in a cross-sectional analysis of the Aerobics Center Longitudinal Study (N=38,659). MetSyn z-score decreased across higher fitness levels for males ($p<0.001$) as well as for females ($p<0.007$) [9]. It is apparent from these data that cardiorespiratory fitness level is associated with MetSyn prevalence and MetSyn z-score.

Metabolic Syndrome Prevalence Reduction with Aerobic Exercise Training

Similar to the epidemiological research concerning fitness level, exercise training has been observed to reduce the prevalence of MetSyn [13, 14]. Tjønna et al. [14] randomly assigned 32 participants with MetSyn to one of three groups: moderate continuous aerobic exercise, aerobic interval training, or control. Maximal aerobic capacity (VO_2max), blood draws, and muscle biopsies were performed before and after the intervention which was designed to be performed three times per week for sixteen weeks. MetSyn was determined categorically according to World Health Organization criteria [14]. Aerobic interval training was significantly

associated with reduction in the number of risk factors for MetSyn from 6 to 4 ($p<0.001$). The continuous moderate exercise group produced favorable but non-significant changes from 6 to 5 risk factors. Participants in the control group increased the number of risk factors from 5 to 6. It was observed that aerobic exercise training produced favorable improvements in MetSyn risk factors [14].

Katzmaryzk et al. [13] investigated the effect of an aerobic exercise training intervention on MetSyn prevalence using data from the HERITAGE family study. MetSyn was determined as 3 or more risk factors as according to the NCEP-ATP III criteria. Participants with MetSyn present (N=105) performed 20 weeks of supervised aerobic exercise training which included three sessions per week using a cycle ergometer. The overall prevalence of MetSyn significantly decreased from 16.9% at baseline to 11.8% post training intervention ($p<0.05$). It appears that aerobic exercise training is an effective tool in reducing MetSyn prevalence.

MetSyn z-score Reduction with Aerobic Exercise Training

As current data suggests that exercise training aids in reduction of MetSyn prevalence, it has also been observed to aid in reduction of MetSyn z-score. It has been proposed that a dose-response relationship exists between energy expenditure and MetSyn z-score reductions, such that moderate intensity aerobic exercise is sufficient in producing favorable changes [10, 11, 15]. Much of the present research on the effect of exercise training on MetSyn z-score is focused on special populations, such as women [10] and those with type 2 diabetes [11] that also have MetSyn. Earnest et al. [10] utilized data from the Dose-Response to Exercise in Women (DREW) aged 45 to 75 years study and observed that a cardiorespiratory exercise intervention for 6-months resulted in the reduction of MetSyn z-score in postmenopausal women (N=408).

Participants were randomly assigned to one of four groups: control, low intensity (4 kilocalories per kg per week or KKW), moderate intensity (8 KKW), or high intensity (12 KKW). The reduction in MetSyn z-score was significant only in the moderate and high intensity groups (-3%, -9%, respectively). The control group had a 4% increase in MetSyn z-score. Those who expended more energy per week presented greater reductions in MetSyn z-score. Overall, the reductions in MetSyn z-score were positively associated with the total exercise energy expenditure.

Similarly, Earnest et al. [11] observed a significant dose-response effect of cardiorespiratory exercise on MetSyn z-score in an ancillary analysis of the Health Benefits of Aerobic and Resistance Training in individuals with type 2 diabetes (HART-D) study (N=208). MetSyn was defined by NCEP-ATP III criteria and was determined by prevalence and z-score. Participants in the aerobic exercise training group expended ~12 KKW for 9-months by means of moderate to vigorous intensity on a treadmill. Participants in the non-exercise control group were asked to maintain their normal daily physical activity throughout the intervention; however, they were offered stretching and relaxing classes. The aerobic exercise training group had a baseline MetSyn z-score of 2.99±0.91 with a significant -0.5 reduction in MetSyn z-score following the intervention. The non-exercise control group had a baseline MetSyn z-score of 2.63±0.86 and a +0.03 increase in MetSyn z-score following the intervention. The authors observed that moderate intensity aerobic exercise was effective in reducing MetSyn z-score in those with type 2 diabetes [11].

The Studies of a Targeted Risk Reduction Intervention through Defined Exercise (STRRIDE) evaluated the effects of 8 months of different volumes and intensities of exercise training. The differing volume and intensity exercise training groups included low

amount/moderate intensity, low amount/vigorous intensity, and high amount, vigorous intensity. Participants in the control group were asked to remain inactive and maintain their current dietary intake for 6 months. Johnson et al. [15] reported a significant improvement in MetSyn z-scores with low amounts of moderate intensity aerobic exercise (walking ~ 19 kilometers per week) compared to the control group ($p<0.05$). However, the change in MetSyn z-score was not different between the low amount/vigorous intensities group and control group. The authors also observed that the higher amount/vigorous intensity group improved MetSyn z-scores relative to the controls ($p<0.0001$), as well as the low amount/vigorous intensity group ($p=0.001$) and low amount/moderate intensity ($p=0.007$). This suggests that there is possibly a dose-response relationship [15]. It has been proposed that moderate intensities of aerobic exercise are sufficient reducing an individual's MetSyn z-score.

Summary: Metabolic Syndrome and Exercise Training

Previous studies have observed that cardiorespiratory fitness level is inversely associated with MetSyn prevalence and z-score [8, 9]. Individuals can improve their MetSyn risk factors by improving their cardiorespiratory fitness through exercise training [8-15], which in turn reduces MetSyn prevalence and z-score. Current data suggests that moderate intensities may be sufficient to improve MetSyn in special and sedentary populations [11, 15]. This has important and practical implications for public health recommendations and messages.

Metabolic Syndrome and C - reactive protein

C- reactive protein (CRP) is measured to examine systemic inflammation, as inflammation is an inaugural step in multiple disease processes especially atherosclerosis [25].

Evidence has recently emerged that CRP may be a strong predictor of MetSyn prevalence, cardiovascular disease and type 2 diabetes [21, 22]. According to the National Institute of Health, CRP levels are classified as low, average, and high risk with values < 1.0 mg/L, 1.0 to 3 mg/L, and >3.0 mg/L, respectively [25]. It has been observed in few studies that individuals with multiple MetSyn risk factors tend to have higher CRP values compared to those with fewer MetSyn risk factors [19, 21-23]. Therefore, reductions in MetSyn z-score and reduction systemic inflammation may be associated.

Ridker et al. [22] (N=14,179) examined apparently healthy women who completed a follow up evaluation after an 8-year period for myocardial infarction, coronary revascularization, stroke, cardiovascular event death, and MetSyn. At baseline, 24.4% of the participants had MetSyn based on a categorical approach using NCEP ATP III criteria. Participants with no characteristics of the MetSyn had median CRP levels of 0.68 mg/L while those with 5 characteristics had median CRP levels of 5.75 mg/L. For those with 3 or more MetSyn characteristics CRP levels after the 8-year follow up were 3.38 mg/L, whereas those with no MetSyn characteristics CRP levels were 1.08 ($p<0.0001$).

Sattar et al. [23] evaluated baseline and follow-up assessments completed over a 4.9 year period of men from the West Scotland Coronary Prevention Study to determine the association between CRP and MetSyn. There was a 3.7 fold increase risk for coronary heart disease and 24.5 fold increased risk for diabetes with the presence of 4-5 MetSyn risk factors when compared with those men who did not have 4-5 MetSyn risk factors. It was observed that CRP was higher at baseline (2.36±2.68 mg/L) in the 26% of men with MetSyn as compared to those without MetSyn (1.56±2.97 mg/L), ($p<0.0001$). Higher CRP levels with the presence of MetSyn predicted higher risk for cardiovascular disease (Hazard Ratio (HR): 2.75; 95% CI: 2.1, 3.6) and

diabetes (HR: 5.3; 95% CI: 3.3, 8.3). [23]. It appears that CRP is an appropriate addition to prediction methods for risk of cardiovascular disease, diabetes, and MetSyn.

Effect of Exercise Training on C –reactive protein

As fitness level and exercise training plays a role in MetSyn, it has also been observed to have an effect on CRP levels. Ford et al. [27] analyzed data from the National Health and Nutrition Examination Survey III (1988-1994) (N=13,748) to evaluate the relationship between physical activity and inflammation as represented by CRP. Elevated CRP levels were recorded in 21% of participants that reported no physical activity, 17% that reported light physical activity, 13% that reported moderate physical activity, and 8% that reported vigorous physical activity ($p<0.001$). It was proposed that there is an inverse relationship between physical activity and CRP levels [27].

Numerous studies have evaluated the effect of exercise training on CRP levels in varying populations such as those with coronary heart disease, older adults, and adults with elevated CRP levels [18, 24, 28-30]. Goldhammer et al. [30] (N=28) observed reductions in CRP following a 12-week aerobic exercise training program in participants with coronary heart disease. The exercise intensity was set at 70-80% of their individual maximal heart rate for 45 minute sessions 3 times per week. Additionally, the participants attended 2 half hour calisthenics group exercise sessions per week. It was observed that exercise training induced favorable improvements in CRP levels by 48% from baseline (7.5±4.2 mg/L) to post training (3.9±3.5 mg/L) ($p<0.01$). These reductions were independent of changes in body weight [30]. Milani et al. [28] (N=35) examined the effect of exercise training on CRP through a three-month formal phase II cardiac rehabilitation program in patients with coronary heart disease. Exercise training produced

significant reductions from baseline (5.9±7.7 mg/l) to post exercise training (3.8±5.8 mg/l) ($p<0.0001$). These data suggest that exercise training elicits favorable improvements in CRP for participants with coronary heart disease [28, 30].

Recent data suggests that CRP reduction following exercise training is dependent on weight loss and fat reduction [18, 24, 29]. Vieira et al. [29] randomized 127 community residents ages 60-83 into a flexibility group (n=61) and a cardio group (n=66). The participants were sedentary for 6 months prior to the 10 month exercise intervention. The flexibility group participated in 2 supervised exercise sessions for 75 minutes per week during which they completed flexibility and balance exercises. The cardio group participated in 3 supervised cardiovascular exercise sessions for 45-60 minutes at 60-70% VO_2max. A significant reduction in CRP following exercise was observed for the cardio group (-0.5 mg/L) ($p=0.009$). Reductions in CRP were also significantly correlated with reductions in both body fat percentage ($r=0.193$) and trunk fat mass ($r=0.222$) ($p<0.05$) [29].

Few randomized controlled exercise training trials have observed that reductions in CRP were positively associated with reductions in weight [18, 24]. Stewart et al. [24] (N=421) concluded that a 6 month aerobic exercise training intervention yielded CRP reductions that were significantly and positively associated with weight ($r=0.15$; $p<0.001$) and waist circumference ($r=0.12$; $p=0.01$). Participants that expended 12 KKW lost 5.9 (±3.0) kg and had a reduction in CRP of 1.0 (±2.8) mg/L. Church et al. [18] utilized data from the Inflammation and Exercise (INFLAME) study to evaluate the effect of exercise training on CRP. It was observed that reduction in CRP was associated with weight loss ($r=0.34$) and change in body fat mass ($r=0.35$) following 4 months of aerobic exercise training reductions ($p<0.01$). It is reasonable that reductions in waist circumference and weight reduction may perhaps be related, such that this

may be the mechanism that explains the association between MetSyn z-score and CRP [18, 24, 29].

Summary

It is probable that there is an association between MetSyn z-score and CRP level following exercise training [22, 23]; however, there is currently no available research on this topic. Current research supports that CRP levels are associated with an increased risk for MetSyn [19, 21-23]. It has been established that aerobic exercise training has beneficial effects in reducing MetSyn z-score, as well as waist circumference [8-15]. Few studies have observed that exercise training yields favorable improvements in CRP level [18, 24, 28-30] . Weight loss, which may be represented by improvements in waist circumference, has been positively correlated with reduction in CRP levels following an aerobic exercise training intervention [18, 24, 29] . This association between MetSyn z-score and CRP may exist due to the observation that exercise training aids in reduction of waist circumference, an individual risk factor of MetSyn, as well as weight reduction that yields CRP reductions. These exercise related reductions in MetSyn z-score could possibly mediate cardiovascular disease risk by reduction in inflammation. This potential association could hold important implications in the understanding of the mechanism for improvement in health risk with MetSyn z-score reduction.

Chapter III: Methods

Participants

The present study was a secondary analysis of the Inflammation and Exercise (INFLAME) study (N=162). The main methodology and primary outcomes of INFLAME has been previously published [18, 31]. The INFLAME study was designed to determine the effect of exercise training on elevated CRP levels in sedentary men and women [31]. All participants provided a written informed consent prior to enrollment in the study and the INFLAME study was approved for analysis by the Institutional Review Board at the Cooper Institute. Participants in the INFLAME study were healthy, sedentary men and women between the ages of 30 and 75 years old with elevated CRP levels (≥2.0 mg/L but <10 mg/L). Sedentary behavior was defined as exercising less than 20 minutes per day on less than 3 days per week. Other major inclusion criteria included body mass index (BMI) > 18.5 kg/m^2 and < 40.0 kg/m^2, fasting glucose < 126 mg/dL, resting blood pressure < 140 mmHg systolic and/or 90 mmHg diastolic, no current tobacco use in the past 6 months, and not currently taking specific medications that had confounding effects on inflammation (statins, angiotensin converting enzyme inhibitors, oral contraceptives, multi-vitamins, aspirin, ibuprofen, and other anti-inflammatory medications). Major exclusion criteria included specific medications (hormone replacement therapy, beta blockers, allergy shot, or systemic corticosteroids), significant cardiovascular disease or disorders (serious arrhythmias, cardiomyopathy, congestive heart failure, stroke, peripheral vascular disease with intermittent claudication, acute, chronic, or recurrent thrombophlebitis, stage II or III hypertension, myocardial infarction, or an abnormal exercise stress test), total cholesterol ≥ 240 mg/dL with LDL-C ≥190 mg/dL or triglyceride levels > 300 mg/dL, and other significant medical conditions (chronic or recurrent respiratory, gastrointestinal, neuromuscular,

neurological, or psychiatric conditions, musculoskeletal problems interfering with exercise, or any other medical condition or disease that may be life-threatening, may interfere with, or be aggravated by exercise activities). Only participants with adherence levels above 75% were included in the analysis. Participants without data for all components for MetSyn data were excluded from the analysis.

Outcome Measures

Weight was measured to the nearest 0.1 kg using a calibrated electronic scale (Siemens Medical Solutions, Malvern, PA) with the participant wearing a hospital gown. Height was measured using a standard stadiometer. Body composition was measured via dual energy x-ray absorptiometry (DXA) using a Hologic Bone Densitometer (Hologic Inc., Bedford, MA). Abdominal circumference was determined by standard measuring techniques at the level of the umbilicus [32]. A 10-12 hour fasting blood sample was drawn to measure CRP and lipids. Fasting plasma glucose was measured by the hexokinase-glucose-6-phosphate dehydrogenase method. Insulin was measured using electrochemiluminescence. Blood pressure measurements were collected following a 20 minute supine rest period with a Colin STBP-780 automated BP unit (Colin Medical Instruments Corp., San Antonio, TX). Homeostasis Model Assessment-Insulin Resistance (HOMA-IR) was used to determine the insulin: glucose interaction using a computer model [33]. All assessment measures were performed at baseline and follow-up. The assessment staff was blinded from group randomization.

C- reactive protein Analysis

Prior to blood draws to determine CRP levels participants fasted for 10-12 hours, abstained from alcohol consumption and exercise for 24 hours, and refrained from acute usage of

aspirin or other anti-inflammatory medications for 48 hours. Those participants who reported use of anti-inflammatory medications at the orientation visit were asked to abstain from use of the medication prior to the blood draw.

Blood was drawn baseline and follow-up, and then stored at -80° C. CRP assays were performed only after both baseline and follow-up samples are available such that the measurement was completed with the same assay kit. CRP was measured by a solid-phase, chemiluminescent immunometric assay (Immulite 2000, High-Sensitivity CRP, Diagnostic Products Corporation, Los Angeles, CA).

Metabolic Syndrome z-score

MetSyn z-score was computed by computing a z-score from the study population mean and standard deviation of each risk factor (waist circumference, fasting glucose, HDL, triglycerides, and mean arterial pressure). Mean arterial pressure was determined through the equation: MAP= diastolic blood pressure- 1/3 (systolic blood pressure-diastolic blood pressure). The individual z-scores were summed together to create a composite MetSyn z-score.

Intervention

Following baseline assessments participants were randomized to an exercise group or a control group. Individuals randomized into the exercise group participated in supervised aerobic exercise for four months. The total energy expenditure was set at 16 kcal/kg per week (KKW) which was divided into 3-5 sessions per week. A two-week ramping period was used to ease participants into the exercise intervention to minimize injury, soreness, and dropout. The first week began at 10 KKW and the second week at 12KKW. Exercise intensity was 60-80% of maximal oxygen consumption (VO_2max) which was determined from their baseline exercise test.

Participants alternated modes of exercise between the treadmill and cycle ergometers to prevent injury occurrence. Heart rates, blood pressure, rate of perceived exertion, mode, and speed/grade or watts were recorded during each exercise session. The control group remained physically inactive throughout the duration of the intervention. Non-exercise physical activity during the intervention was measured through the use of step counters (Accusplit Eagle, Japan) in both groups. The exercise group was allowed up to 10 exercise sessions out of the laboratory and were provided with heart rate monitors and exercise prescriptions. Participants recorded the duration and intensity of the exercise to be included in their weekly energy expenditure. Dietary intake was tracked and participants were asked to not make any changes to their diet. No changes in diet or non-exercise physical activity were observed between the aerobic and control group.

Statistical Analysis

The primary outcomes of this analysis were the change in MetSyn z-score, as well as the association of changes in CRP following an aerobic exercise intervention. Baseline data was tabulated in means and standard deviations. Group differences in baseline data was evaluated with a t-test. Adherence was determined by dividing each participant's actual energy expenditure by the study prescribed energy expenditure of 16 KKW. Change in MetSyn z-score was analyzed using an analysis of covariance (ANCOVA) between the control and exercise group. Covariates in the analysis were designated and included a priori baseline value of the respective variable and age. Change in CRP was analyzed using an ANCOVA. The covariate in this analysis was designated as a priori baseline value. Results are presented in adjusted least squared means with 95% confidence intervals. Pearson correlations were utilized to evaluate the association between change in MetSyn z-score and change in CRP prior to and following exercise training. The association of HOMA-IR and MetSyn z-score was assessed in a post-hoc analysis by Pearson

correlation. Statistical significance for all statistics was defined as $p<0.05$. These analyses were completed with SPSS software (SPSS version 17.1, Chicago, 2009).

Chapter IV: Results

The control group population had a mean (SD) age of 50.9 (1.4) years, a mean BMI of 32.0 (3.9) kg/m^2, with 9.1 % African American and 66.7 % female. Baseline CRP was 4.5 (2.6) mg/L. The exercise group had a mean (SD) age of 50.6 (1.3) years, a mean BMI of 30.9 (4.4) kg/m^2, with 10.5 % African American and 78.9 % female, and baseline CRP was 5.1 (4.2) mg/L. Significant differences between the groups existed at baseline for HDL, waist circumference, fasting glucose, plasma insulin, and HOMA-IR. Baseline characteristics for both control and exercise groups are summarized in Table 1. As described in Table 1, the control and exercise groups were significantly different at baseline for MetSyn z-score ($p<0.004$). There was a significant association between MetSyn z-score and CRP at baseline ($r=0.193$, $p=0.34$). There was a significant association at baseline for MetSyn z-score and HOMA-IR for both groups ($r=0.561$, $p=0.000$).

Table 2 presents the change in z-score for each MetSyn component following the intervention for the control group and exercise group. There were no significant differences between the control and exercise groups for changes in z-score for triglycerides, fasting glucose, mean arterial pressure, or HDL-cholesterol ($p>0.05$). However, the change in waist circumference z-score was significantly different (control: 0.1 [.01, 0.3] versus exercise: -0.08 [-0.2, 0.05], $p< 0.022$). The adjusted mean change in CRP was similar in the control and exercise groups with no significant difference between groups (control: 0.5 [-0.6, 1.5] versus exercise: 0.4 [-0.7, 1.6], $p=0.922$).

As represented in Figure 1, there were small, non-significant differences observed in z-score changes between the control and exercise group following the intervention (0.2 [CI: -0.2, 0.6] versus -0.3 [CI:-0.7, 0.2], p=0.114).

Figure 2 depicts the change in MetSyn z-score and change in CRP in the in exercise group only. Changes in MetSyn z-score were not significantly associated with change in CRP (r=-0.15, p=0.914).

Figure 3.A. depicts the positive and significant association between MetSyn z-score change and change in HOMA-IR in the exercise group (r=0.286, p=0.036). Figure 3.B. depicts the significant association of change in MetSyn z-score and change in insulin in the exercise group (r=0.305, p=0.025). Figure 3.C. represents a significant association of change in MetSyn z-score and change in glucose in the exercise group (r=0.445, p=0.001).

Chapter V: Discussion

The primary findings of the present study were that 4 months of aerobic exercise training resulted in no change in MetSyn z-score and no association was observed between MetSyn z-score and systemic inflammation as measured by CRP. However, insulin resistance as assessed by HOMA-IR was positively and significantly associated with changes in MetSyn z-score following exercise training. These data suggest that a reduction in cardiovascular disease risk as represented by MetSyn z-score may not be mediated through a pro-inflammatory pathway but through improvements in insulin resistance. The present study is the first to our knowledge to examine the relationship between changes in MetSyn z-score with inflammation indicated by CRP following exercise training.

Categorical MetSyn determines the presence of elevated cardiovascular and type 2 diabetes risk by identifying cut-off points. MetSyn z-score determines the presence and severity of that elevated risk through a continuous risk score. Utilization of MetSyn z-score by healthcare providers ensures that improvements in individual risk factors following exercise training can be identified even if they are not below the recommended values. As discussed in previous literature, exercise training has been shown to promote a reduction of MetSyn z-score based on data from several randomized controlled trials [10, 11, 15]. In contrast to these findings, we observed no change in MetSyn z-score following exercise training. This discrepancy in results may be due to differences in intervention duration and total energy expenditure during the exercise intervention. In the present study, participants exercised for 4 months while the interventions in previous studies lasted between 6-9 months [10, 11, 15]. Moreover, the accumulation of total energy expenditure of 16 KKW after 4 months in the present study may not have been enough to elicit a reduction in MetSyn z-score. Previous studies had varying energy

expenditures throughout their interventions (ranging from 8 to 23 KKW) in which greater energy expenditure accumulations were achieved compared to the present study [10, 11, 15]. It is feasible that the duration and total energy expenditure of the present study's exercise intervention was not extensive enough to yield a significant reduction in MetSyn z-score and multiple components of MetSyn.

No studies to date have examined the relationship between MetSyn z-score and inflammation following exercise training; however, epidemiological research has suggested that a greater number of risk factors for MetSyn is associated with elevated CRP [21-23]. Additionally, previous literature has observed that reductions in CRP are dependent upon weight and fat loss following exercise training ranging from 4-10 months in duration [18, 24, 29]. Although the results of the present study suggest that there were significant changes in waist circumference z-score following the exercise intervention, we did not observe an association between change in MetSyn z-score and CRP. As with change in total MetSyn z-score, the 4 month duration and total energy expenditure may not have been extensive enough to elicit reductions in waist circumference of a necessary magnitude to demonstrate a relationship between MetSyn z-score and CRP. Although it is possible that the reduction in disease risk as represented by MetSyn z-score could be mediated through a pro-inflammatory pathway, this is not supported by the data in the present study.

In the present analysis, the effects of aerobic exercise training on MetSyn z-score were driven by improvements in glucose control and insulin resistance as measured by HOMA-IR. Both glucose and insulin were positively and moderately associated with MetSyn z-score change following the exercise intervention. As glucose is a component of MetSyn and HOMA-IR, it was expected that it would be a probable link between MetSyn z-score and HOMA-IR. However, we

observed a positive association between change in insulin and change in MetSyn z-score. MetSyn was defined upon characteristics of insulin resistance such that risk factors for MetSyn are generally similar to those of insulin resistance [6]. Based upon that definition, it would be expected that insulin resistance measurements are associated with changes in MetSyn z-score. The present results suggest that small, incremental changes in MetSyn z-score may be beneficial to improvement in insulin resistance.

The present study had several strengths. INFLAME was a randomized, controlled trial. The exercise dose during the intervention was controlled with extensive monitoring of energy expenditure during training sessions. The aerobic exercise program was consistent with public health recommendations. Exercise adherence levels were excellent (>90%) in the exercise group and we excluded participants with low adherence levels from the present analysis. Thus, the lack of significant findings for the primary analyses of the present study are not due to inadequate levels of exercise. Additionally, the inclusion/exclusion criteria of the present study were designed with intention to evaluate individuals with chronically elevated CRP while excluding individuals with acute inflammatory conditions. To control for confounding of medication on CRP, entry into the study was restricted by usage of anti-inflammatory medications and medications that may attenuate reductions in CRP. However, the study also had several limitations. The results cannot be generalized to other populations such as adults with normal CRP levels (as the study population included adults with elevated CRP). Additionally, the MetSyn z-score was determined using the study population compared to using population based data. The intervention was limited to only aerobic exercise training and did not investigate the effect of other exercise modalities such as resistance or circuit training. Lastly, the intervention duration for INFLAME was only four months and it is plausible that a more extended exercise

intervention and greater accumulation of total energy expenditure may have resulted in more favorable changes in MetSyn z-score.

Conclusion

The results of the present study suggest that reductions in MetSyn z-score following 4 months of aerobic exercise training are not mediated through an inflammatory pathway, but rather through improvements in insulin resistance for individuals with elevated CRP. MetSyn z-score is a valuable tool that can be used by healthcare and exercise professionals to evaluate the change in cardiovascular disease risk and type 2 diabetes risk to develop exercise interventions to target improvement in insulin resistance. Future studies should investigate if a relationship between MetSyn z-score and systemic inflammation are observed following a longer aerobic exercise training intervention, following different training modalities, or using more sensitive measures of insulin resistance.

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Tables and Figures

Table 1. Baseline characteristics for the Control and Exercise Groups. Results presented as Mean (SD). * represents significance between groups, $p<0.05$.

	Control (n=66)	**Exercise (n=57)**
Age (years)	50.9 (1.4)	50.6 (1.3)
BMI (kg/m^2)	32.0 (3.9)	30.9 (4.4)
Ethnicity African American (%), n	(9.1%), 6.0	(10.5%), 6.0
Sex Female (%), n	(66.7%), 44.0	(78.9%), 45.0
High Density Lipoprotein (mg/dL)	51.7 (11.7)	58.1 (15.4)*
Triglycerides (mg/dL)	117.0 (42.0)	110.0 (46.3)
Mean Arterial Pressure (mmHg)	97.8 (12.0)	95.7 (12.8)
Waist Circumference (cm)	100.2 (12.5)	95.7 (12.6)*
Fasting Glucose (mg/dL)	97.0 (10.2)	93.0 (9.7)*
Plasma Insulin (pmol/L)	84.7 (41.4)	68.2(34.2)*
VO_2 (L/min)	1.61 (0.7)	1.60 (0.7)
C-reactive Protein (mg/L)	4.5 (2.6)	5.1 (4.2)
HOMA-IR	3.4 (1.9)	2.7(1.5)*
MetSyn z-score	0.7 (2.9)	-0.9 (3.0)*

Table 2. Change in z-score for each MetSyn component and CRP. Results presented as Mean (95% Confidence Interval). Each component was adjusted for baseline z-score. * represents significance between groups, $p<0.05$.

MetSyn component, z-score	**Control**	**Exercise**	**p-value**
High Density Lipoprotein	0.02 (-0.1, 0.2)	-0.08 (-0.2, 0.07)	0.325
Triglycerides	-0.06 (-0.2, 0.1)	-0.04 (-0.2, 0.1)	0.868
Mean Arterial Pressure	0.08 (-0.1, 0.3)	-0.1 (-0.3, 0.1)	0.193
Waist Circumference	0.1 (.01, 0.3)	-0.08 (-0.2, 0.05)	0.022*
Fasting Glucose	0.07 (-0.1, 0.3)	-0.1 (-0.4, 0.06)	0.136
C- reactive protein	0.5 (-0.5, 1.5)	0.4 (-0.7, 1.5)	0.922

Figure 1. Change in MetSyn z-score for Control and Exercise group. Z-scores adjusted for baseline z-score and age. The data represents the least-squares means and 95% confidence intervals. (0.2 [CI: -0.2, 0.6] versus -0.3 [CI:-0.7, 0.2], p=0.114).

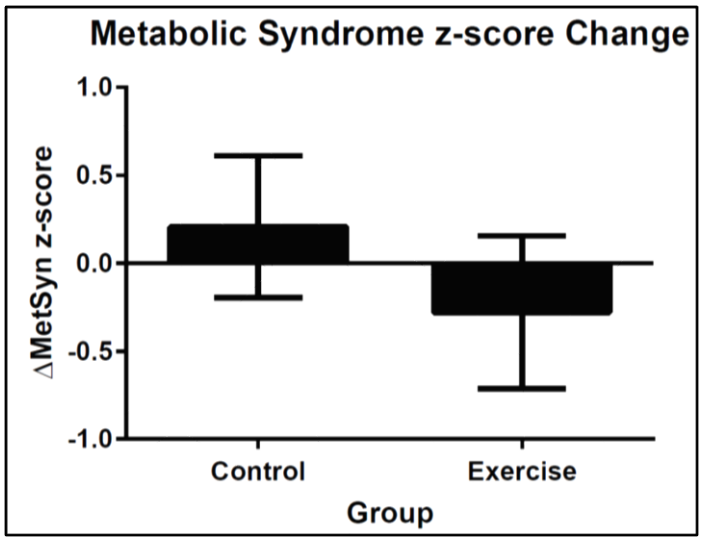


Figure 2. Change in MetSyn z-score and CRP for Exercise Group. (r=-0.15, p=0.914).

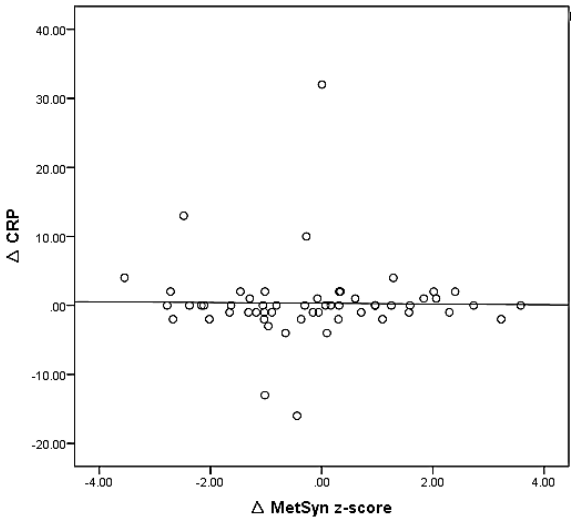
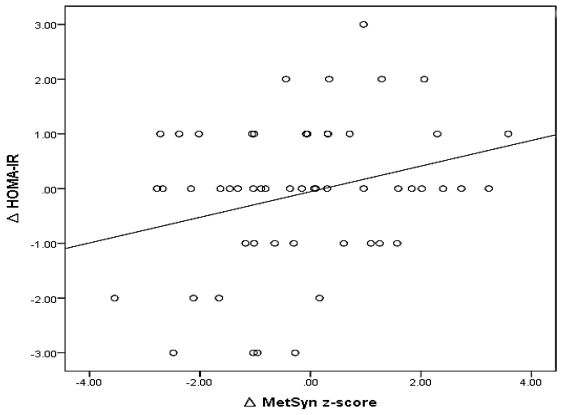


Figure 3.A. Change in MetSyn z-score and HOMA-IR for Exercise Group (r=0.286, p=-.036). **3.B.** Change in MetSyn z-score and Insulin for Exercise Group (r=0.305, p=0.025). **3.C.** Change in MetSyn z-score and Glucose for Exercise Group (r=0.445, p=0.001).

A)



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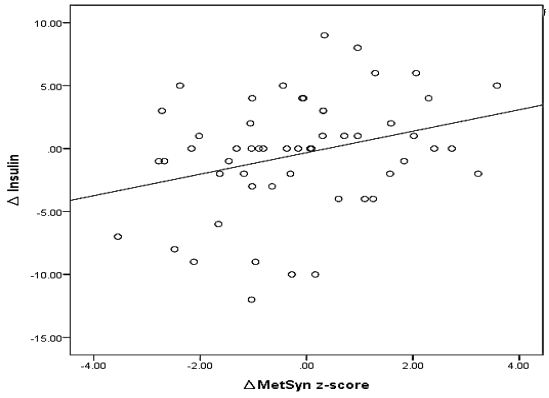

C)