EFFECTS OF EXERCISE AND DIET-INDUCED WEIGHT LOSS IN OVERWEIGHT/OBESE WOMEN ON CHARACTERIZATION OF SERUM/WHITE BLOOD CELLS, microRNAs AND CYTOKINE GENE TRANSCRIPTION

A Dissertation

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ABSTRACT

This study examined the effects of exercise and diet-induced weight loss on markers of inflammation in obese females. Forty-nine obese women (age 48.2±10.5 years, height 63.5±2.7cm; weight 203.3±30.5 kg; BMI 35.6±5.6 kg/m^2; 45.9±4.4 % body fat) completed a 12-week study (exercise group (EX): n=29; control group (C): n=20). Participants followed an energy-restricted diet (1,200 kcal/d for 1-week and 1,500 kcal/d for 11 weeks; 30% CHO, 45% P, and 25% F) while participating in a 30-min circuit resistance-training (3 days/wk) and 30 min walking program on non-workout days. DEXA body composition, fitness, and serum/white blood cells samples were obtained at 0, 4, 8 and 12 wks. The expression of microRNA (21 and 146a) and the expression levels of IL-6, TNF-α, (PTEN, TRAF6)/PI3k/AKT/NF-kB were measured by real-time RT-PCR at 0 and 12 wks. Data were analyzed by MANOVA and presented as changes from baseline after 12 wks for the EX and C groups. Overall MANOVA analysis revealed a significant time effect (p=0.004) and group x time effect (p=0.004) for body composition measures. Participants in the EX group experienced significant changes in body weight (EX -4.0±4.4 kg; C 0.1±3.0 kg, p=0.001), fat mass (EX -3.8±4.0 kg; C -0.03±2.0 kg, p<0.001), and percent body fat (EX -2.7±3.4%; C -0.1±1.7%, p=0.002). Overall MANOVA analysis revealed a significant time effect (p<0.001) and group x time effect (p=0.003) for measures of fitness. Overall significant MANOVA interaction was observed among EX and C groups (Wilks' Lambda p<0.001) on markers of inflammation. Significant interactions were observed among groups in microRNA 21 (EX -1.5±2.34; C 0.13±2.2, p=0.03), mRNA expression levels of PTEN (EX -4.5±3.2; C

-1.6±3.4, p=0.005), IL-6 (EX -2.8±3.6; C 2.8±2.2, p=0.00); and, TNF-α (EX -0.52±2.5; C 2.3±1.9, p=0.00). Changes in microRNA (21 and 146a) were positively and significantly correlated to body weight, total weight, fat mass, and body fat percent, with circulatory levels of IL-6 and TNF-α. Results indicate that 12-wks of participation in an exercise and weight loss program significantly affects microRNA 21 and its target gene PTEN, mRNA TNF-α, and mRNA IL-6 levels suggesting an anti-inflammatory response compared to a control group.

DEDICATION

To my parents Grace and Stephen Simbo, who demonstrated the importance of scholarly pursuits and the benefits of hard work; to my wife, Pamela Ndi and two boys Sunnybright and Mcleonel Simbo. They served as a constant source of support whose encouragement and patience were instrumental in my successful completion of this challenge and to the rest of my siblings and relatives in Cameroon.

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CHAPTER I

INTRODUCTION

Overweight/obesity is the most common chronic disease situation in the world. Overweight is a problem that complicates and exacerbates the growth process in human life all over the world (67). The rising incidence of overweight in the United States and the world population has brought about intense concern in the exercise, nutrition and medical community (108, 169). Approximately 68.5% of the United States adult population is considered either overweight or obese, and this percentage has increased over the last few decades (46, 48). Multiple factors including various genetic, environmental and physiological changes within the human body can lead to overweight and its consequences (108, 169). The adverse effects physical of inactivity and poor dieting include; overweight, obesity, diabetes, metabolic syndrome and even death (169). The complication of obesity includes medical problems such as: metabolic abnormalities, diabetes, cardiovascular diseases, arthritis, pulmonary abnormalities, urinary incontinence, cataracts and certain types of cancer (85). Other major concerns in the obese population are physical inability, poor lifestyle, overeating, increased body composition and high risk of stress (179). Many research studies have shown that exercise and diet are highly effective in promoting weight loss and improving markers of health and enhancing fitness (71, 72, 81, 169). However, the major mechanisms underlying the pathogenesis of overweight remain obscure and need more research (100, 144, 169).

Exercise and diet plays a critical role in weight loss, metabolism, physiological function and weight maintenance in obesity (29). Physical inactivity and excess body weight in the obese population may increase or exacerbate treatment related decrements in the quality of life due to increasing body weight. Prior research findings in our lab showed that overweight women participating in a 14-week resistance-based circuit training and diet program lost 10-14 pounds, a 10-14% increase in muscular strength/endurance, and a 17% and 15% decrease in fasting leptin and insulin, respectively, while insulin sensitivity improved by 19% (71, 72, 80-82). Exercise and diet-induced weight loss have the ability to improve overall body health by reducing total body weight and can have a positive effect on body composition (71, 72, 74, 81). Exercise and diet-induced weight loss may influence gene expression (27, 28). Also the expression of some cytokines and proteins (116, 118, 121), as well as alterations of mRNAs and microRNAs gene transcription (87, 88).

Cytokines mediate communication between cells in the human body and are used extensively in cellular communication between the endocrine, immune, nervous and circulatory system in the development, growth and gene regulation (117, 122). Cytokines are produced from muscles and adipose tissues (113, 120-123). Cytokines regulate local defense reactions in tissues, organs and systems. The expression of cytokine genes in research may help to determine functional qualities of metabolism in physiological systems. Research has shown that contracting muscles release IL-6 into circulation during exercise (116, 120). The plasma levels of IL-6 increased from about 20 to 100 fold in response to intense exercise (119, 121). The IL-6 released during

exercise from contracting muscles may affect many other tissues and organs of the human body (123, 124). For example, Keller and colleagues (68, 69) isolated nuclei from muscles biopsies obtained before and during exercise and demonstrated that transcription for Il-6 increases rapidly after the onset of exercise and decreases post exercise. Weight reduction through exercise training decreases serum concentration of these inflammatory makers (IL-6 and TNF-α) (160). Tsukui and associates (160) conducted a 5 month exercise training program, 30-45 min/day for 4-5 days /week at 40-50% VO_{2max} and concluded that weight loss significantly decreased serum concentration of TNF-α These findings were negatively correlated with HDL cholesterol levels, and reductions in BMI and percent body fat (160). For example, Buford and colleagues (18) evaluated the effects of eccentric exercise on inflammatory gene expression on 29 males for 45 min downhill treadmill running at a 10^{o} for 45 min at 60% of the VO_{2max}. The researchers reported a significant up-regulation ($p < 0.05$) in IL6 expression compared with baseline and significant increases in IL-6 mRNA at 3 h ($p < 0.001$) and 24 h ($p=0.043$) post exercise, and no significant changes were observed for TNF-α and NF-kB (p105/p50) expression (18).

The first microRNA was discovered in 1993 (35, 75). MicroRNAs (microRNAs) are small (21–25 nucleotides in length), non-coding RNAs (54, 58, 75), that play an important regulatory role targeting mRNA transcripts for translational repression. MicroRNAs play a very important role in a variety of biological processes, including development, differentiation, apoptosis, lipid metabolism and cancer (75). They are present in all body fluids and are active moieties that can influence gene expression and

physiology activity (54, 58). MicroRNA are regulated and transcribed like protein coding genes and are emerging as highly specific serum biomarkers with a potential clinical applicability (54, 58). They have been reported to modulate adipocyte differentiation as well as insulin secretion (75). Research done on microRNAs showed that the overexpression of specific microRNAs in human cells may regulate many transcripts predicted to bind the microRNA molecule (86, 143). For example, Esau et al (39) found that reducing microR-143 by transfecting antisense oligonucleotides inhibited adipocyte differentiation in vitro, suggesting that microRNAs may play a role in the regulation of key adipose tissue processes and could therefore represent novel targets for the treatment of overweight and obesity. MicroRNAs have also been shown to regulate metabolic and physiological processes associated with type 2 diabetes including insulin signaling and glucose homeostasis (54, 58, 75, 90). MicroRNA play a very important role in the regulation of glucose and lipid metabolism through adipocyte proliferation and differentiation as well as in cholesterol biosynthesis (58). The emergence of microRNA (92) in research provides an opportunity to understand the molecular factors that control metabolism and physiological regeneration and function. There is evidence that exercise and nutrition may impact microRNA gene regulation (54, 58, 90, 92). However, more research is needed to understand the impact of exercise training and weight loss on microRNAs.

The nuclear factor kappa beta (NF-kB) signaling pathway is another important molecular pathway affected by exercise that is not well-understood. NF-kB is found in almost all animal cell types and is involved in cellular responses to stimuli such as stress

and inflammation (151, 153). The nuclear factor-Kb (NF-kB) p50/p65 is a heterodimer of the rel (NF-kB1, NF-kB2, relA, relB and c-Rel) family that regulates a variety of cellular functions (151, 153, 165). NF-κB controls many genes involved in inflammation e,g. (TNF-α, IL-6) (165) The NF-kB activity includes a large variety of factors linked to inflammatory cytokines and may be very important in understanding the effects of exercise on different genes (165). Protein modulators such as phosphatase and tensin homologue deleted from Chromosome-10 (PTEN), TNF-receptor-associated factor6 (TRAF6), phosphatidylinositol-3-kinase (PI3k), protein kinase B (AKT or PKB), nuclear factor kappa beta (NF-kB) can induce changes that are beneficial to whole organism depending on whether they are modulated in an up-stream or downstream manner. Exercise and diet-induced weight loss could be one of those essential ways of regulating cellular gene functions (87, 88, 90, 165). Physical exercise can induce the activation of NF-kB in blood lymphocytes (165). For example, Vider and colleagues (165) conducted a study on 12 healthy male endurance and mixed aerobic athletes on a treadmill for 60min at 80% VO2 max after a 5 min warm-up. They reported that physical exercise caused activation of NF-kB in 8 of 12 subjects by significantly increasing NF-kB activity by greater than 50% ($p=0.002$) and also increased plasma TNF-α ($p = 0.003$ (165).

Consequently, additional research is needed to understand the effects of exercise and diet-induced weight loss on microRNAs expression through target genes (PTEN, TRAF6, PI3K, AKT and NF-kB) (8, 9, 87, 90, 168). Moreover, how exercise and diet-induced changes in microRNA expression may affect secondary responses such as

protein synthesis, cell growth and inflammation (IL-6 and TNF-α) (126, 129, 130, 133) Thus, the main foci of this study was to examine whether obesity is associated with increases in inflammation; whether exercise and diet-induced weight loss reduces inflammation; and, whether changes in expression levels may explain how exercise and diet-induced weight loss can be beneficial in improving markers of health, fitness and inflammation.

Statement of the Problem

Does exercise and diet-induced weight loss affect the inflammatory responses associated with obesity?

Purpose of the Study

The purpose of the study was to examine how exercise and diet-induced weight loss influences markers of inflammation and regulate genes associated with obesity.

General Study Design

This study was conducted as a randomized, repeated measure, prospective clinical trial in an obese female population ages 18-65 years. Participants meeting entrance criteria were randomly assigned to a 12-week exercise and diet-induced weight loss group designed to improve fitness and promote fat loss, regulate genes related to inflammation and reduce inflammatory markers or a non-diet, non-exercise control group. The independent variable was exercise and diet intervention. The dependent variables were: body composition (weight, fat mass, fat free mass, percent body fat); markers of fitness; inflammatory cytokines IL-6 and TNF-α; and microRNAs (21 and

146a) and their target genes and the NF-kB signaling pathway (PTEN, TRAF6)/AKT/PI3K/NF-kB.

Hypotheses

The following hypotheses have been based on the existing literature:

H_1-The exercise and diet intervention will promote significant differences in body composition compared to controls.

H_2-The exercise and diet intervention will promote significant differences in markers of health and fitness compared to controls.

H_3-The exercise and diet intervention will promote significant differences in mRNA IL-6 expression compared to the controls

H_4-The exercise and diet intervention will promote significant differences in mRNA TNF-a expression compared to the control

Null Hypotheses

Based on Previous research, the following relationships have not demonstrated. Therefore the following null hypotheses were stated.

Ho_1-The exercise and diet intervention will not result in significant differences in mRNA TNF-a expression compared to the controls.

Ho_2-There will be no significant differences between groups in changes in microRNAs (21, 146a) expression.

Ho_3-There will be no significant differences between groups in changes in mRNA PTEN expression.

Ho_4-There will be no significant differences between groups in changes in mRNA TRAF6 expression.

Ho_5-There will be no significant differences between groups in changes in mRNA PI3k expression.

Ho_6-There will be no significant differences between groups in changes in mRNA AKT expression.

Ho_7-There will be no significant differences between groups in changes in mRNA NF-kB expression.

Delimitations

The research study followed the guidelines listed below:

1. There were approximately 50 sedentary overweight female participants (BMI > 27) ages 18-65 that participated in the study.
2. Participants were recruited with flyers posted on campus, physician offices/clinics, and retirement facilities. Advertisements were run in the local newspapers and Radio.
3. Familiarizations and testing sessions were conducted in the Exercise and Sport Nutrition Laboratory (ESNL) at Texas A&M University.
4. Participants were randomly assigned to one of two treatment groups, (exercise and diet-induced weight loss group and a control group).
5. Participants followed the exercise prescription guidelines in a supervised exercise program three times per week throughout the investigation.

Limitations

1. Participants were sedentary and obese females (BMI > 27) between the ages of 18-65 years, met medical clearance and qualifying criteria.
2. Participants in the exercise and diet-induced weight loss group were required to adhere to an exercise program three times per week throughout the investigation.

Assumptions

1. Participants followed the exercise and diet-induced weight loss program as specified by the assigned high protein diet regimen only.
2. Participants adhered to verbal and written instructions, on more than one occasion to refrain from exercise for 48 hours prior to baseline testing.
3. Participants fasted for 12 hours prior to lab collection.
4. Participants followed the intensity guidelines for all work outs per instructions.

CHAPTER II

LITERATURE REVIEW

Background

There is evidence that exercise can both cause and attenuate inflammation. Acute, unaccustomed exercise can cause muscle and connective tissue damage, especially if done at high intensities and for prolonged durations. This typically manifests as delayed onset muscle soreness which is preceded by microstructural skeletal muscle damage (e.g. streaming z disks), inflammatory cell infiltration, and elevation of muscle-specific creatine kinase isoforms (50, 51). In some cases, inflammatory cytokines (IL-6 and TNF-α) and target genes can be detected in peripheral blood of people after high intensity, unaccustomed exercise, especially if lengthening contractions are performed (45). This damaging response is attenuated if exercise is done repeatedly as the tissue adapts to the new overload stress. Indeed, blocking the inflammatory response using broad spectrum anti-inflammatory drugs that can reduce muscle adaptation and, ultimately, increase muscle performance induced by exercise may be beneficial (102).

On the other hand, some studies have demonstrated that regularly performed cardiovascular exercise training may reduce markers of systemic inflammation (172, 174, 175). It is this literature that will be the focus of this review because of the strong association between elevated systemic inflammatory markers and chronic diseases of the aged and obese (172). Evidence regarding non-pharmaceutical means to reduce chronic inflammation would be important due to the potentially harmful side effects of

medications. Weight loss induces marked alterations in whole body metabolism and physiological function and this may result in cellular alteration that can be very beneficial during an exercise and diet-induced weight loss program and reducing diseases situation such as cardiovascular diseases, insulin resistance, diabetes, overweight and obesity.

Prevalence of Obesity in Women

Approximately 68.5% of the United States adult population is considered either overweight or obese (47), and this percentage has increased over the last few decades. The prevalence of overweight in the United States has been steadily on the rise for several decades; growing from 44.8% of our population in the early 1960s to 68.5% in 2010 (46, 48, 67, 127). Women have experienced a particularly sharp increase in the prevalence of obesity going from 40.2% to 63.9% over that same time frame (127). The World Health Organization (WHO) estimates that globally 1.6 billion adults are overweight and at least 400 million adults are obese (1). The frequency of high BMI also varies between racial groups and somewhat between different income brackets; white females have the lowest percentage (60.2%) whereas Black or African American women have a much higher occurrence (80.3%). All socioeconomic levels showed reasonably high frequencies of overweight/obesity. BMI readings however the highest percentage occurs with persons living above the poverty level, but not making more than 200% of the poverty level income (70.9%) and the lowest percentage was observed in persons making more than 400% the poverty level income (66.7%) (67).

Multiple factors including various genetic, environmental and physiological changes within the human body can lead to becoming obese. Obesity has been generally believed to be a direct result of the imbalance between energy intake and energy expenditure although we now know obesity is much more complicated than these factors. Obesity increases excess fat storage in adipose tissue, liver and muscle and can result in physical inactivity and increase mortality. The complications of obesity can include medical problems such as: metabolic abnormalities, arthritis, pulmonary abnormalities, urinary incontinence, cataracts and certain types of cancer (85). The accumulation of adipose tissue associated with obesity is clinically associated with diseases such diabetes, hypertension, insulin resistance and cardiovascular diseases (43). Obesity has been associated with inflammation and obesity-induced inflammation has been reported to adversely affect the function of organs and tissues of the human body such as the pancreas, liver, skeletal muscle, heart, and joints. Obesity has also been reported to increase the activity of NF-kB in the liver, skeletal muscle and the transcription of its target genes primarily responsible for the activation of NF-kB and reduction of obesity-induced inflammatory cytokines (IL-6, TNF-a) (19, 107, 169, 175).

Most obese individuals have large muscle mass. However, traditional weight loss programs (diet only or diet and aerobic exercise) typically promote loss in muscle mass leading to a reduction in resting energy expenditure (REE) which is associated with weight regain. A decrease in muscle mass in obesity not only impacts metabolic disorders (i.e., insulin resistance) but significantly affects physiological and metabolic function. Adherence to a higher protein diet and/or incorporation of resistance-exercise

into a training program can increase energy expenditure, maintain or increase muscle mass during weight loss, and minimize reductions in REE (71, 72, 79). The above beneficial effects on the human body can help to maintain body weight and improve metabolic functions, regulate gene expression, reduce obesity-induced inflammation and improve on markers of health and fitness. However, the major mechanisms underlying the pathogenesis of obesity remain obscure and more research is needed to understand obesity related induced inflammation and its consequences in humans.

Defining Inflammation and Markers

Inflammation can be defined as the body's natural means of stimulating healing or repair (59). Inflammation is a reaction of microcirculation that is characterized by the migration of serum proteins and leukocytes from blood to extravascular tissues (32). This migration is regulated by the release of cytokines such as interleukins (IL-6, IL-1, IL-10, IL-8), tumor necrosis factor-alpha (TNF-α), and monocyte chemo-attractant protein-1 (MCP-1). The release of these metabolites is sometimes accompanied by increases in temperature, pain, redness and loss of tissue function (59). The human body uses acute inflammation as a means to repair, heal and regenerate itself following events such as pain, redness, swelling and heat and tissue damage. Chronic inflammation and oxidative stress may be caused by environmental factors, lifestyle and diet depending on where the individual lives.

Relationship of Obesity to Inflammation

Obesity is associated with inflammation and serum concentrations of various cytokines are increased in obese individuals (34). Obesity is also associated with

alteration of the immune system and function, the effect of obesity on the immune system may vary (34). Obesity can be defined as an overabundance of free fatty acids secreted from a large reservoir of adipose tissue and can also be characterized as a state of chronic low-grade inflammation (34, 60, 109, 110). The metabolism and physiological effects of obesity can affect the entire body system. The deposition of abdominal fat can alter metabolic pathways and lead to excess energy (fat) redirected towards peripheral organs (115). Obesity is a disease that predisposes humans to increasing risk of many other diseases including, diabetes, fatty liver, cancer and immune disorders (164). Obesity has been characterized as a state of low grade or chronic systemic inflammation due to the abnormal circulating levels of inflammatory markers released from increased size of adipocytes caused by excess lipid storage in the obese (41, 174, 176, 179). Obesity is associated to chronic inflammation characterized by increased production of pro-inflammatory cytokine (IL-6 and TNF-α) and C-reactive protein (CRP) (156, 158, 159). IL-6 and TNF-α level from adipose tissue and in circulation are highly elevated in obese and correlates to fasting glucose, insulin, triglycerides, free fatty acids levels (62, 87-90). These markers of inflammation have been associated with increases in the risk of many diseases associated with obesity such, heart disease, diabetes, and mortality among older adults (37, 45, 83, 88, 89). IL-6 and TNF-a expression levels are very high in obese adipose tissues about 10-fold than in adipose tissues in lean individuals (38, 65-69).

The expression of IL-6 is higher in visceral than in peripheral adipocytes and the plasma concentrations of IL-6 increases with obesity as much as 30% of total body IL-6

production humans (56, 76, 125, 136, 180). This tremendous increase in IL-6 increases lipolysis and fat oxidation in humans (56, 76, 125, 136, 180). A few studies have also shown that IL-6 and TNF-α (159, 160) are expressed in adipose tissue with in vitro studies proving great evidence of their release from visceral fat cells than subcutaneous fat cells (159, 160). For example, Yudkin et al (179) reported that about 20% of IL-6 levels could be used to explain anthropometric measures of obesity and its distribution. They and another group concluded that it was unclear whether adipose tissue is the major source of IL-6 production (179, 180). Trujillo et al (159) conducted a study to test the effects IL-6 on leptin and lipolysis at 5 h, 24 h and 48 h by obtaining adipose tissue samples from some obese subjects with BMI > 57 kg/m^2 and reported that IL-6 increase lipolysis by about 79% and leptin by about 90% at 48 h and no significant effects at 5h and 24 h. In obesity blood cell expression levels of pro-inflammatory cytokines such as Il-6 and TNF-α are elevated and may contribute to the increased levels of inflammation and previous studies have shown that these cytokines can be reduces via exercise and diet (174). You et al (175) conducted a 6 month study to show that exercise 20 min/d 3 d/wk at 50- 55% heart rate reserve for one week and changing to 45-60 min at 65-70% heart rate reserve for the rest of the time on a 250-350 kcal/d hypocaloric diet had significant changes on IL-6 and TNF-a overtime with $p<0.05$ and $p<0.001$ respectively.

To understanding the link between obesity and inflammation and why obesity is a state of low grade to chronic inflammation, is important to conduct studies that are aimed at identifying those molecular mechanisms that are involved in regulation in the obese (156). In obesity treatments such as dieting, exercising and losing weight can

trigger released of these molecules into circulation to various target organs and can cross-regulate each other to reduce higher levels of pro-inflammatory cytokines (156). The inflammatory cytokine, IL-6 levels from adipose tissue in circulation are highly elevated in obese and correlates to fasting triglycerides, free fatty acids levels (159). IL-6 expression is very high in obese adipose tissues to about 10-fold that in adipose tissues in lean individuals (56, 76, 125, 136, 180). Other studies confirm that elevated levels of IL-6 and TNF-α are a strong predictor for the development of myocardial infarction and type 2 diabetes and has also been shown to be directly linked to overweight, obesity and insulin resistance in the liver (85-88, 98). Adipose tissue plays a role in regulating body weight and secretes products such as leptin, adiponectin, interluekin-6, TNF-α, angiotensinogen, sex steroids, glucocorticoids and many other genes. Many of these products have been present in the literature since the mid-1990s but were not always connected to overweight or obesity (120, 121).

Effects of Diet-Induced Weight Loss on Inflammatory Markers

Prior research has shown that macronutrients and obesity can activate inflammation (100, 144). For example, increase in energy intake and energy imbalance have both been shown to induce and increase inflammatory markers levels potentially through increase in the activities of NF-kB and oxidative stress and other accompanied disease situation (100). Shoelson et al (144) demonstrated that intravenous lipid infusion of triglycerides plus heparin in normal subjects raised the levels of free fatty acids (FFA) compared to those seen in obese subjects, which consequently led to inflammation as determined by inflammatory markers response (144). Excess adipose tissue and obesity

is associated with increases in inflammatory cytokines Il-6 and TNF-α (60, 73); whereas, weight loss decreases the levels of these inflammatory makers (60, 73, 102, 111). For example a study (2) conducted by a different group reported that macronutrient intake in obese subjects by caloric restriction to about 1000 kcal/day for a period of 4 wks including a 48 h fasting period led to a reduced level of oxidative stress and inflammatory mediators (2). Ferrante Jr (43) reported that in obese individuals, weight loss following bariatric surgery reduces adipose tissue macrophage content and this reduction may lead to a reduction in inflammatory gene expression and other associated molecules. This was also confirmed by other studies (43, 178).

Dahlman and associates (31) conducted a study on 40 obese individuals assigned to a moderate-fat, moderate carbohydrate or low-fat, high carbohydrate hypoenergetic (-600 kcal/d) diet for 10 wks. They reported that both diets resulted in about 7.5% of baseline weight loss and a total of 52 genes were significantly up-regulated and 44 genes were down-regulated (31). Another study of same kind was investigated on 25 obese subjects following a 10 wk hypocaloric (low-fat and high-fat diets) programs with either 20-25 or 40-45% of total energy derived from fat, they again reported that both groups lost 7kg and 10 genes showed a significantly different response to the diets (167). They all concluded that macronutrients have a secondary role in changes in gene expression after energy-restricted diets and weight loss same as a few other studies listed on the table below (31, 167). Mello and coworkers (34) also showed that weight reduction for a period of 33 wk on the American Heart Association diet with a mean deficit caloric intake of about 500 kj/d on obese women significantly reduced TNF-α by 37.6% and

increased IL-6 by 21.4% but not significant. Table 1 provides a summary of some other studies that have been done to look at the effect of weight loss including weight loss through surgery on inflammatory makers following calorie and diet restriction on obese men, women and children.

Effects of Acute Exercise Training on Inflammatory Markers

Exercise is a safe and inexpensive anti-inflammatory medicine that could be used by obese individuals to reduce obesity and its consequences (68). Exercise training depending on its duration and intensity engages whole body movements and may be the best means of attaining the anti-inflammatory effects of exercise. As already mentioned above, IL-6 is one of the most important inflammatory cytokines released during exercise and others include TNF-α, IL-1, Il-8, IL-10 (23, 69, 116, 118). Exercise and training adaptations affect the human body by producing beneficial effects that will improve and regulate whole body metabolic and physiological function (119, 120). Rising exercise intensity or full body muscle contraction and glycogen depletion during exercise are the major exercise elements involved in the release of IL-6 from contracting muscle and adipose tissue from about 20 to 100 fold compared to resting levels of IL-6 (17, 42, 96, 116, 120, 138, 145, 146) (68). For example following exercise, the basal IL-6 level may increase up to 100 fold during a marathon race (122). Margeli et al (94) reported that in a race that consisted of a continuous, prolonged and brisk exercise; a 246 km spartathlon race in less than 36 h IL-6 levels increased up to about 8000-fold. Other studies conducted by Keller et al and Steensberg et al (146) in response to exercise, they showed that an increase in IL-6 mRNA content from contracting muscles could be

detected after 30 min of exercise and up to about 100-fold increase of IL-6 mRNA content at the end of the exercise bout (146).

Steensberg et al (145) reported on a study they conducted that a correlation exist between the exercise intensity and the high increase in plasma IL-6 and the appearance of IL-6 in blood. They showed that when adrenaline was infused in some volunteers during 2.5 h of running, plasma IL-6 increased only by 4-fold with rhIL-6 infusion and increased to about 30-fold without rhIL-6 infusion during exercise. A few other studies have shown that IL-6 level depends on the duration, intensity and type of exercise and at the onset of exercise compared to basal levels (116, 118-124). For instance, a study (138) conducted on overweight/obese children and adults who performed a 30 min intermittent exercise test consisting of ten, 2 min bouts of constant work on a cycle ergometer with 1 min rest interval at a VO2max of about 80%. They reported that, throughout the exercise in the obese group, IL-6 concentration were quantitatively greater (range 20.5-24.3 pg/ml) than in the control group (range 10.9-11.7 pg/ml). That TNF-α was significantly greater in the obese group (range 3.2-3.6 pg/ml than in the control group (range 2.1-2.3 pg/ml) (138). Table 2 shows some selected studies on the effects of physical activity on inflammatory biomarkers.

Table 1: Some published data on the effects of weight loss on inflammatory markers

Author	Participants /BMI (kg/m^2)	Intervention	Duration	weight loss	Effects on inflammation
Xydakis et al, 2004 (173)	40 obese adults BMI 38.9 ± 1.0	600-800 kcal/day Very-low-calorie diet	4---6 weeks	7.0% of weight	CRP ↓14%; no change in TNF-α
Gallistl et al, 2001 (52)	49 obese children BMI 26.7 ± 1.4	908-1194 kcal/day energy-restricted diet	3 weeks	5.2% of BMI 3.1% of fat mass	IL-6 ↓49%
Bastard et al, 2000 (11)	14 obese women BMI 39.5 ± 1.1	941 kcal/day Very-low-calorie diet	3 weeks	5.3% of BMI 8.5% of fat mass	IL-6 ↓17% no change in CRP, TNF-α
Long-term behavioral changes:					
Marfella et al, 2004 (93)	67 obese BMI 36.5 ± 1.8	1300 kcal/day energy-restricted diet, increased exercise	12 months	13.4% of weight	CRP ↓44%; IL-6 ↓ 62% TNF-α ↓ 31%; IL-18 ↓ 30%
Monzillo et al, 2003 (101)	24 obese BMI 36.7 ± 0.9	500 kcal/day deficit energy-restricted diet and moderate-intensity exercise	26 weeks	7.0% of weight	IL-6 ↓ 41% no change in TNF-α
Bruun et al, 2003 (16)	19 obese men, BMI 38.7 ± 0.7	1000-1480 kcal/day energy-restricted diet	16 weeks	14.7% of weight	IL-6 ↓ 24%; TNF-α ↓ 29% IL-8 ↓ 30%
Ziccardi et al, 2002 (181)	56 obese women BMI 37.2 ± 2.2	1300 kcal/day energy-restricted diet, increased exercise	12 months	12.6% of BMI	Il-6 ↓ 47%; TNF-α ↓ 31%
Dandona et al, 1998 (33)	38 obese women BMI 35.7 ± 0.9	925-1150 kcal/day energy-restricted diet and increased aerobic exercise	1-2 years	12.3% of weight	TNF---α ↓ 24%
Weightloss surgery:					
Vendrell et al, 2004 (163)	34 morbidly obese BMI 49.6 ± 1.0	Gastric bypass	6 months	30% of weight, 62% of fat mass	IL-6 ↓ 59%; sTNFR1 ↓ 15%; no change in sTNFR2
Laimer et al, 2002 (83)	20 obese women BMI 41.6 ± 1.2	Adjustable gastric banding	1 years	27% of weight 45% of fat mass	CRP ↓ 70%; no change in IL-6
Randomized, controlled trials:					
Esposito et al, 2003 (40)	120 obese BMI 35.0 ± 2.3 (v. BMI 34.7 ± 2.4)	1300-1500 kcal/day energyrestricted Mediterranean-style AHA step I diet (v. normal diet)	2 years	14.7% of weight (v. 3.2% of weight)	CRP ↓ 34%; IL-6 ↓ 33% (v. no changes in CRP, IL-6)
Nicklas et al, 2004 (102)	316 older adults BMI 34.5 ±5.4 b± 5.0)	Behavioral counseling to achieve and keep a 5% weight loss (v. normal diet)	18 months	5.1% of weight (v. 1.8% of weight)	CRP ↓ 3%; IL-6 ↓ 11%; no change in TNF-α

↓ = significant reduction; CRP = C-reactive protein; IL=interleukin; TNFα = tumor necrosis factor alpha; kcal = kilocalorie, BMI = basal metabolic; AHA= American Heart Association

Effects of Chronic Exercise on Inflammatory Markers

Inflammation is a factor of many diseases such as insulin resistance, obesity, diabetes and cardiovascular diseases (116). Exercises (aerobic and resistance) has been demonstrated to significantly reduce and improve inflammatory cytokine levels (21, 23, 24, 45, 50, 133). Exercises of this nature remain the most safe, inexpensive, under-utilized modality of treatment, readily accessible despite its well-known beneficial effects to all humans especially to the obese population.

Table 2: Studies demonstrating the association between increased physical activity or physical fitness and inflammatory biomarkers in the elderly

Reference	N	Age	Activity or Fitness Measure	Inflammatory Markers
Taaffe et all, 2000 (149)	880	74.3 ±2.7	Time in moderate or strenuous activity (hr/yr)	↓CRP (43.8%), ↓IL-6 (39.1%)
Reuben et al., 2003 (135)	870	74.3	Modified Yale Physical Activity Survey (kcal/min)	↓ CRP (47.4%), ↓IL-6 (44.2%)
Colbert et al., 2004 (26)	2964	70-79	Time and duration spent activity over previous year (kcal/kg/wk)	↓ CRP (27.8%), ↓ IL-6 (21.1%), ↓ TNFα (6%)
Elosua et al., 2005 (38)	1004	65 or older	Time spent in light and moderate activity	↓CRP (70%), ↓ IL-6 (20%) Men ↓TNFα Women = TNFα
Yu et al., 2009 (177)	3289	50-70	International Physical Activity Questionnaire (MET-hr/wk)	↓CRP (19.6%), = TNFαRII, = IL-6
Valentine et al., 2009 (162)	132	70 ± 5.4	VO_2 Peak (ml/kg/min)	↓ CRP men (6.6%) = CRP women

↓ = significant reduction; = means no change; CRP = C-reactive protein; IL=interleukin; TNFα = tumor necrosis factor alpha; kcal = kilocalorie, MET = metabolic equivalent; wk = week; ml = milliliter, kg = kilogram

There is limited research on the effects of exercise on inflammatory marker such as IL-6 and TNF-α. However the mechanisms by which exercise training influences changes in inflammatory marker are not fully known and needs to be studied. The initial cytokines in the cytokine cascade are IL-6, IL-1, and TNF-α (124). These cytokines may increase up to 100 fold during intense exercise (116, 119, 124). A study (22) was conducted on subjects who completed a 30-min cycle ergometer at 60% of peak oxygen

up-take, 3 day/wk for 8 wk and reported that IL-6 concentration following the 30 bouts of cycle ergometer increased significantly 30 min post-exercise by 13%, stayed elevated 2 h post-exercise by 9% and returned to baseline 3 h post exercise and TNF-α concentration decreased significant by -16% 2 h and -39.5% 3 h post-exercise (22). Another study done by Keller and colleagues (68, 69) isolated nuclei from muscle biopsies obtained from subjects who completed a 60-min cycling exercise at about 70% of their maximum work load on day 1 and two-legged knee extensor at about 60% of their maximum work load for 180 min of cycling exercise on day 2 demonstrated that the transcription rate for IL-6 rapidly increases 40 fold after 90-min and about 60 fold after 180 min of exercise and concluded that exercise activates the transcription of the IL-6 gene in skeletal muscles (68). Niebauer et al (104) evaluated the effects of 8 weeks of exercise training consisting of a 5 d/wk submaximal cycle ergometer (30 min/d) and 9 min/d of calisthenics exercises on cytockines. They reported that exercise program had no effect on IL-6 but VO_2max was increased by about 3ml/kg/min. Timmerman et al (157) conducted a 12-week training study involving endurance exercise (3 d/wk for 20 min at 70-80% heart rate reserve) and resistance exercise (8 exercises, two sets at 70-80% of one repetition maximum). They reported that there was a significant time effect driven by a 41% decrease TNF-a production and a 64% reduction in inflammatory monocyte in the physical inactive group.

Obese individuals usually have high levels of some these cytokines which has been associated with chronic low-grade inflammation (36, 91, 95). Exercising regularly helps reduced body weight and pro-inflammatory cytokines (113). For example,

Ostrowski et al (113) investigated the time course of prolonged strenuous exercise on inflammation in marathon runners with a median running time of 3 h and 26 min and a median VO_2max of 61.2ml/min/kg. They reported that the mean plasma concentration of IL-6 decreased significantly to about 20-fold 4 h post exercise. They concluded that strenuous exercise (marathon race) induces anti-inflammatory cytokine action during exercise. Other studies have also shown same anti-inflammatory effects during exercise (113, 116). For example Dekker et al (36) examined obese men who performed a 60 min aerobic exercise (jogging, walking on treadmill at about 60% VO_2max), 5 times/wk for 12 wk. They reported a 17% reduction of IL-6 in the obese as a result of exercise training. During exercise IL-6 is produced by the muscles and adipose tissue and can stimulate the production of other anti-inflammatory cytokines such as IL-10, IL-1ra and turns to inhibit the production of TNF-α a pro-inflammatory cytokine (124, 137, 146). Another study demonstrated that exercise induces the transcription of metabolic genes in skeletal muscles (120). The exercise-induced production of IL-6 that may be involved in mediating the reduction of TNF-α production and other plasma cytokines, suggesting that exercise induces a strong anti-inflammatory effect in human subjects (42). Another study conducted by Keller and associates (69) evaluate the transcription levels of IL-6 during exercise. They measured IL-6 level on subjects who performed an incremental cycle ergometer maximal exercise test. The researchers found that IL-6 levels increased 6.49-fold throughout the exercise bout and decreased during recovery (69).

Other studies have shown that the IL-6 gene transcription located in the muscles and adipose tissue is released during exercise in high amounts into blood (42, 68, 69,

116, 121). The IL-6 level during exercise increases dramatically (up to 100 fold) and the increased levels of IL-6 has been consistently shown in many research studies (17, 21, 23, 24, 45, 66, 68, 89, 105, 106, 116, 118, 120-124, 145, 146). Endurance training can be very helpful in reducing inflammation. Scheett et al, (142) evaluated the effects of 90-min of aerobic-type training consisting of running, jumping, aerobic dance, 50% team sports and 50% running games interspersed through 3 h each day, 5 d/wk on inflammatory markers. The researchers reported that training resulted in a significant increase in TNF-α33.9% compared to control and an increase in IL-6 15.3% in training but not significant. Adamapoulos et al (5) also showed that exercise training (cycling 5 d/wk for 30 min/d at 60% to 80% maximal heart rate) resulted in a significant reduction in TNF-a by -38.7% (2.9 pg/ml) and IL-6 by -28.9% (2.4 pg/ml). Rall et al, (134) conducted another study to examine the effects of resistance training (strength training) (3 sets of 8 repetitions on 5 different machines (abdominal and back extension), upper body (chest press), and lower body (leg press and leg extension) strength with a 2 min rest between each set for about 45 min 2 times a wk). Each session was preceded by a warm-up period consisting of approximately 15 min of water exercises (calisthenics and 10 min of water walking). They reported that resistance training had no significantly difference on IL-6 and production of secreted TNF-α at baseline was 50% greater among exercisers.

Fischer et al, (45) evaluated the effect of glycogen mediated exercise-induced increase in IL-6 mRNA on subjects who performed a power max test starting with a 60 knee extensions/min at 30 W followed by an incremental increase of workload by 10 W

every 2 min until volitional exhaustion. A second test evaluated at a workload of about 90% of power max for 10 wk, 5 times/wk during a 1 h training session and reported that mRNA IL-6 increased from 20-210-fold following their endurance training compared to no change in control. Their data was consistent with an earlier theory developed that the transcription of the IL-6 gene is regulated by glycogen depletion since carbohydrate ingestion during exercise does not attenuate the rate of muscle glycogenolysis (69, 130, 131). Ho et al, (64) conducted a study to look at the effects of chronic exercise training on markers of inflammation in overweight and obese individuals. Their exercise intervention involved a 30 min aerobic exercise on a treadmill at 60% heart rate reserve. A 30 min resistance exercise, 4 sets of 8-12 repetitions at 10-RM level of leg press, leg curl, bench press and rear deltoid row with each set completed in about 30 s with 1 min rest in between each set or a combination of 15 resistance (2 sets of the above) and 15 min aerobic exercises for a duration of12 weeks. From their evaluation, they reported that exercise significantly decreased TNF-α by 20.8% in the aerobic group and 32.6% in the resistance group and by -22.6% in the combination group and no significant changes in IL-6 levels after 12 weeks in all groups. Physical exercise training has been shown to lower levels of inflammatory makers (63, 64, 172, 174, 175). Aerobic exercise training has been demonstrated to significantly reduce and improve inflammatory cytokine levels (10, 36, 64, 102, 172, 174, 175). A study performed by Pedersen and associates (115) done on young healthy subjects who performed a 3 h dynamic two-legged knee extensor exercise at about 50% individual maximum power output showed a moderate increase in heart rate from about 113-122 beat/min and a 16-fold increase in mRNA IL-6 and a 20-

fold increase in plasma IL-6 (115). Same model was applied to older healthy untrained adults and they showed an even higher amount of IL-6 at same relative intensity (115). A training exercise program that combines two or more different forms of exercises in one movement to stimulate large amounts of muscles, increase VO_2max, change body composition and induce changes in inflammatory markers can be very beneficial for the obese population. Table 3 shows some selected studies on the effects of exercise training on inflammatory biomarkers. Table 3 shows evidence from some intervention studies showing the association between physical activity and inflammatory markers.

Table 3: Selected studies showing the effect of exercise training on inflammatory biomarkers in the elderly

Reference	N	Age	Duration	Exercise Intervention	Inflammatory Marker
Nicklas et al., 2004 (102)	67 70	69±6 69±6	18month	1 hour, walking 50-75% HRR and lower body resistance training with weighted cuff, 3x/wk	↓CRP 3% ↓IL-6 11%, ↓TNFα 2%
Hammett et al., 2004 (61)	30 31	66±4 67±5	6month	45min, aerobic training estimated 80% of VO2 Max, 4x/wk (3x supervised, 1x unsupervised)	= CRP
Kohut et al., 2006 (78)	48 49	69.8±5.5 70.3±4.6	10month	45min, aerobic exercise class at up to 65-80% VO2 peak, 3x/wk	↓ CRP (35.3%), ↓ IL-6 (33.3%)
Nicklas et al., 2008 (103)	183 186	76.4±4.1 77±4.4	12month	150min/wk, walking at RPE 12-13, resistance training RPE 15-16	↓ CRP (32%), ↓IL-6 (16%)
Vieira et al., 2009 (166)	66 61	70±.8 70±.6	10month	45-60 min, 60-70% VO2 Max, 3x/wk	↓ CRP
Campbell et al., 2009 (20)	53 62	60.5±7 60.9±6.8	12month	45min, 60-75% HR max 5x/wk (3x supervised, 2x unsupervised)	↓ CRP (10%), = IL-6
Beavers et al., 2010 (12)	182 186	76.4±4.1 77±4.4	12month	150 min/wk walking goal at RPE 12-13, resistance training at RPE 15-16	=TNFα = IL-6sR

↓ = significant reduction; = means no change; CRP = C-reactive protein; IL=interleukin; TNFα = tumor necrosis factor alpha; kcal = kilocalorie, MET = metabolic equivalent; RPE = rate of perceive exertion; wk = week; kg = kilogram

Effects of exercise training and diet-induced weight loss on these markers

Despite the complexity of inflammatory cytokine gene transcription, the key interventions in treating obese patients remains to moderate caloric restriction, moderate

exercise, 5%-10% weight loss, prevention and treatment of co-morbid conditions associated with obesity (114, 139, 140). Numerous studies have shown the high increase of IL-6 during at the onset exercise and how the IL-6 gene expression from contracting muscles can affect metabolic and physiological function during training (17, 18, 116, 118, 171). Exercise and diet increases the transcription of inflammatory markers (102). A study was conducted by Nicklas and colleagues (53) on 316 overweight or obese subjects for 18 months. These subjects were randomized into four 18-mo treatments (a healthy control, diet-induced weight loss, exercise and diet plus exercise groups). These subjects participated in a 3 d/wk exercise program consisting of 15 min aerobic exercise and a 15 resistance exercise portion consisting of 2 sets of 12 repetitions of the following exercises. The exercises they performed were leg curl, leg extensions, heel raise, and step ups at a 50-75% heart rate reserve. Cuff weights and weights vest were used to provide resistance. They reported that the diet-induced weight loss group observed significantly greater reduction in IL-6 by 11% ($p=0.009$) and TNF-α 2% ($p=0.007$) than all the other groups with no significant changes (53). A study conducted by You et al (175) sought to determine whether a hypocaloric diet with and without exercise training is effective in reducing IL-6 and TNF-a levels and whether these markers are related to changes in regional lipolysis in obesity for 6-mo on 50 women. They reported that diet plus exercise and not diet alone significantly decreased plasma levels of IL-6 27.1% and TNF-a decreased by -6.4% and that changes in IL-6 ($r = 0.37$) were correlated to changes in body weight. There were no significant relationships between changes in inflammatory markers and changes in fat, fat free mass percent body fat and visceral fat

area. They then concluded that a modification of chronic inflammation is associated with changes in local adipose tissue metabolism in response to diet and exercise (175). Tsukui and co-workers (41) conducted a 5-month longitudinal intervention training program (30-45 min/d/wk, 4-5 d/wk at 40-50% of their VO_2max) on 41 women. They demonstrated that regular exercise significantly decreased body weight, decreased BMI, percent body fat, serum TNF-α from 9.0 to 1.5 pg/ml (41). Table 4 summarizes some studies that had been done to show that effects of weight loss and exercise training on inflammatory marker.

Table 4: Shows a summary of studies on the effects of weight loss and exercise training on markers of inflammation.

Study, year	No. of participants	Duration	Effects on body composition and aerobic fitness/physical function	Effects on Inflammation
Kohut et al, 2006 (78)	97 older adults	10 months	Aerobic exercise versus control = body weight ↑maximal METs	↓ CRP (-35.3%), ↓ IL-6 –(33.3%),
Olson et al, 2007 (111)	28 overweight or obese women	12 months	Control: =body weight, =fat mass, =lean mass, exercise: =body weight =fat mass, ↑ lean body mass	=CRP, =IL-6 ↓ CRP (9%), =IL-6
Nicklas et al, 2008 (103)	369 older adults	12 months	Exercise versus control: =body weight, =fat mass	↓CRP (32%), ↓ IL-6 (16%)
Campbell et al, 2009 (20)	115 overweight or obese	12 months	Exercise versus control: ↓ body fat, ↑ VO_{2max}	↓ CRP (10%), =IL-6
Donges et al, 2010 (37)	102 adults	10 weeks	Aerobic exercise versus control: ↓ body weight; resistance exercise versus control: =body weight	↓CRP (16.1%), =IL-6 ↓CRP (32.8%), =IL-6

↓ = significant reduction; ↑= significant increase = means no change; CRP = C-reactive protein; IL=interleukin; TNFα = tumor necrosis factor alpha; MET = metabolic equivalent.

Effect of Exercise and Diet on microRNAs and NF-kB Expression

MicroRNAs remain potent targets to many cells that are very sensitive to up or down regulation of microRNAs expression (54). The microRNAs in NF-kB signaling pathway (90) in cells of obese individual may provide a potential progression and maintenance that might pave the way towards the translation of microRNA-based therapeutic (90) and clinical chemistry to improve on obesity and other metabolic diseases that are affecting millions of people around the world. Chen and colleagues (25) reported in a research study that the level of microRNA in serum are stable, reproducible and consistent among individual of the same species (25). They sequenced all serum microRNA from healthy Chinese subjects and reported that 100 and 91 serum microRNAs are found in male and female subjects respectively (25). They also identified specific expression patterns among serum microRNAs for diabetes and cancer, providing evidence that serum microRNAs contain fingerprints of numerous diseases (25). microRNAs may be very related to an individual's body immune system and their alteration may very much and may reflect an inflammatory response shared by various diseases such as in overweight, obesity, diabetes and cancer (25, 39, 54, 90). It is unclear whether insulin-resistant and obese human subjects have abnormal NF-kB activity in muscle (153).

The nuclear factor-kB (NF-kB) a transcriptional factor that regulates genes is a protein dimer reticuloendotheliosis (Rel) (90) that can bind on a DNA sequence on the kB site. The transcription factor NF-kB was first discovered by David Baltimore in 1986. The NF-kB pathway is known to be activated by many stimuli including cytokines

(13, 59). Pro-inflammatory cytokines such as IL-6, TNF-α, IL-1 and IL-8 are produced upon the activation of NF-kB. The activation of NF-kB plays a major role in the inflammatory response and regulation to inflammatory stimulus. It is an effector present in the cytoplasm and when activated, it is translocated to the nucleus where it induces gene transcription (13). The microRNAs target genes encoding NF-kB regulators and effectors in the NF-kB signaling pathway with the microRNAs participating in a positive or negative feedback (90). It is very challenging targeting microRNAs that are effectors of NF-kB using chemical inhibitors but it remains to be ascertained whether dysregulation of microRNAs (25) rather than NF-kB activation is a causal to obesity and other metabolic diseases and their progression.

The effect of exercise on inflammation can't be very well understood without studying the molecular pathways linking exercise training to inflammation (96, 130, 153, 165). Every time the body moves, the muscles releases signaling molecules that communicate with the rest of the body to coordinate and regulate different mechanisms associated with exercise (151, 153). Tantiwong et al (153) measured NF-kB DNA-binding activity and the mRNA level of putative NF-kB-regulated myokines such as IL-6 and MCP-1 in muscle samples from type 2 diabetes mellitus (T2DM), obese, and lean subjects before (0 min), during (40 min), and after (210 min) bout of cycling exercise training. At baseline, NF-kB activity was elevated 2.1-fold and 2.7-fold in obese, nondiabetic and T2DM subjects, respectively. NF-kB activity was increased significantly at 210 min following exercise training in lean (1.9-fold) and obese (2.6-fold) subjects, but NF-kB activity did not change in T2DM. Exercise training increased

MCP-1 mRNA levels significantly in the three groups, whereas IL-6 gene expression increased significantly only in lean and obese subjects. MCP-1 and IL-6 gene expression also peaked at the 40-min exercise time point (153). Their result indicated that exercise affect changes in signaling molecules and inflammatory cytokines. Inflammation resulting from oxidative stress, free radical species may be attenuated by chronic exercise through the activation of factors such as NF-kB mediated gene expression during exercise training (59, 90). During exercise training signaling molecules are released that stimulate unique healing responses that couples both pro-inflammation and anti-inflammation mechanisms to regenerate and repair and grow stronger tissues and communicates with to the rest of the body and provides instructions to the body how to function, control and regulate inflammation (116, 118-121).

Exercise that is normally performed at high intensities has an anabolic stimulus that may increases the synthesis of contractile and structural proteins and also specific protein that may affect microRNA gene regulation (57). The recent identification of microRNAs has been helped to show the role of microRNA expression during exercise (87, 133, 168). Lui et al (87) investigated the microRNAs associated with PTEN signaling pathway on cycling exercise training. They conducted a study on subjects performed a cycling exercise training 5 d after spinal cord transfection. The training included a 5 d/wk of 30 min per session at 45 rpm for either 10 d or 31 d. They reported changes in the expression of microRNAs and target mRNA. Their conclusion was that exercise significantly increased microRNA-21 at 10 d ($p<0.0001$) with a significant decrease in PTEN ($p<0.05$). They also reported a 50% decrease in microRNA 15b with

no change of microRNA 21 at 31days (87). Another study (9) investigated the role of microRNA 146a on inflammation on 20 type 2 diabetes subjects. They reported that microRNA 146a level was significantly decreased by 37.5% and the target genes TRAF6 and NF-kB significantly increased by 61.2% and 59.8% respectively with an increase trend in IL-6 and TNF-a levels in these patients with no statistical significance (9).

Baggish et al (8) conducted a study to investigate the effect of microRNA in response to acute exhaustive cycling (a 1 min of unloaded exercise 0 W/min and workload was increased by 25 W/min until volitional exhaustion) before and after a 90 d training period at a respiratory exchange ratio value > 1.1 and at a heart rate of at least 90% of age-predicted maximum and sustained rowing exercise training on inflammatory microRNA 21 and 146a for a period of 90 d. These subjects also performed a 5 km distance of team-based rowing, an indoor ergometer session for 1-3 h at low stroke rates (20-24 strokes/min between the 9 d period. They reported a microRNA-146a 3.05-fold and microRNA 21 2.63-fold changes with the sustained exercise training and about a 7.5-fold and 3.16-fold change in microRNA-146a and microRNA-21 respectively with acute exercise. MicroRNA expression of human adipose tissue can be correlated with adipocyte phenotype, and obesity parameters (35). A global microRNA expression micro array of about 723 human and 76 viral mature microRNAs in human adipocytes were studied in subcutaneous fat and during differentiation from non-obese and obese women with and without type 2 diabetes. Study report by Ortega and associates (112) revealed that 50 microRNAs (6.2%) were significantly different between lean and obese subjects, 70 microRNAs (8.8%) were significantly and highly up or down-regulated in

adipocyte compared to pre-adipocyte, 17 microRNAs (2.1%) were correlated to anthropometrical and metabolic parameters (BMI and fasting glucose and triglycerides) and 11 microRNAs (1.4%) were significantly down-regulated in subcutaneous fat from obese subject with and without type 2 diabetes. They concluded that microRNAs may represent biomarkers and therapeutic targets for obesity and obesity-related complications (112).

Relationship between microRNAs and Inflammation (microRNA-inflammation)

The response to inflammation comprises of complex physiological and metabolic reactions that requires integration between all human systems and biomolecules including cytokines (109, 110). The evidence of circulating inflammatory cytokines and microRNAs provides a tissue specific area in research that can help understand the intercellular and intra-organ biochemical mechanisms or pathways (14, 109, 110, 128, 150). Evidence also indicate that microRNAs (9, 14, 75, 109, 110, 128, 150-152) can activate or inhibit inflammatory cytokines which relates to activating anti-inflammatory mechanisms to counter pro-inflammatory signals such as the NF-kB pathway to prevent cell and tissue damage (110). The microRNA based anti-inflammatory mechanisms in research may provide evidence that microRNAs are involved in regulating inflammation with prototype such as microRNA-21 and microRNA-146a (128). In physiological conditions, the transcription of microRNA 21 and 146a is at baseline levels and can initiate pro-inflammatory signals which can immediately result in a co-induction of their expression largely dependent on NF-kB pathway (14).

Evidence from a few studies indicates that microRNAs are delivered into blood and might be susceptible to degradation by extra-cellular RNAses and some studies have shown that microRNAs are secreted into the bloodstream either through micro-vesicles or in exosomes (14, 109, 110, 128). Olivieri et al (109) showed that there is a positive correlation between microRNA 21 and markers of inflammation such as CRP. Balasubramanyam et al (9) showed in one of their studies that reduced microRNA 146a levels were reported in blood cell of Asian Indian patients with type 2 diabetes in association with insulin resistance, poor glycemic control, specific pro-inflammatory cytokine genes and high levels of TNF-a and IL-6. Most microRNAs targeting NF-kB pathway affects the NF-kB signaling through a negative feedback mechanism that is aimed at restraining or reducing the excessive pro-inflammatory response induced by signaling activation (14).

MicroRNA are not only important for normal development and physiology but also for important in the pathologies of obesity, diabetes, cardiovascular diseases and inflammation. An altered expression of microRNA targeting the NF-kB pathway may contribute to the dysregulation of anti-inflammation and microRNA are an important modulator of the immune function. For example microRNA-146a has been shown to inhibit the expression of its target gene TRAF6 , impair NF-kB activity and suppress the expression of NF-kB target genes such as IL-6, Il-8, and TNF-α (65, 109, 110, 150). A study was conducted by Balasubramanyam et al (9) on 20 subjects in South India. The study comprised of two groups of subjects who were confirmed by oral glucose tolerance test (OGTT) to have a 2 h plasma glucose value >11.1 mmol/l (200 mg/dl)

based on WHO and were diagnosed with type 2 diabetes and reported that microRNA 146a was significantly decreased ($p<0.001$) with type 2 diabetes, TRAF6 mRNA levels was significantly increased ($p<0.015$) with type 2 diabetes and NF-kB expression was also significantly increased ($P=0.007$) with type 2 diabetes with an increase trend in TNF-α and IL-6 levels in patients with type 2 diabetes. They reported that the microRNA 146a was negatively correlated to TRAF6, NF-kB mRNA levels and circulating levels of TNF-α and IL-6. Emerging evidence suggest that microRNAs play a significant role in adipocyte differentiation and the identification of tissue specific microRNAs implicated in obesity might help in future studies to understand and develop new strategies for early diagnosis and therapeutic prevention and intervention of obesity-related complication (35). Targeting microRNAs such as microRNA 21 and 146a and many others that are related to obesity, their target genes such as PTEN and TRAF6 and the mRNAs of (PI3K/AKT/NF-kB), TNF-α and IL-6 will provide a break-through in solving complicated diseases such as obesity.

Conclusion

The obese population is currently one of the fastest growing segments in United States and around the world. Due to the high prevalence of obesity, it has been estimated that almost a third of health care expenditures is for overweight and obesity treatment. There are numerous research studies about obesity and weight maintenance, but it has yet to be determined what the ideal weight loss and exercise program, diet and caloric percentages is needed for the obese population. Obesity is related to inflammation. Exercise causes an acute increase in inflammatory markers that is mediated to some

degree during training. Exercise and strenuous activity have been shown to increase blood IL-6 and TNF-α, but regular exercise is believed to attenuate the increase in these inflammatory markers. Weight loss has also been shown to reduce markers of obesity. The purpose of this study is to examine the combined effects of exercise and diet-induced weight loss on markers of health and fitness, inflammation, microRNA, and gene expression related to NF-kB signaling pathway. The results of this study will provide additional data evaluating whether exercise and diet-induced weight loss affects inflammatory cytokine gene transcription, microRNAs gene expression and NF-kB signaling pathway. Obesity is associated with inflammation and we believe that our exercise and diet-induced weight loss program may reduce these makers. This study will compare results of the overweight /obese population to control group only.

CHAPTER III

METHODS AND MATERIALS

Experimental Design

Table 5 below shows the general research design and time course for assessments and an overview of the research design and the testing schedule for our 12 wks study program. Data for this study were obtained from a large comparative effectiveness study evaluating different weight loss program approaches. Participants were randomized into a non-exercise and non-diet control or an exercise and weight loss program for 90-days. The study included a baseline testing session followed by 3 additional testing sessions (0, 4, 8, 12 wk). Exercise testing occurred during the baseline and 12 wk sessions. The independent variable was exercise and diet-induced weight loss intervention program. Dependent variables were: body composition (body weight, fat mass, fat free mass and body fat percent), clinical white blood cells and serum profiles for microRNAs (miR21 and miR146a) and mRNA of IL-6, TNF-α, and the mRNAs of PTEN, TRAF6, PI3k, AKT and NF-kB; maximum repetition 1(RM) and 80% 1RM isotonic strength.

Participants

Eighty-two (82) sedentary, overweight/obese women participants (BMI > 27) between the ages of 18-65 were recruited to participate in this portion of the study. Participants were not be allowed to participate in the study if they had any uncontrolled metabolic disorder, known electrolyte abnormalities, heart disease, arrhythmias,

Table 5: Effects exercise and diet-Induced weight loss in overweight women on characterization of serum/white blood cells on microRNAs and cytokine gene transcription–Overview of research design and testing schedule

Familiarization and Entry (T0)	Baseline (T1)	4 weeks (T2)	8 weeks (T3)	12 weeks (T4)
Phone interview Familiarization session Complete of paper work Review medical history Randomization into exercise and diet-induced weight loss and control programs	High Protein Diet Review Body Weight Blood collection (white blood cells/serum) Dual Energy X-ray Absorptiometry (DEXA) Graded Exercise Testing (GXT) 1RM at 80% 1RM Isotonic Bench and Isotonic Leg Press 30 minutes Resistance Exercise/Zumba 4 times/week	High Protein Diet Review Body Weight Blood collection (white blood cells/serum) Dual Energy X-ray Absorptiometry (DEXA) 30 minutes Resistance Exercise/Zumba 4 times/week	High Protein Diet Review Body Weight Blood collection (white blood cells/serum) Dual Energy X-ray Absorptiometry (DEXA) 30 minutes Resistance Exercise/Zumba 4 times/week	High Protein Diet Review Body Weight Blood collection (white blood cells/serum) Dual Energy X-ray Absorptiometry (DEXA) Graded Exercise Testing (GXT) 1RM at80% 1RM Isotonic Bench and Isotonic Leg Press 30 minutes Resistance Exercise/Zumba 4 times/week

diabetes, or thyroid disease; a history of hypertension, hepatorenal, musculoskeletal, autoimmune, or neurological disease. Participants were not allowed if they were taking any hypoglycemic, or androgenic medications; and/or, if they had taken ergogenic levels of nutritional supplements that may affect muscle mass (e.g., creatine, HMB), anabolic/catabolic hormone levels (e.g., DHEA), or weight loss (e.g., thermogenics) within three months prior to the start of the study. Participants with a controlled medical

condition had their physician complete and sign a physician consent form prior to participation in the study. Participants that meet eligibility criteria into our study program were informed of the requirements of the study and sign consent statements in compliance with the Human Participants Guidelines of Texas A&M University and the American College of Sports Medicine.

Study Site

All familiarization and testing was conducted at the Exercise & Sport Nutrition Laboratory (ESNL) in the Department of Health, and Kinesiology at Texas A&M University. Exercise training was conducted at the Exercise & Sport Nutrition Laboratory (ESNL) in the Department of Health and Kinesiology at Texas A&M University. Assays were conducted at the Institute of Obesity and Cancer research Laboratory at Texas A&M University.

Entry and Familiarization Session

Participants expressing interest in the study were interviewed by phone to determine whether they are qualified to participate. The participants who met the eligibility criteria were invited to attend a familiarization session. During this session participants received verbal and written explanation of the study protocol and design. Participants also received testing procedures, blood collection procedures and were introduced to the equipment that would be used throughout the entire study 12 wk.

Randomization and Dietary Intervention

Participants were then randomized according to body mass index (BMI) and age into either an exercise and diet-induced weight loss (E) group or a control (C) group.

Participants were given an appointment time to perform baseline assessments and three other sessions as summarized in table 5.

Training Protocol

Participants randomized into the exercise and diet-induced weight loss group (EX) followed the Curves™ training program while participants in the control group (C) followed their normal eating and daily activities with no exercise. The physical training protocol included three regular resistance workouts each week for 12 weeks and one Zumba dance workout, while maintaining a greater than 90% compliance record (43 out of 48 workouts). The circuit training utilized the computerized CurvesSmart system (*Curves International, Waco, TX, USA*) equipped with software designed by MYTRAK (*version 4.2.1, copyright 2004-2011, MYTRAK Health System Inc, Mississauga, Ontario, Canada*). The circuit training consisted of 13 bi-directional hydraulic resistance exercise machines that worked all major muscle groups (i.e., elbow flexion/extension, knee flexion/extension, shoulder press/lat pull, hip abductor/adductor, chest press/seated row, horizontal leg press, squat, abdominal crunch/back extension, chest flies, oblique, shoulder shrug/dip, hip extension, and side bends). During the circuit training, subjects were instructed to complete as many repetitions as possible during a 30 second time period on each resistance machine. Between machines, subjects performed floor-based aerobic exercises or stepping exercise designed to maintain an elevated heart rate. Subjects performed the entire circuit twice during the 26 minute regular circuit workout. During the Curves Zumba workout, subjects performed 1 minute of Zumba dance moves taught by a certified Zumba instructor and all circuit training sessions were supervised

by trained fitness instructors who assisted subjects with proper exercise technique and self-monitoring of heart rate in order to maintain an exercise heart rate between 60-80% of target heart rate using age-predicted maximal heart rate (220-age). They were also encouraged to walk for 30 min at a brisk pace at 60-80% of heart rate reserve on one of the days they were not using the curves equipment. Subjects also performed 4 minutes of whole body stretching following circuit workouts. The circuit training equipment was located in the Exercise and Sports Nutrition Laboratory at Texas A&M University.

Diet Intervention

In concurrence with this plan, subjects followed the Curves Phase I higher protein weight loss diet (1,200kcals/day) for one week and the phase II higher protein weight loss diet (1,500kcals/day) for 11 weeks. The macronutrient content for both phases I and II diets was 45% protein, 30% carbohydrate, 25% fat. A registered dietitian at the Exercise and Sports Nutrition Lab of Texas A&M University reviewed the higher protein diet (HPD) and exercise plan with subjects and provided them with the Curves Fitness and Weight Management Plan (62), the Curves Food & Exercise Diary (30) and Curves Essentials 2 go dietary supplements. Participants were encouraged to consume of Curves multivitamins and minerals containing 800 mg of calcium and 520 mg of Omega-3 fatty acids daily with food (*Curves International, Inc. Waco, TX*). The dietitian also met with subjects weekly throughout the study to discuss any dietary challenges or concerns. The Curves Fitness and Weight Management Plan dietary intervention protocol is summarized in Table 6.

Table 6: Overview of diet assignments

Diet Period	Group		Macro-nutrient	Grams/ Day	Kilocals/ Day	Percent Daily Diet (%)
Dietary Phase	**Exercise and Diet-Induced Weight Loss (EX) Group)** n = 29					
Phase I (1 Week)	1,200 kcals/d	HPD + Exercise	PRO CHO FAT	135 90 33	540 360 300	45 30 25
Phase II (11 weeks)	1,500 kcals/d	HPD + Exercise	PRO CHO FAT	169 113 42	675 450 375	45 30 25
	Control (C) Group n = 20					
Baseline to 12 Weeks	Normal Diet Normal Activity No Exercise		None	None	None	None

Testing Methods

Test sessions

Participants were instructed to refrain from exercise for 48 hours and fast for 12 hours prior to baseline testing. Baseline (0 wk) testing sessions and the 12 wk testing sessions were identical and different from the testing sessions of 4 wk and 8 wk that were also identical as shown in Table 5. Participants reported to the ESNL for body composition and clinical assessments. Once they reported to the lab, participants completed a radiation questionnaire. Participants were weighed, had body composition determined using Dual Energy X-ray Absorptiometry (DEXA); donated blood samples, performed a cardiopulmonary symptom-limited maximal exercise stress test; and,

performed 1 repetition maximum (RM) and 80% of 1RM on the isotonic bench press and leg press.

Body composition

Height was measured using standard anthropometry. Total body weight was measured using a Healthometer (*Bridgeview, IL, USA*) self-calibrating digital scale with a precision of +/-0.02 kg. Body composition (excluding the cranium) was determined using a Hologic Discovery W QDR series DEXA (*Hologic Inc, Waltham, MA, USA*) equipped with APEX software (*APEX Corporation Software, Pittsburgh, PA, USA*). Participants were informed of any or all inherent risks that could come from the radiation exposure and completed a radiation exposure questionnaire prior to all scans. Quality control (QC) calibration procedures were performed on a spine phantom (*Discovery W-CALIBER Model DPA/QDR-1 anthropometric spine phantom*) prior to each testing session. The DEXA has been validated as an accurate method for body composition assessment (55, 77, 97, 147). The mean test-retest reliability of studies performed on some male athletes with this Hologic system yielded mean coefficients of variation for total bone mineral content and total fat free/soft tissue mass of 0.31% to 0.45% with a mean intra-class correlation of 0.985 (6).

Cardiopulmonary exercise tests

Cardiopulmonary exercise tests were performed at baseline and 12 weeks in accordance to standard procedures described by the American College of Sports Medicine's (ACSM) *Guidelines for Exercise Testing and Prescription* (4). The Nasiff Cardio Card electrocardiograph (*Nasiff Associates, Inc, Central Square, NY, USA*) was

used to assess heart function using a standard 12-lead arrangement (4). Cardiopulmonary measurements were assessed using a Parvo Medics TrueMax 2400 Metabolic Measurement System (*ParvoMedics, Inc., Sandy, UT*). The participants performed the Bruce or modified Bruce treadmill protocol (148) following the speeds and inclination grades of the Parvo Medics TrueMax 2400 Metabolic Measurement System (*ParvoMedics, Inc., Sandy, UT*). Heart rate (HR), electrocardiograhic tracings, and expired gases were monitored continuously throughout the exercise test. Blood pressure (BP) and ratings of perceived exertion (RPE) were obtained toward the end of each stage. The participant were encouraged to exercise to their maximum unless they experienced clinical signs that required test termination as stated by the ACSM's *Guidelines for Exercise Testing and Prescription* (3). The mean coefficient of variation for assessing peak Vo_2 utilizing the Bruce or modified Bruce protocol has been shown to be 6.5% (range, 2.0-14%) (44). This test was performed to determine maximal aerobic capacity and anaerobic threshold to measure the effects of exercise and diet-induced weight loss training on makers of health and fitness on exercise capacity.

Isotonic strength tests

Participants in the exercise and diet-induced weight loos program had their 1RM determined using an isotonic Olympic bench press (*Nebula Fitness, Versailles, OH, USA)* and a standard hip sled/leg press (*Nebula Fitness, Versailles, OH, USA*) to determine changes in maximal strength according to standardized procedures. Hand positioning on the bench press and foot and seat position on the hip sled/leg press were standardized between trails. Muscular endurance was assessed by having participants

perform as many repetitions as possible at 80% of their predetermined 1RM on the bench press and leg press using standard lifting techniques and testing criteria (7).

All strength/exercise tests were conducted using standard procedures and were supervised by certified lab assistants experienced in conducting strength/anaerobic exercise testing. We tested for upper body strength and endurance; participants performed one repetition maximum (1RM) test on the isotonic bench press and the Nebula Fitness (*Versailles, OH, USA*) Olympic Power Station (*#1005*). Participants performed a warm-up (2 sets of 10 repetitions at approximately 50% of anticipated 1RM) followed by progressive lifts starting at about 70% of anticipated 1RM and increasing by 5–10 lbs. until 1 RM is reached. Once the 1RM was attained, subjects performed as many repetitions as possible with 80% of their 1 RM efforts. Participants rested for 4 minutes, then performed a warm up of 8-10 repetitions at approximately 50% of anticipated maximum on the Nebula 45° Leg press. Participants performed successive lifts on the leg press starting at about 70% of anticipated 1RM and increasing by 10–25 lbs. until 1RM was be attained. Once the 1RM was achieved, subjects performed as many repetitions as possible with 80% of their 1 RM efforts. Test-retest reliability of performing these strength tests on resistance-trained subjects in the ESNL have yielded low mean coefficients of variation and high reliability for the bench press (1.9%, intraclass $r = 0.94$) and leg press/hip sled (0.7%, intraclass $r = 0.91$) (170).

Blood collection and analysis

Participants donated approximately 2 teaspoons of fasting venous blood (10 milliliters). Blood samples were obtained using standard phlebotomy procedures using

standard sterile venipuncture of an antecubital vein by ESNL laboratory technicians or graduate research assistance trained in phlebotomy in compliance with guidelines established by the Texas Department of Health and Human Services. The phlebotomists and lab technicians wore personal protective clothing (gloves, lab coats, etc.) when handling blood samples. About 10 ml of whole blood was collected from each participant in EDTA tubes. The EDTA tube contains a gel that forms a physical barrier between serum and white blood cells (buffy coat) and red blood cells during centrifugation. After collection, BD Vacutainer® SST™ Serum Separation Tubes were centrifuged immediately after collection at 1,200g for 15min at 4^{o}C to spin down the blood cells, and the supernatant (serum) were transferred into two 1.5ml microcentrifuge tubes, followed by transferring the intermediate white layer or WBC (buffy coat) using a plastic 3 ml transfer pipette into appropriately labeled 1.5 ml Eppendorf tubes. Serum blood samples were sent to Quest Diagnostics (*Houston, TX*) for comprehensive metabolic panel analysis using an Olympus AAU 5400 Chemistry Immuno Analyzer (*Olympus America Inc., Center Valley, PA*). Whole blood samples were analyzed for complete blood counts with platelet differentials using an Abbott Cell Dyn 3500 automated hematology analyzer (*Abbott Laboratories, Abbott Park, IL*). Reported test to test reliability of performing these assays generally range from 2 to 6% for individual assays. Samples were run in duplicate to verify results if the observed values were outside control values and/or clinical norms according to standard procedures. The serum and white blood cells (buffy coat) were then aliquoted and stored at -80C until analyzed for the blood lipid panel (Triglyceride, HDL, LDL and total cholesterol),

microRNAs (21 and 146a), cytokines expression IL-6 and TNF-α), PTEN, TRAF6, PI3K, AKT, NF-kB signaling pathway.

RNA extraction and real-time PCR analysis of cytokines, mRNAs and microRNAs

Total RNA was isolated from 200ul serum and white layer according to the manufacturer's recommended protocol using the mirVana *PARIS kit (Ambion, USA*) for mRNA analysis and the mirVana™ miRNA isolation kit *(Applied Biosystems, Foster City, CA)* for micro-RNA analysis. Samples were evaluated for nucleic acid quality and quantity using the NanoDrop® ND-1000 Spectrophotometer (*NanoDrop Technologies, Wilmington, DE*). Isolated RNAs were synthesized to cDNA using a Reverse Transcription Kit (*Invitrogen Corp., Grand Island, NY*) according to the manufacturer's protocol. RT-PCR was carried out with the SYBR Green PCR Master Mix from *Applied Biosystems (Foster City, CA)* on an ABI Prism 7900 HT Sequence Detection System (*Applied Biosystems Inc, Foster City, CA*) with appropriate cycle conditions. Primers were designed using Primer Express software (*Applied Biosystems, Foster City, CA).*

Each primer was a homology-searched by an NCBI BLAST search to ensure that it is specific for the target mRNA transcript. The pairs of forward and reverse primers will be purchased from Integrated DNA Technologies, Inc. *(San Diego, CA*). Product specificity was examined by dissociation curve analysis. Table 7 shows the microRNA and mRNA primers used for RT-PCR in the exercise and diet-induced weight loss study program for the duration of the study.

Table 7. **microRNA and mRNA primers used in RT-PCR genes expression analysis**

microRNAs		
microRNA-21	UAGCUUAUCAGACUGAUGUUGA	
microRNA-146a	GGGTGAGAACTGAATTCCA	
microRNA-U6B	ACG CAA ATT CGT GAA GCG TT	
mRNAs	Sense sequence (5' → 3')	Antisense sequence (3' → 5')
PTEN	ACCAGGACCAGAGGAAACAT	TTTGTCAGGGTGAGCACAAG
TRAF6	TGGCATTACGAGAAGCAGTG	GTACCATCTTGTGCAAACAACC
PI3K	GCTTGTTCTGTGGTTGCAGA	TAGTGGAGCACCAGCTCCTT
AKT	GGGCCTAGGTGTTGTCATGT	AAGAGAGTGTTCGGGGGAAT
	AAGAGAGTGTTCGGGGGAAT	
NF-kB	TGGGAATGGTGAGGTCACTCT	TCCTGAACTCCAGCACTCTCTTC
IL-6	AGGGCTCTTCGGCAAATGTA	GAAGGAATGCCCATTAACAACAA
TNF-α	CCCAGGCAGTCAGATCATCTTC	AGCTGCCCCTCAGCTTGA
GAPDH	CCATCCCAGACCACCTAAC	GCAGCGAACTTTATTGATGA

Quantification of microR-NU6B, microR-146a, and microR-21 were performed using the Taqman® MicroRNA reverse transcription kit (*Applied Biosystems, Foster City, CA*). For the PCR reaction, the reverse transcription product will be diluted as 1:15 and amplified using TapMan® 2× Universal PCR Master Mix (No AmpErase® UNG) (*Applied Biosystems, Foster City, CA*) according to the manufacturer's specifications. The expression levels of the microRNAs were determined using $2^{-\Delta\Delta Ct}$ and normalized to microR NU6B small nuclear RNA. The expression level of mRNA was determined using $2^{-\Delta\Delta Ct}$ and normalized to GADPH for mRNAs (IL-6, TNF-α, PTEN, TRAF6, AKT, PI3K and NF-kB) expressions.

Luminex inflammatory cytokine analysis

The Luminex® assay was performed using the Multiplex L200 *(Austin, TX)* with the high sensitive human inflammatory marker kit (*Millipore, Billerica, MA*) following the manufacturer's protocol. Data were analyzed using Luminex xPonent 3.0 software

(*Millipore, Billerica, MA*). This quantitative measure is considered a kinetic enzymatic method to measure gene expression in serum and white blood cells. The analyses of these blood parameters were performed to determine the exercise and diet-induced weight loss intervention on markers of clinical health, inflammatory cytokines gene expression and microRNAs expression.

Statistical analysis

Related data were analyzed by Multivariate Analysis of Variance (MANOVA) with repeated measures (*PASW Statistics version 20, 2011, SPSS Inc, Chicago, IL*.). Overall MANOVA effects were examined using Wilks' Lamda time and group x time p-levels as well as MANOVA univariate ANOVA group effects. Greenhouse-Geisser univariate tests of within of within-subjects time and group x time effects and between-subjects univariate group effects were reported for each variable analyzed within the MANOVA model. In some instances, repeated measures ANOVA was run on variables not included in a MANOVA design with univariate group, time, and group x time interaction effects reported. Variables with baseline differences determined by ANOVA were analyzed using analysis of covariance (ANCOVA).

Delta values or percent difference were calculated and analyzed on select variables by ANOVA for repeated measures to assess changes from baseline values. Delta values were calculated by subtracting the first testing session (T1) from later testing sessions (T4-T1). Percent differences were calculated by subtracting T1 from the later testing session, then performing division by T1 followed by multiplication by 100 [(T4-T1)/T1·100]. Data were considered statistically significant when the probability of

type I error was 0.05 or less and statistical trends were considered when the probability error ranged between $p > 0.05$ to $p < 0.10$. If a significant group, treatment and/or interaction alpha level was observed, Tukey's least significant difference (LSD) post hoc analyses were performed to determine where significance was obtained. Power analysis of previous studies using similar designs and subject populations indicated that a sample size of 30 subjects per group yielded high power (>0.8) for delta values of 0.75 to 1.25 for weight and fat loss. All data are presented as means ± standard deviation for baseline to 12 wk data analyses and as means ±standard error of the mean for group data analyses. Pearson product correlations were performed to examine relationship of changes in body composition to markers of inflammation.

CHAPTER IV

RESULTS

Participants

Figure 1 presents a Consort Diagram for participant recruitment, entry, and completion observed in this portion of the study. Eighty-two (82) out of 125 subjects who called to participate in our 12 wk study program met phone interview entrance criteria and were invited to attend familiarizations sessions. All 82 women showed up for familiarization sessions and met entrance criteria to participate in the study after evaluation of medical history and obtaining consent. All 82 obese women (BMI 35.6±5.6 kg/m^2) completed baseline testing and were cleared to participate in the study. Of these women, 49 women completed the 12 week study. The primary reasons participants dropped out of the study were due to time constraints, work, moving, falling sick, Complaints about the exercise program (that it was too intense) and did not want to participate in the control group once they found out they were in the control group.

Table 8 presents the participant demographics data. Forty-nine obese women (age 48.2±10.5 years, height 63.5±2.7 cm; weight 203.3±30.5 kg; BMI 35.6±5.6 kg/m^2; 45.9±4.4 % body fat) completed the 12-week study (EX group n=29; C group n=20) with greater than 80% compliance. One-way ANOVA of baseline demographic data revealed no significant differences were observed between groups in baseline age, height, weight, BMI, or percent body fat.

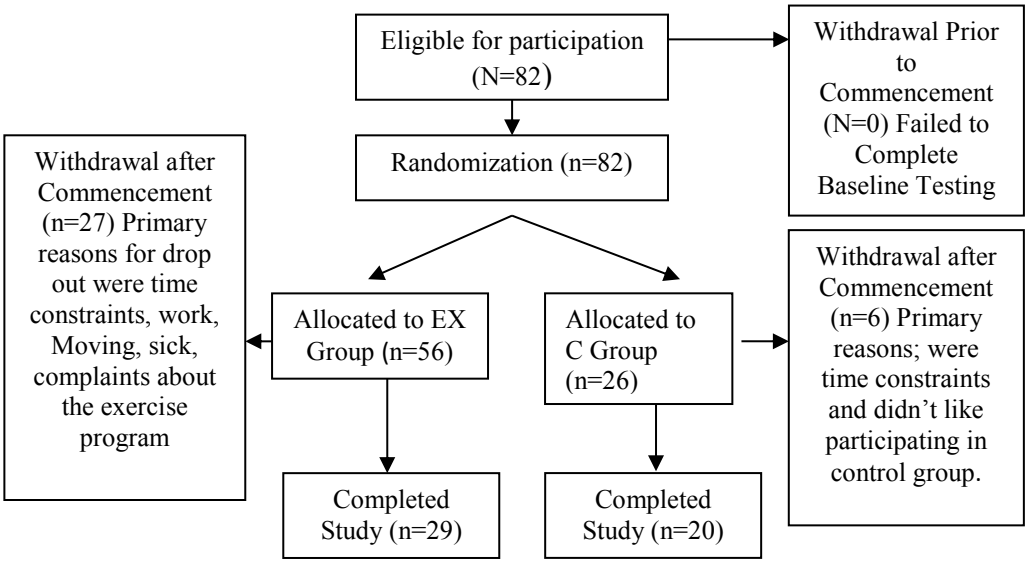

Figure 1: Consort diagram for participation.

Table 8. Baseline demographics for the exercise and diet-induced weight loss (EX) and control (C) groups

	Exercise Group (N=29)	Control Group (N=20)	P-value
Age (years)	46.5±10.1	50.6±10.9	0.191
Height (cm)	63.6±2.6	63.4±3.0	0.874
Weight (kg)	199.4±30.0	209.0±31	0.288
BMI[a] (kg/m²)	34.9±5.9	36.6±5.2	0.307
DEXA[b] BF (%)	45.6±3.7	46.3±5.2	0.571

All data is presented as means ± SD at baseline.
[a]Body mass Index
[b]Dual Energy X-ray Absorptiometry

Results

Energy intake

Table 9 presents changes in nutritional intake at baseline and 12 weeks of program intervention. The completed food records were obtained on all participants completing the study. MANOVA's were run on energy intake and macronutrient intake expressed in kcals/kg/day, g/kg/day, and percentage of total calories consumed. MANOVA analysis revealed significant time (Wilks' Lamda p=0.001), overall group (Wilks' Lamda p=0.001), and an overall time by diet interaction (Wilks' Lamda p=0.003) effects. MANOVA analysis of percent dietary intake data revealed an overall time (Wilks' Lamda p=0.000), and an overall time by diet interaction (Wilks' Lamda p=0.000).

MANOVA univariate analysis revealed that the Ex group had a greater reduction in energy intake per kilogram body weight than the C group (EX 1,748±515, 1,351±228, 1,397±311, 1,445±430; C 2,153±725, 1,715±502, 1,783±599, 1,778±555g/kg/d) with a significant time (p=0.001) and group (p=0.001) effect observed. Carbohydrate intake decreased overtime with a greater reduction in the EX group than in the C group (EX 187±80.0 129.8±33.5, 127.6±38.6, 124±50.9; C 227±92.8, 188.2±46.5, 187.9±62.1, 190±71.9) with a significant time (p=0.001) and group (p=0.000) effect observed. Univariate analysis revealed a significant time by diet effect (p=0.01) with no significant time or group effect. Fat intake resulted in a significant (EX 74.3±29.9, 50.2±18.85, 53.6±19.2, 58.5±23.77; C 82.5±33.8, 69.4±26.20, 71.8±28.3, 73.9±26.54) time (p=0.01)

Table 9. **Nutritional intake changes obtained at baseline, 4, 8 and 12 weeks of the program participation for the exercise/diet-induce weight loss (EX) and control (C) groups[a]**

Variable	Group	Baseline	4-weeks	8-weeks	12-weeks	Group SEM	P values
Energy	Exercise	1,748±515	1,351±228[g]	1,397±311 [g]	1,445±430[g]	1,485±71.03[g]	G[c] = 0.001
Intake	Control	2,153±725	1,715±502	1783±599	1,778±555	1,848±76.43	T[d] = 0.00
(kcals/kg/d)	Total	1,930±643	1,515±414	1571±496	1,594±512		I[e] = 0.97
CHO	Exercise	187±80.0	129.8±33.5[g]	127.6±38.6[g]	124±50.9[g]	141.9±8.30[g]	G[gh] = 0.00
Intake	Control	227±92.8	188.2±46.5	187.9±62.1	190±71.9	196.9±8.93	T[gh] = 0.001
(g/kg/d)	Total	205±87.3	156.1±49.1	154.7±58.3	154±69.1[g]		I = 0.59
Protein	Exercise	74.5±17.7	83.1±26.36	84.7±18.47	83.8±22.27	81.5±3.32	G = 0.15
Intake[f]	Control	86.3±30.3	68.6±20.46	69.3±28.01	74.5±22.06[g]	74.3±3.58	T = 0.61
(g/kg/d)	Total	79.8±24.6	76.6±24.70	77.8±24.21	79.6±22.39		I[g]= 0.01
Fat Intake	Exercise	74.3±29.9	50.2±18.85	53.6±19.2	58.5±23.77	59.1±3.84[g]	G [g]= 0.01
(g/kg/d)	Control	82.5±33.8	69.4±26.20	71.8±28.3	73.9±26.54	74.2±4.13	T[h] = 0.002
	Total	78.0±31.6	58.8±24.18	61.8±25.1	65.5±25.91[g]		I= 0.57
CHO	Exercise	42.4±9.12	40.0±8.93	37.9±7.00	35.6±8.05	39.0±1.00[g]	G[gh] = 0.001
Intake	Control	43.6±5.17	45.8±4.89	44.5±6.31	43.6±7.36	44.4±1.12	T = 0.08
(%)	Total	42.9±7.54	42.6±7.87	40.8±7.40	39.2±8.66		I = 0.13
Protein	Exercise	18.4±5.09	25.5±7.00	26.1±6.09	25.3±6.69	23.8±0.82[g]	G [gh]= 0.00
Intake	Control	17.2±4.07	16.7±3.55[g]	16.5±3.33[g]	17.7±3.82[g]	17.0±0.91	T[gh] =0.001
(%)	Total	17.8±4.64	21.6±7.19[g]	21.8±6.95[g]	21.9±6.73[g]		I[gh]= 0.00
Fat Intake	Exercise	38.4±7.31	34.0±9.69	35.4±7.21	37.7±7.42	36.3±1.07	G = 0.71
(%)	Control	37.1±6.13	36.6±4.96	36.0±5.51	38.1±5.18	36.9±1.18	T = 0.09
	Total	37.7±6.75	35.2±7.94	35.7±6.43	37.9±6.44		I = 0.47

[a]Complete food intake records were obtained at Baseline and 12 weeks for 29 participants in the group EX and 20 participants in the group C (N=49). Data were analyzed using ESHA Food Processor Nutritional Analysis Software (Version 8.6).
[b]p<0.05 difference from baseline.
[c]G=group alpha level.
[d]T=time alpha level.
[e]I=group x time interaction alpha level.
[f]Protein intake
Group SEM=group standard error of the mean
[g]p<0.01 difference between EX and C groups.
[h]p<0.05 difference between EX and C groups.

and group (p=0.002) effect observed. Our results also revealed a significant increase percent protein intake in the EX group compared to the C group (EX 18.4±5.09, 25.5±7.00, 26.1±6.09, 25.3±6.69; C 17.2±4.07, 16.7±3.55, 16.5±3.33, 17.7±3.82 with a significant time (p=0.001) and group (p=0.000) and time by diet (p=0.000) effect observed. Percent carbohydrate intake revealed an increased effect in the Ex group compared to the C group (EX 42.4±9.12, 40.0±8.93, 37.9±7.00, 35.6±8.05; C 43.6±5.17, 45.8±4.89, 44.5±6.31, 43.6±7.36) with a significant group (p=0.001) effect and no significant time (p=0.08) effect observed. There was no significant effect observed with

percent fat intake. These findings indicate that protein intake was greater in the exercise and diet intervention group.

Body composition

Table 10 presents changes in body composition observed during the study. MANOVA analysis of body composition data revealed overall time (Wilks' Lamda p=0.000) and time by diet effects (Wilks' Lamda p=0.000). Univariate analysis revealed significant time effects for body weight (p=0.001), fat mass (p=<0.001), and percent body fat (p=0.001).

Table 10. Changes in body composition and fitness related data values observed at baseline, 4, 8 and 12 weeks of program participation for the exercise/diet-induced weight loss (EX) and control (C) groups[a]

Variable	Group	Baseline	4-weeks	8-weeks	12-weeks	Group SEM	p-value
Total Mass (kg)	Exercise	84.18±13.05	82.17±13.51	81.04±13.4[b]	80.18±13.51[b]	81.90±2.44	G[cb] =0.09
	Control	88.44±13.58	88.31±12.88	88.50±12.85	88.55±12.82	88.45±2.94	T[db]=0.001
	Total	85.92±13.30	84.68±13.47	84.08±13.57	83.60±13.74[b]		I[eb]= 0.00
Fat Mass (kg)	Exercise	38.59±7.97	36.58±8.74	35.21±8.40[b]	34.78±8.87[b]	36.29±1.56[b]	G[b] = 0.05
	Control	41.12±9.15	41.08±8.18	41.26±7.98	41.09±8.33	41.14±1.87	T[b] =0.00
	Total	39.62±8.47	38.42±8.72	38.68±8.69	37.36±9.12[b]		I[b] = 0.00
Fat-free Mass (kg)	Exercise	45.60±6.47	45.60±6.35	45.83±6.83	45.40±6.34	45.60±1.26	G =0.39
	Control	47.32±7.72	47.22±7.30	47.21±7.46	47.46±7.22	47.31±1.52	T = 0.96
	Total	46.29±6.99	46.26±6.73	46.40±7.05	46.24±6.72		I = 0.58
Body Fat (%)	Exercise	45.60±3.73	44.10±4.69	43.07±4.94[b]	42.85±5.38[b]	43.91±0.84	G[b] = 0.07
	Control	46.33±5.24	46.42±4.51	46.60±4.54	46.27±4.52	46.41±1.02	T[b]= 0.00
	Total	45.90±4.37	45.05±4.71	44.51±4.05	44.25±5.28[b]		I[b]= 0.00

[a]Data are from 29 participants in the EX group and 20 participants in the C group (n=49) who completed the 12-week study.
[b]p<0.05 difference from baseline.
[c]G=group alpha level.
[d]T=time alpha level.
[e]I=group x time interaction alpha level.
Group SEM=group standard error of the mean

Participants in the EX group experienced greater changes in body weight (EX -4.0±4.4 kg; C 0.1±3.0 kg, p=0.001), fat mass (EX -3.8±4.0 kg; C -0.03±2.0 kg, p<0.001), and

percent body fat (EX -2.7±3.4%; C -0.1±1.7%, p=0.002). No differences among groups were observed in FFM (EX -0.2±2.0 kg; C 0.1±2.3 kg, p=0.59). Review of body composition data lead to acceptance hypothesis H_1 that the exercise and diet intervention would promote statistically significant differences in body composition measures compared to controls and thus, based on our results data we therefore accepted our H_1 hypothesis. Figure 2 represents changes from baseline to 12-wk between our exercise and diet-induced weight loss and control group on body composition.

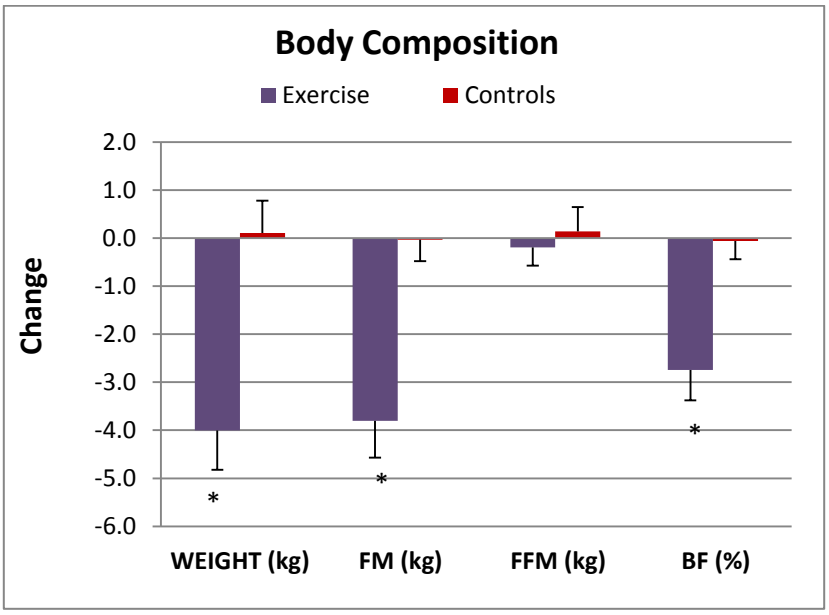

Figure 2. Data are presented as means ±SD of body composition. *P <0.05 significant differences from baseline to 12-wk between the exercise and diet-induced weight loss and control groups.

Fitness assessment

Table 11 presents changes in markers of fitness observed during the study. MANOVA analysis revealed a significant time effect (p<0.001) and group x time effect (p=0.002) for measures of fitness. Univariate analysis revealed significant VO_{2max}

(p=0.014) and lower body 1-RM (p<0.001). Participants in the EX group experienced greater changes in VO_{2max} (EX 13.6±17.0%; C -2.2±10.3%, p=0.001) and upper body (EX 8.7±12.5%; C -1.2±13.9%, p=0.016) 1-RM while no differences in lower body 1-RM strength were observed between groups (EX 15.0±21.9%; C 13.8±23.7%, p=0.86) after 12 weeks of program participation.

Table 11. **Changes in general health and fitness values obtained at baseline and 12 weeks of program participation for the exercise/diet-induced weight loss (EX) and control (C) groups**[a]

Variable	Group	Baseline	12 -weeks	Group SEM	p-value
Peak VO_2[g] (L/min)	Exercise	1.84±0.2	1.94±0.2[b]	1.89±0.05[b]	G[b] = 0.05
	Control	1.77±0.3	1.72±0.3	1.74±0.06	T = 0.19
	Total	1.81±0.3	1.86±0.3		I[jb] = 0.004
Peak VO_2[g] (ml/kg/min)	Exercise	20.5±3.1	23.1±3.4[jb]	21.77±0.57[jb]	G[jb] = 0.001
	Control	18.7±3.1	18.2±3.3	18.49±0.71	T[j] = 0.008
	Total	19.8±3.2	21.1±4.1[jb]		I[jb] = 0.00
Treadmill Time (min)	Exercise	7.6 ±0.9	8.4±0.8[b]	8.02±0.19[bj]	G[jb]=0.00
	Control	6.7±1.4	6.8±1.4	6.73±0.25	T[jb]=0.00
	Total	7.2±1.2	7.8±1.3[bj]		I[b]=0.004
Bench Press 1 RM[h] (kg)	Exercise	67.89±13.58	73.27±14.28[j]	70.58±3.18	G = 0.91
	Control	70.56±19.55	69.44±20.78	70.00±3.83	T = 0.09
	Total	68.97±16.12	71.70±17.12		I[j] = 0.01
Bench Lifting Volume[k] (repetitions*kg)	Exercise	204.08±91.14	205.83±116.69	204.95±17.47	G = 0.08
	Control	160.49±115.94	152.28±55.04	156.39±20.99	T = 0.81
	Total	186.25±103.02	183.92±99.12		I = 0.71
Leg Press 1 RM (kg)	Exercise	366.92±113.68	420.77±153.57	393.85±27.14	G = 0.62
	Control	392.78±145.39	437.50±171.41	415.14±32.61	T[jb] = 0.001
	Total	377.50±126.64	427.61±159.37[j]		I = 0.74
Leg Lifting Volume[k] (repetitions*kg)	Exercise	1,931±1,531	2,595±2,624	2,263±297.81	G = 0.37
	Control	1,838±758	1,850±760	1,844±357.92	T = 0.19
	Total	1,893±1,262	2,290±2,090		I = 0.20

[a]Data are from 29 participants in the EX group and 20 participants in the C group (n=49) who completed the 12-week study.
[b]p<0.05 difference from baseline.
[c]G=group alpha level
[d]T=time alpha level
[e]I=group x time interaction alpha level
[g]Peak VO_2 = peak oxygen uptake.
[h]1 RM = 1 repetition maximum
[i]Bench press lifting volume at 80% 1 RM (kg)
[j]p<0.01 difference between EX and C groups.
[k]Leg Press lifting volume at 80% 1 RM (kg)
Group SEM=group standard error of the mean

Consideration of peak $V0_2$, fitness and upper extremity muscular strength data led to acceptance of H_2 that the exercise and diet intervention would promote statistically significant differences in marker of health and fitness compared to controls. The graph in Figure 3 below illustrates changes from baseline to 12-wk between our exercise and diet-induced weight loss and control groups in measures of fitness assesses during our 12-wk program.

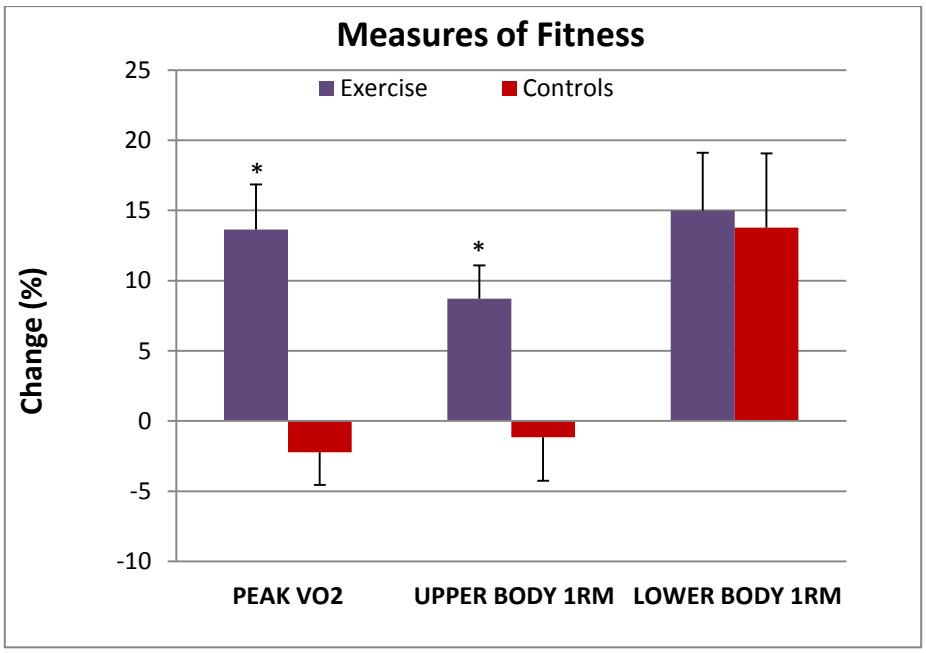

Figure 3. Data are presented as means ±SD of markers of fitness. *P<0.05 significant differences from baseline to 12-wk between the exercise and diet-induced weight loss and control groups.

Biochemical markers of health

Table 12 presents changes in fasting blood lipid and blood glucose values observed at Baseline, 4, 8 and 12 weeks of program participation. Overall MANOVA analysis revealed a non-significant time effect (Wilks' Lambda p=0.23) and a very

significant group x time effect (Wilks' Lambda p=0.04) for blood lipid markers. Univariate analysis revealed significant time effects for low-density lipoprotein cholesterol (p=0.037). No differences for participants in the EX group were observed for triglycerides (EX -6.7±26.4%; C 0.1±24.4%, p=0.37), total cholesterol (EX -3.6±10.0%; C -2.2±10.7%, p=0.65), high density lipoprotein cholesterol (EX 2.5±15.1%; C -5.0±10.5%, p=0.06); low-density lipoprotein cholesterol (EX -4.7±11.5%; C -4.0±16.8%, p=0.87) or blood glucose (EX -0.6±14.5%; C -1.3±8.4%, p=0.847).

Table 12. **Fasting blood lipid and blood glucose values obtained at baseline, 4, 8 and 12 weeks of program participation for the exercise and diet-induced weight loss (EX) and control (C) groups**[a]

Variable	Group	Baseline	4-weeks	8-weeks	12-weeks	Group SEM	p-value
Total Cholesterol (mg/dl)[b]	Exercise	191.3±30.7	178.7±27.9	183.1±32.9	184.2±33.0	184.3±5.6	G= 0.45
	Control	192.9±33.0	192.4±32.4	190.5±31.8	188.2±35.6	191.0±6.7	T=0.08
	Total	192.0±31.4	184.4±30.3	186.2±32.3	185.8±33.8		I= 0.16
HDLc (mg/dl)	Exercise	54.7±13.2	52.4±11.5	54.7±15.6	55.82±14.2	54.4±2.3	G = 0.27
	Control	51.4±10.5	51.7±11.9	49.5±105	49.20±12.1	50.4±2.7	T = 0.68
	Total	53.3±12.1	52.1±11.5	52.6±13.8	53.0±13.7		I = 0.15
LDLc (mg/dl)	Exercise	111.1±22.7	101.7±21.8	103.1±25.2	105.3±22.6	105.3±5.0	G = 0.66
	Control	122.3±50.6	114.9±32.6	116.5±26.6	112.4±30.6	116.5±5.9	T[c]=0 .04
	Total	115.7±36.9	107.2±27.3	108.7±26.4	108.2±26.2		I= 0.59
Triglycerides[h] (mg/dl)	Exercise	127.8±64.6	122.8±60.7	118.3±68.1	114.9±58.7	121.0±9.9	G = 0.52
	Control	139.8±58.4	128.0±43.1	112.4±40.1	133.2±43.9	130.8±11.7	T = 0.09
	Total	132.8±61.7	125.0±53.6	120.0±57.6	122.5±53.3		I= 0.50
Blood glucose (mg/dl)	Exercise	93.5±16.1	94.0±16.7	94.2±14.1	91.5±11.1	93.1±2.0	G = 0.18
	Control	96.9±9.9	98.5±12.5	99.1±9.1	93.3±10.5	97.4±2.4	T = 0.24
	Total	94.9±13.8	95.8±15.1	96.2±12.4	93.1±10.9		I= 0.96

[a]Lipid and glucose data were obtained at 0, 4, 8 and 12 weeks on 29 participants in the EX group and 20 participants in the C group (n=49) completing the 12week weight loss program.
[b]To convert mg/dL cholesterol to mmol/L, multiply mg/dL by 0.026. To convert mmol/L cholesterol to mg/dL, multiply mmol/L by 38.6. Cholesterol of 194 mg/dl=5.04 mmol/L.
[c]p<0.05 difference from baseline and between EX and C groups
G=group alpha level.
[e]T=time alpha level.
I=group x time interaction alpha level.
Group SEM=group standard error of the mean
[h]To convert mg/dL triglyceride to mmol/L, multiply mg/dL by 0.0113. To convert mmol/L triglyceride to mg/dL, multiply mmol/L by 88.6. Triglyceride of 137 mg/dl=1.55 mmol/L.

Results indicate that participation in a structured exercise and diet-induced weight loss program promotes effective weight loss and improvement in some markers of health in support of H_2. Figure 4 illustrates changes from baseline to 12-wk between our exercise and diet-induced weight loss and control groups in changes in markers of health assesses during our 12-wk program. We observed a significant difference from baseline to 12-wk with triglycerides.

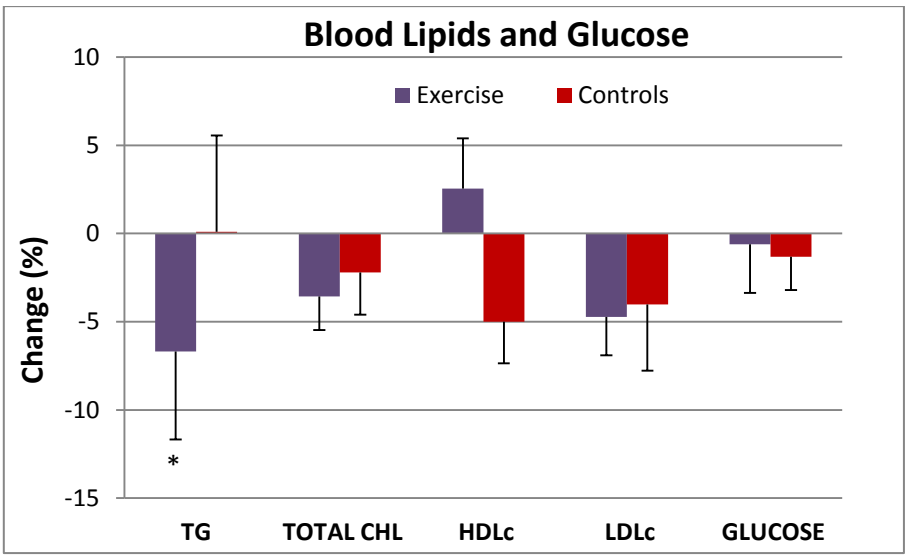

Figure 4. Data are presented as means ±SD of markers of health. *P<0.05 significant differences from baseline to 12-wk between the exercise and diet-induced weight loss and control groups with triglycerides.

Biochemical markers of inflammation and gene expression

Table 13 represents changes in overweight women (48.16±10.5 yr, 45.9±4.4% body fat, BMI 35.6±5.6 kg/m^2) in microRNAs (21 and 146a) and mRNAs of IL-6, TNF-α, (PTEN, TRAF6)/PI3k/AKT/NF-kB genes expression analysis values observed at Baseline and 12 weeks of program participation. MANOVA analysis of genes

expression data revealed a significant overall time (Wilks' Lamda p=0.000), group effect (Wilks' Lambda p=0.000) and time by group effects (Wilks' Lamda p=0.000). MANOVA univariate analysis revealed no significant interactions among groups in changes in microRNA 146a (EX -0.73±2.0; C -0.28±2.1, p=0.46); TRAF6 (EX –1.35±2.7; C -0.74±3.5, p=0.52); mRNA expression levels of PI3K (EX -2.4±4.5; C -1.8±2.9, p=0.66); AKT (EX -1.34±4.2; C -0.67±7.4, p=0.70); or, mRNA NF-kB (EX -1.6±3.2; C -0.73±3.2, p=0.40). Significant interactions were observed among groups in changes in microRNA 21 (EX -1.5±2.34; C 0.13±2.2, p=0.03); mRNA expression level of its target gene PTEN (EX -4.5±3.2; C -1.6±3.4, p=0.005); mRNA IL-6 (EX -2.8±3.6; C 2.8±2.2, p=0.000); and, mRNA TNF-α expression levels (EX -0.52±2.5; C 2.3±1.9, p=0.000). Decreased microRNA (21 and 146a) were positively and significantly correlated to body weight, total weight, fat mass and body fat percent with circulatory levels of IL-6 and TNF-α.

Review of our H_{01} hypothesis initially state that the exercise and diet intervention will not result in a statistically significant differences in mRNA TNF-α expression compared to controls. Based on our TNF-α ($P<0.000$) data observed, we therefore reject our H_{01}. We accepted partially accept our Ho_2 because microRNA-21 ($p<0.03$) was significantly different from baseline and microRNA-146a ($p<0.46$) was not. We had previously hypothesized that there will be no statistically significant differences between groups in changes in mRNA PTEN. We found based on our results that mRNA PTEN was statistically significant ($p< 0.005$). Therefore, we reject our H_{03}. Also we have previously hypothesized that there will be no statistically significant differences between

groups in changes in mRNA TRAF6. We found based on our results that mRNA TRAF6 was not statistically significant ($p< 0.52$). Therefore, we accept our $H_{04.}$ At the beginning of our study we hypothesized that there will be no statistically significant differences between groups in changes in mRNA PI3K.

Table 13. **Changes in microRNAs and mRNAs values obtained at baseline and 12 weeks of program participation for the exercise/diet-induced weight loss (EX) and control (C) groups[a].**

Variable	Group	Baseline	12-weeks	Group SEM	p-value
microRNA21/micro RNAU6B (AU)	Exercise	5.30±2.56[b]	3.84±1.45[b]	4.57±0.29[b]	G[c] = 0.00
	Control	2.43±1.87	2.56±1.27	2.49±0.36	T[d]=0.06
	Total	4.15±2.69	3.33±1.51		I[e]= 0.03
microRNA146a/micro RNAU6B (AU)	Exercise	4.53±2.41	3.79±1.65	4.17±0.29	G = 0.08
	Control	3.52±1.27	3.23±1.22	3.38±0.35	T= 0.10
	Total	4.13±2.07	3.57±1.51		I=0.46
PTEN /GAPDH(AU)	Exercise	7.79±3.10	3.27±1.54[b]	5.53±0.36[b]	G[b] = 0.00
	Control	8.68±2.02	7.09±2.88	7.89±0.44	T[b] = 0.00
	Total	8.15±2.73	4.80±2.86[b]		I[b]= 0.005
TRAF6 /GAPDH (AU)	Exercise	8.45±2.63[b]	7.09±2.72[b]	7.77±0.41[b]	G[b] = 0.00
	Control	10.64±2.60	9.89±2.46	10.27±0.50	T[b] = 0.03
	Total	9.32±2.81	8.22±2.94[b]		I = 0.52
PI3K/ GAPDH (AU)	Exercise	11.23±3.16[b]	8.84±3.13	10.04±0.39[b]	G[b] = 0.008
	Control	9.21±2.85	7.37±1.78	8.29±0.49	T[b] = 0.001
	Total	10.42±3.17	8.26±2.75[b]		I = 0.66
(AKT/PKB)/GAPDH (AU)	Exercise	13.45±2.04	12.10±3.76	12.77±0.61	G[b]=0.028
	Control	15.30±4.87	14.63±6.29	14.97±0.74	T=0.25
	Total	14.19±3.53	13.11±5.03		I=0.69
NF-kB /GAPDH (AU)	Exercise	9.56±2.83[b]	7.98±2.66[b]	8.77±0.41[b]	G[b] = 0.00
	Control	6.31±2.81	5.57±2.33	5.94±0.51	T[b] = 0.023
	Total	8.26±3.22	7.02±2.78[b]		I =0.40
IL-6 /GAPDH (AU)	Exercise	7.18±2.87[b]	4.40±2.43[b]	5.79±0.37	G = 0.11
	Control	3.42±1.59	6.22±2.75	4.82±0.46	T = 0.98
	Total	5.68±3.05	5.13±2.69		I [b]= 0.00
TNF-α/GAPDH (AU)	Exercise	7.40±2.05	6.88±2.05[b]	7.14±0.28[b]	G [b]= 0.03
	Control	5.01±1.90	7.29±0.82	6.15±0.34	T[b] = 0.02
	Total	6.44±2.30	7.04±1.67[b]		I[b] = 0.00

[a]Data are from 29 participants in the EX group and 20 participants in the C group (N=49) who completed the 12-week study.
[b]$p<0.05$ difference from baseline and between EX and C groups
Group SEM=group standard error of the mean
[c]G=group alpha level
[d]T=time alpha level
[e]I=group x time interaction alpha level
AU= arbitrary units

We found based on our results that mRNA PI3K was not statistically significant ($p<0.66$). This led us to acceptance of our H_{05}. We again hypothesized earlier that there will be no statistically significant differences between groups in changes in mRNA AKT. We also found based on our results that mRNA AKT was not statistically significant ($p< 0.70$). We therefore accept H_{06}. We initially that, there will be no statistically significant differences between groups in changes in mRNA NF-kB. We found based on our results that mRNA NF-kB was not statistically significant ($p< 0.40$). Therefore, we accept our H_{07}.

Based on our results data, review of mRNAs of IL-6 and TNF-a genes expression analysis data resulted in acceptance of H_3 ($p<0.000$). We have previously stated that the exercise and diet intervention will promote a statistically significant difference in mRNA IL-6 (H_3) and mRNA TNF-α (H_4). Also based on our result data we therefore accept of H_4 ($p<0.000$) and reject our H_{01} in which we stated that the exercise and diet intervention will not result in statistically significant differences in mRNA TNF-α expression compared to control. Figures 5a and 5b presents changes from baseline to 12-wk between our exercise and diet-induced weight loss and control group on markers of inflammation.

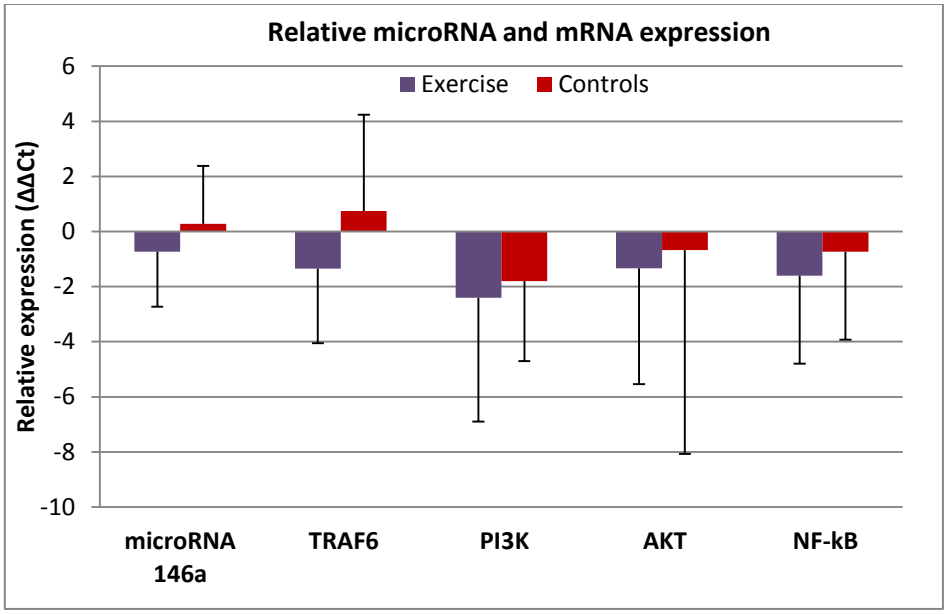

Figure 5a. Data are presented as means ±SD of markers of inflammation from baseline to 12-wk between the exercise and diet-induced weight loss and control groups.

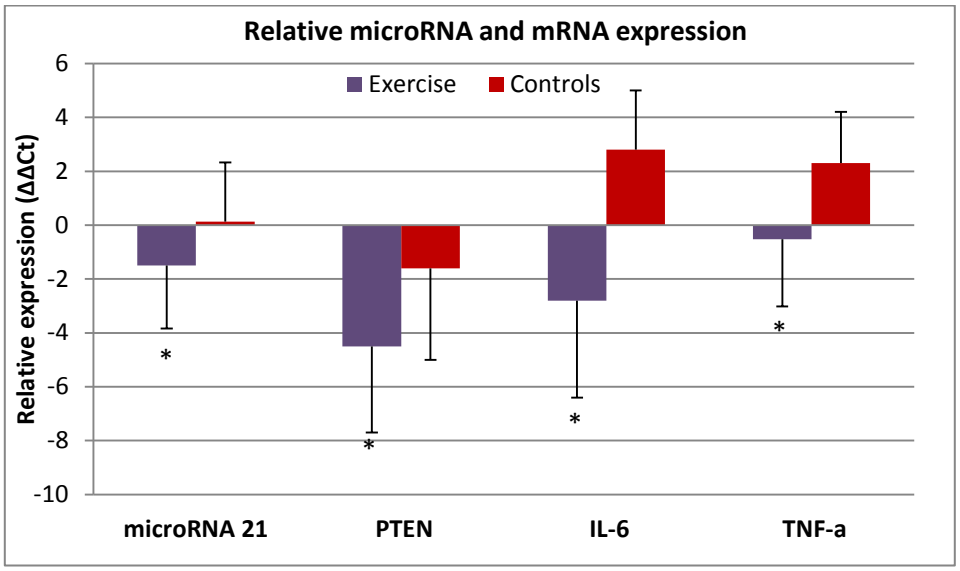

Figure 5b. Data are presented as means ±SD of markers of inflammation and microRNA 21.
*P<0.05 significant differences from baseline to 12-wk between the exercise and diet-induced weight loss and control groups.

CHAPTER V

DISCUSSION AND CONCLUSION

Obesity has been on the rise for decades especially with increasing rise in fast food restaurants, food processing industries, over nutrition and most of all the high rate of physical inactivity around the world. Obesity has been associated with increases in markers of inflammation (144). Intense exercise increases markers of inflammation (68, 122). However, chronic exercise and diet-induced weight loss has been suggested to decrease makers of inflammation in obese populations (124, 154, 155, 157). Additional research is needed to examine gene expression and molecular changes related to weight loss.

Our investigation looked at the effects of exercise and diet-induced weight loss in overweight/obese women on the characterization of serum/plasma microRNAs and cytokine gene transcription on body composition and markers of health and fitness. We also looked at microRNA-21, microRNA-146a and the mRNAs of IL-6, TNF-α, (PTEN, TRAF6)/PI3k/AKT/NF-kB genes expression analysis in previously sedentary obese women. Some studies have investigated the efficacy of exercise and diet-induced weight loss on markers of fitness and inflammation but not connected to microRNAs and their regulatory role with respect to exercise and diet-induced weight loss.

In this study, forty-nine obese women were assigned to an exercise and diet group or a non-exercise and non-diet control group. No significant differences were observed between groups in baseline age, height, weight, BMI, or percent body fat as determined by ANOVA. Results revealed that participants in the exercise and diet

intervention group experienced significant reductions in body weight and fat mass with no changes observed in FFM in comparison to controls. Participants also improved their fitness, some markers of health, and experienced a significant reduction in some markers of inflammation. These finding support previous research that has shown that exercise and/or diet induced weight loss can reduce markers of inflammation in obese individuals.

While these findings are not novel, our investigation extended previous research by examining the effects of exercise and diet-induced weight loss on microRNAs (21 and 146a) and mRNAs of IL-6, TNF-α, (PTEN, TRAF6)/PI3k/AKT/NF-kB genes expression (see Figure 6). We found that exercise and diet-induced weight loss resulted in an overall effect on these markers with more influence on some markers than others. More specifically, we found no significant interaction effects among groups in changes in pathway #2 microRNA 146a (EX -0.73±2.0; C -0.28±2.1, p=0.46); TRAF6 (EX – 1.35±2.7; C -0.74±3.5, p=0.52); mRNA expression levels of PI3K (EX -2.4±4.5; C -1.8±2.9, p=0.66); AKT (EX -1.34±4.2; C -0.67±7.4, p=0.70); or, mRNA NF-kB (EX -1.6±3.2; C -0.73±3.2, p=0.40). However, significant interactions were observed among groups in changes in pathway #1 microRNA 21 (EX -1.5±2.34; C 0.13±2.2, p=0.03); mRNA expression level of its target gene PTEN (EX -4.5±3.2; C -1.6±3.4, p=0.005); mRNA IL-6 (EX -2.8±3.6; C 2.8±2.2, p=0.000); and, mRNA TNF-α expression levels (EX -0.52±2.5; C 2.3±1.9, p=0.000). Decreased microRNA (21 and 146a) were positively and significantly correlated to body weight, total weight, fat mass and body fat percent with circulatory levels of IL-6 and TNF-α (175). Many investigations looking at

the effects of exercise on gene expression in human peripheral blood mononuclear cells, on microRNAs observed significant effects demonstrating the relationships between NF-kB and how this transcription factor regulates many genes involved in inflammation (8, 27, 90, 133, 138, 146, 151, 153, 168).

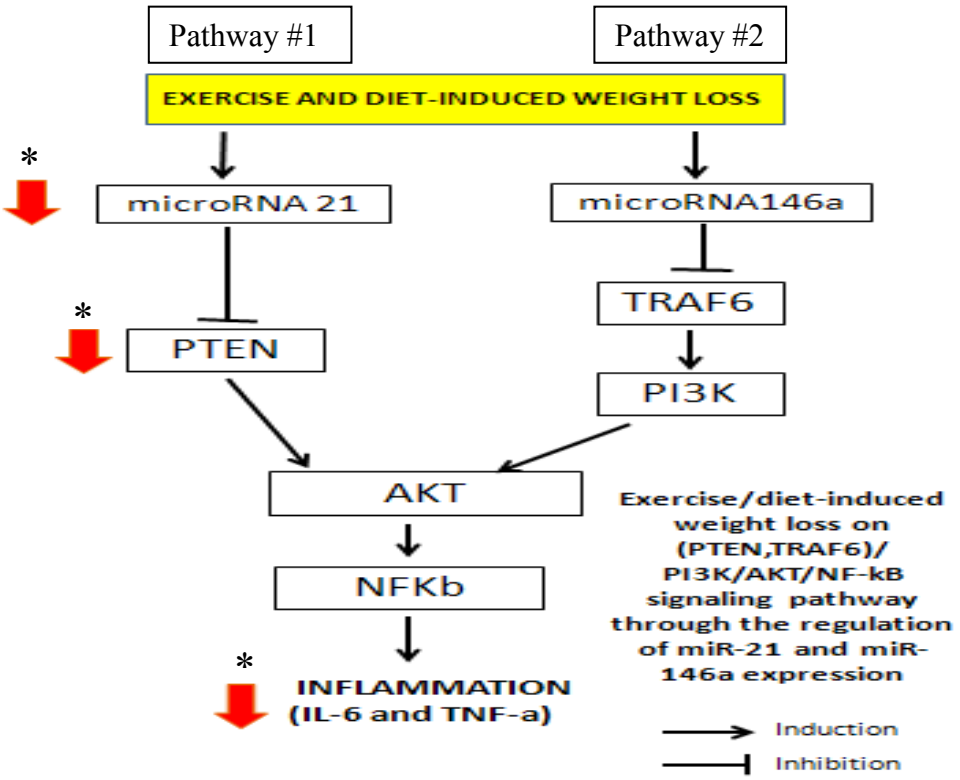

Figure 6: The diagram represents significant reductions and changes from baseline after 12 weeks. Figure illustrates gene levels from blood samples collected from 49 obese women (BMI 35.6±5.6 kg/m^2; 45.9±4.4 % body fat) analyzed using real time-PCR. * $P < 0.05$ significant effect of the exercise and diet-induced weight loss intervention.

Our main finding showed that microRNA-21, PTEN, IL-6 and TNF-α decreased significantly during our exercise and diet-induced weight loss program. Our study

showed that IL-6 and TNF-α were significantly reduced through our exercise program on obese women with BMI 35.6±5.6 kg/m^2. In a study conducted by Nicklas et al (102) looking at the effects of exercise training (lower body resistance training with weighted cuff 3 times/wk and walking at 50 to 70% heart rate reserve for 18 months) in obese individuals on IL-6 and TNF-α levels, showed that IL-6 and TNF-α decreased significant by 11% and 2% respectively. Their results agrees to our results obtained during our exercise and diet-induced weight loss study in which we showed that IL-6 and TNF-a decreased significant during our 12 wk study program. Another study conducted by Tsukui et al (160) confirm our results obtained on IL-6 and TNF-α. They conducted a 5 month training intervention program, 30-45 min/d/wk, 4-5 d/wk at 40-50% VO_{2max}. Their result demonstrated that their exercise program intervention significantly decreases body weight, percent body and TNF-α from 9.0 to 1.5 pg/ml. Interestingly, a study conducted by Lambert et al (84) evaluated the effect of exercise (12 wk aerobic 30-40 min and resistance 30-40 min, 3 d/wk at 75% peak heart rate) but not diet-induced weight loss decreases inflammation gene expression in frail obese persons. They reported that exercise resulted in a significant decrease in IL-6 and TNF-α by 50% and 50% respectively whereas weight loss had no significant effect on IL-6 and TNF-α expression.

In our study, we found a significant decrease in IL-6 and TNF-α. Our results agree with observation as observed by Lambert et al (84) in their study program that lasted 12 wk (84). Bruun et al (15) also reported a similar effect that a combination of exercise and caloric restriction led to a reduction in IL-6. Gielen et al (53) also reported

that aerobic exercise training at about 70% VO_{2max} for 20 min/d for 60 min/wk also significantly reduced IL-6 levels by 42%. Important we have shown that combining exercise and diet significantly reduced Il-6 and TNF-α with other inflammatory target genes. Mingrone et al (99) reported that a 23.6% decrease in body weight via gastric bypass resulted in an 82% decrease in TNF-α mRNA. All these studies confirm with our investigation that a decrease in body weight caused by exercise and diet led to a reduction in microRNAs and inflammatory cytokines. A reduction in body weight (fat mass) led a reduction in pro-inflammation level which led to an improvement in immune, hormonal and metabolism function.

Our results suggest (IL-6 and TNF-α) in circulation may be involved in lipolysis (56, 136) and may promote physiological changes directly activate linked to NF-kB which has a direct effect on reducing inflammation (17, 18, 137). Each of these microRNA (21 and 146a) and mRNAs of IL-6, TNF-α, (PTEN, TRAF6)/PI3k/AKT/NF-kB contributed to our inflammation response (90, 151-153, 161, 165, 168). The significant expression changes observed on inflammatory cytokines (IL-6 and TNF-α) (16, 93, 181) in our study program provides sufficient evidence to conclude that these observations are due to our exercise and diet-induced weight loss program and that exercise training protects against low-grade systemic inflammation link to obesity.

Initially, we proposed two mechanisms that could be affected by exercise and diet-induced weight loss. The first pathway (pathway #1) microRNA-21, and PTEN/AKT/NF-kB and IL-6, TNF-α and our second pathway (pathway #2) microRNA-146a and TRAF6/PI3K/AKT/NF-kB and IL-6 and TNF-α. We proposed both pathways

could be affect by our exercise and diet-induced weight loss program. Our result data indicate the pathway (pathway #1) with microRNA-21 (p=0.03) and PTEN (p=0.005) /AKT(p=0.70) /NF-kB (p=0.40) and IL-6 (p=0.00) and TNF-α (p=0.00) produced a significant change resulting in the reduction of inflammation than the pathway #2 with microRNA-146a (p=0.46) and TRAF6 (p=0.52) /PI3K (p=0.66) /AKT (p=0.70) /NF-kB (p=0.40) which produced some changes but not significant changes and shown on table 14 above. Our findings on microRNA-21, mRNA PTEN, mRNA TNF-α and mRNA IL-6 (pathway #1) led us to suggest that our exercise and diet intervention program promoted a reduction in inflammation and that microRNA-21 played a significant role in this biological process and greatly helped to be identified as a new intervention and therapeutic target in the obese population

In general, our results indicate that exercise and diet-induced weight loss affects molecular changes in circulating microRNAs, significantly affects microRNA-21 and its target gene PTEN, mRNA TNF-α, and mRNA IL-6 levels suggesting an anti-inflammatory response compared to a control group. Our result was in conformity with other research studies done on microRNA-21 and its target gen PTEN with significant changes with exercise on inflammation (8, 87, 88). These findings suggest that exercise and diet-induced weight loss is significantly relevant reducing inflammation caused by obesity. Our findings on microRNA-21, mRNA PTEN, mRNA TNF-α and mRNA IL-6 (pathway #1) led us to suggest that our exercise and diet intervention program promoted a reduction in inflammation and that microRNA-21 played a significant role in this

biological process and greatly helped to be identified as a new intervention and therapeutic target in the obese population.

The microRNA-21 has been shown to be involved in promoting inflammation; its overexpression enhances the inflammatory response by increasing the expression of other molecules related to inflammation and also reduces potent human anti-inflammatory molecules (98). Also Olivieri et al (109) recently identified increasing circulating levels of microRNA-21 as a biomarker in inflammation. Keller et al (70) also showed that the microRNA-21 is robustly expressed in human adipose tissue and positively correlated to BMI. On the contrary our exercise and diet-induced weight loss program showed significantly reduction in microRNA-21 with its target gene PTEN and significant reductions in inflammatory cytokines of IL-6 and TNF-α, suggesting that microRNA-21 is linked to obesity and inflammation and could be a potential biomarker or therapeutic means in obesity treatment. These microRNAs modulate the NF-kB pathway related to cellular inflammation regulating several functions relevant to exercise.

Moderate weight loss has been associated with improved health related quality of life (49, 141). Dekker et al (36) conducted a study to look at the effects of exercise and diet intervention on fasting plasma cytokine on subject who were required to participate in a 12 wk aerobic exercise program for 5 min/wk at 60% VO_{2max} and followed a diet of 55-60% carbohydrate, 15-20% proteins and 20-25% fat designed to maintain body weight after a weight loss program. They reported that plasma IL-6 was significantly reduced by 52% in type 2 diabetic followed by 32% and 17% in lean and obese subjects

respectively. This study also confirms with our exercise program that resulted in improved physical activity, body composition, markers of health and fitness and significant reduction in inflammation and genes related to inflammation (130, 132). However, more research is needed to understand microRNA regulation associated with inflammation in response to exercise and diet-induced weight loss.

There are several limitations in conducting an exercise and diet-induced weight loss clinical trials that should be taken into account when interpreting the results of this investigation. First, the participants who volunteered to participate in our exercise and diet-induced weight loss trails were often more motivated to adhere to weight loss programs than the general population or control group. Secondly, the study participants are continually monitored and encouraged to meet program requirements. Thirdly, the results observed in our trial are limited to the population studied, sedentary and overweight/obese women, and may not be applicable to other populations. Other potential limitations to our investigation were that changes in mRNAs and microRNAs expression in blood samples during exercise still needs to be confirmed by protein expression and also the difficulty in isolating RNAs from blood samples. Although our finding on the effects of exercise and diet-induced weight loss on microRNAs and inflammatory cytokines are novel, they should be considered preliminary as this is the first study to investigate the effects of exercise and diet-induced weight loss on microRNA (21 and 146a) and inflammatory cytokine using blood samples collected from sedentary obese women compared to a control only group. Finally, results are

limited to the inherent difficulties in conducting a clinical trial of this nature, compliance to diet and/or exercise protocols, and the accuracy in data collection and analysis.

Conclusion

In our investigation, participants adhering to an exercise and diet intervention designed to promote fat loss experienced clinically significant weight loss, improvements in cardiovascular endurance, positive changes in some markers of health and fitness. We also observed a significant decrease in microRNA-21 that was positively and significantly correlated to changes in body weight, fat mass and body fat percent with significant reduction in circulatory levels of IL-6 and TNF-α. More specifically, we report that exercise and diet-induced weight loss significantly affects changes on microRNA-21 levels, some mRNAs related to inflammation (IL-6 and TNF-α) on our signaling pathway # 1. This could have important implications into the treatment and/or management of obesity treatment because our exercise and diet-induced weight loss program significant reduced markers of inflammation related to obesity in previously sedentary obese women. We therefore conclude that regular exercise training or chronic exercise adaptation reduces inflammation. Future research should evaluate the effect of chronic and acute exercise training on specific microRNAs related to inflammation in obese and insulin resistance individuals as well as the effects of different types of diet and exercise on microRNAs linked to inflammation, obesity, and weight loss.

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APPENDIX A

CONSENT FORM

The effects of the Curves 90-Day Fitness Challenge on health outcomes in women

Introduction
The purpose of this form is to provide you information that may affect your decision as to whether or not to participate in this research study. If you decide to participate in this study, this form will also be used to record your consent.

You have been asked to participate in a research project comparing the Curves International fitness and weight loss program to other popular weight loss programs. The purpose of this study is to determine the effects of the new Curves 90-Day Fitness Challenge on health outcomes in women. You were selected to be a possible participant because you met all entrance criteria for this study. This study is being sponsored/funded by Curves International.

What will I be asked to do?
If you agree to participate in this study, you will first be asked to sign an Informed Consent statement in compliance with the Human Subject's Protection Program (HSPP) at Texas A&M University and the American College of Sports Medicine. You will then be familiarized to the study requirements, food log recording and tests to be conducted during the study. This session will take approximately one hour to complete. Prior to reporting to the lab for baseline testing, you will record all food that you eat on dietary record forms for four days (including one weekend day). You will not exercise for 48 hours nor eat for 12 hours prior to reporting to the lab for baseline testing. You will then undergo a battery of tests as described in Table 1. You will fill out a Demographic Form, a Health History Form, A Radiation Safety Form, a Quality of Life Questionnaire, a Body Image Questionnaire and an eating Satisfaction Questionnaire. You will also be required to report any adverse side effects that you may experience on a weekly basis. .

You will then continue with the tests as described in Table 1. You will first be weighed and have your resting energy expenditure (REE) determined. This will involve lying down on an exam table and having a light blanket placed over you to keep you warm and placing ear plugs in your ears to reduce distractions. A see through plastic canopy will then be placed over your neck and head so that the air that you breathe can be measured for oxygen and carbon dioxide. You should stay motionless without going to sleep for 15-minutes so that your resting energy expenditure can be calculated. You will then donate up to approximately 30 milliliters (6 teaspoons) of venous blood from a vein in your arm. Blood samples will be obtained by standard/sterile procedures using a needle inserted into a vein in your arm and will later be analyzed. Personnel who will be taking your blood are experienced in phlebotomy (procedures to take blood samples) and are qualified to do so under guidelines established by the Texas Department of Health and Human Services. This will take about 5-minutes. You will then have your total body water determined using a bioelectrical impedance analyzer (BIA). The BIA analysis will involve lying down on your back on a table and having two small electrodes placed on your right hand and your right foot. The analyzer wires will be attached and a small and safe current (500 micro-amps at a frequency of 5- kHz) will pass through your body so that the amount of water can be measured. This analyzer is commercially available and has been used in the health care/fitness industry as a means to assess body composition and body water for over 20 years. The use of this device has been approved by the Food and Drug Administration (FDA) to assess total body water and the current to be used has been deemed safe. Your body composition and bone density will then be determined by using a Discovery W dual energy x-ray absorptiometer (DEXA). This will involve lying down on your back on the DEXA exam table in a pair of

shorts or a gown for about 6 minutes. A low dose of radiation will scan your entire body to determine the amount of fat weight, muscle weight, and bone weight. You will be exposed to an x-ray dose that is similar to the amount of natural background radiation a person would receive in one month while living in College Station. After this test, you will have resting blood pressure determined using a blood pressure cuff and stethoscope and heart rate determined by taking your pulse. You will then be prepared to perform a maximal treadmill test. You will have your right and left shoulder, right and left part of your stomach, and several places around your upper chest and below your bra line rubbed with alcohol gauze. Ten (10) electrocardiograph (ECG) electrodes will then be placed on your shoulders, chest, and stomach and you will be attached to an ECG to evaluate your heart. You will then be positioned on the treadmill and a sterile mouthpiece will be placed in your mouth and a mouthpiece holder will be placed on your head. A nose clip will be placed on your nose and that the air you breathe will be measured for oxygen and carbon dioxide content. Once the equipment is attached, you will be given instructions to begin walking on the treadmill. You will then perform an exercise test that involves increasing the speed and grade you are walking on the treadmill until you reach your maximal effort. Heart rate, ECG tracings, blood pressure and your ratings of exertion will be monitored throughout the test. Once you reach your maximum, you will undergo a slow walking and seated recovery period. This test will take about 30 minutes to complete. You will then perform a one repetition maximum (1RM) and 80% of 1RM endurance repetition test on the bench press and hip/leg sled using standard procedures. This will involve warming up and performing successive one repetition lifts on the bench press until you determine your 1 RM. You will then rest for 5-minutes and lift 80% of your 1 RM as many times as you can. You will then rest for 10-minutes and follow the same procedure in determining your 1 RM and 80% of 1 RM on the hip/leg sled. These tests will take about 20 minutes to complete. The same battery of tests will be performed at the post-study assessment 16 weeks into the study protocol. All the assessments minus the exercise tests will also be performed at 4, 6 10 and 12 weeks into the study protocol. Each testing session will take between 1.5 and 3 hours to complete. In the event of an emergency during an exercise test proper emergency response protocols (calling 9-911 for serious injury or a medical emergency, calling Biosafety/EHS for cleanup assistance or spill team response, calling UPD for incidents in public areas, retrieving AED located in the lab, performing CPR or other First Aid techniques, etc.) will be followed by the Exercise & Sport Nutrition Laboratory (ESNL) Staff depending on the severity of the emergency.

After baseline testing, you will be matched based on age, BMI, activity level and eating habits and randomized into one of six intervention groups as described in Table 2. The Operating Systems (OS) Questionnaire (or Initial Assessment) will be used to help determine the group assignments. This will include a high protein/low fat diet group (30% CHO, 45% PRO, 25% FAT, N=40), a high carbohydrate/low fat diet group (45% CHO, 30% PRO, 25% FAT, N=40), a Weight Watchers diet group (N=40), a Jenny Craig diet group (N=40), a Nutrisystem diet group (N=40) and a control group (N=40). If you are randomized into one of the first two groups (N=80 total) you will diet for 7 days at 1,200 kcals/day and then 1,500 kcals/day for the remaining 11 weeks of the study. If you are in one of the first two groups you will meet weekly one on one with your weight loss coach for the duration of the study for weekly weigh-ins. Each meeting will take place in the ESNL and will last approximately 15 minutes. The coach will guide you through each phase of the program, assist with meal planning, assist with goal setting and provide accountability and encouragement in order to meet your fitness and nutrition goals. If you are randomized into the third group (N=40) you will follow the Weight Watchers Momentum Program that is based on their four pillar approach (food, exercise, behavior and support). Every food has a POINTS value, based on its calories, fat and fiber. The Momentum program uses POINTS values to help keep track of what you eat. A POINTS "budget" will be personalized for you at the weekly meetings. You will be required to attend at least one meeting per week at the local Weight Watchers facility located at 4001 E. 29th Street, Suite 112 in the Carter Creek Center in Bryan, Texas. Membership dues/passes to the Weight Watchers program/facility will be covered for you during the duration of the study. If you are randomized into groups four or five (N=80 total), you will follow the dietary guidelines set forth by those respective plans. The sixth group (N=40) will act as a control group. If you are randomized into this group you will not follow a prescribed nutrition program but will continue with your normal daily habits. Everyone,

regardless of group assignment, will keep a food record and food frequency log to monitor dietary compliance.

If you are randomized to participate in the first two groups (N=80 total) you will participate in the Curves 30-minute fitness program three times per week throughout the investigation. The Curves program involves performing thirteen hydraulic resistance exercise machines that utilize bidirectional resistance that work all major muscle groups. These are interspersed with floor-based calisthenics exercises designed to maintain an elevated heart rate. Research has shown that exercise intensity averages 65% of maximal aerobic capacity and that participants generally perform 50 – 75% of 1 repetition maximum on the main exercise machines. The new Curves equipment includes the attached force measurement and feedback system. You will be instructed to push hard enough to generate a green light on the feedback panel for each repetition. You will be instructed to wear heart rate monitors (HR) to access exercise intensity. All exercise sessions will be held in the ESNL. Research Assistants will monitor your exercise sessions and record your attendance. You will also be given a pedometer and will be recording the number of steps taken each day. On the days you do not use the Curves equipment, you will be encouraged to walk for 30-minutes at a brisk pace (60 – 80% of heart rate reserve). If you are randomized to participate in third group (N=40) you will start by focusing on the food plan and then incorporate the specifics of activity a week alter once you have had the chance to get conformable with the eating plan. After a week of reducing sedentary behavior, the POINTS Activity System is introduced. In a way that complements the POINTS values of food, a formula that calculates the POINTS values for activity is used. The formula is based on body weight, the amount of time the activity is done, and the level of intensity. This method enables you to do any exercise or activity that is enjoyable and fits within your lifestyle. If you are assigned to groups four, five or six (N=120 total) you will not follow a prescribed exercise program but will continue your normal daily habits. Everyone, regardless of group assignment, will be required to complete activity logs to monitor exercise frequency and intensity.

Please do your best to: 1) follow the instructions outline by the investigators; 2) show up to all scheduled testing and train ing sessions; and 3) follow the diet prescribed and do not take any other nutritional supplements or performance enhancing aids during this study (i.e., vitamins/minerals, creatine, HMB, androstenedione, DHEA, etc). In addition, please do not take any non-medically prescribed medications and report any medication that is prescribed for you to take during this study. If you take any other nutritional supplements or medications during the course of the study that may affect vitamin/mineral status, body composition, or strength you may be removed from the study.

What are the risks involved in this study?
The risks associated with this study are: You will be exposed to a low level of radiation during the DEXA body composition tests, which is similar to the amount of natural background radiation you would receive in one month while living in College Station. In addition, a very low level of electrical current will be passed through your body using a bioelectrical impedance analyzer (BIA). This analyzer is commercially available and has been used in the health care/fitness industry as a means to assess body composition and body water for over 20 years. The use of the BIA and DEXA analyzers have been shown to be safe methods of assessing body composition and total body water and are approved by the FDA. You will donate about 6 teaspoons (30 milliliters) of venous blood four (4) times during the study using standard phlebotomy procedures. This procedure may cause a small amount of pain when the needle is inserted into the vein as well as some bleeding and bruising. You may also experience some dizziness, nausea, and/or faint if you are unaccustomed to having blood drawn. The exercise tests that will be performed may cause symptoms of fatigue, shortness of breath, and/or muscular fatigue/discomfort. The exercise tests may also cause short-term muscle soreness and moderate fatigue for several days following the tests. You may also experience muscle strains/pulls during the exercise testing and/or training program. However, exercise sessions will be conducted by trained personnel and monitored to ensure you follow appropriate exercise guidelines. You will follow a prescribed dietary regimen involving consuming 1,200 or 1,500 calories per day during various phases of the program. In addition, one group will ingest a high percentage of calories in the form of protein. Although the total amount of total protein is not excessive

(100-220 grams/day or 1.1 - 2.3 grams/kg/day for a 95 kg female) it may be higher than you are accustomed to ingesting and may exceed recommended protein intake for active individuals (i.e., 1-2 grams/kg/day). As a result, you may experience weight loss or gain, feelings of hunger or fullness, and/or changes in appetite and/or mood during various phases of the dietary intervention.

What are the possible benefits of this study?
The possible benefit you may receive from participation in this study is increased physical fitness and improvements in body composition. You may also gain insight about your health and fitness status from the assessments that will be performed.

Do I have to participate?
No. Your participation is voluntary. You may decide not to participate or to withdraw at any time without your current or future relations with Texas A&M University being affected.

Will I be compensated?
You will receive $125 (i.e., $25 for each familiarization and experimental session) upon completion of the study. Disbursement will occur upon completion of all sessions and after all study related materials (food logs, training logs, etc.) are turned in. Those who do not complete the study will be compensated on a pro-rated basis depending on the total number of sessions completed (i.e. a participant who attends the familiarization session and completes only one experimental session is able to receive $50).

Who will know about my participation in this research study?
The records of this study will be kept private. No identifiers linking you to this study will be included in any sort of report that might be published. Research records will be stored securely and only Mr. Christopher Rasmussen and Dr. Richard Kreider will have access to the records.

Whom do I contact with questions about the research?
If you have questions regarding this study, you may contact Dr. Richard Kreider, 945-1333, rkreider@hlkn.tamu.edu or Mr. Christopher Rasmussen, 458-1741, crasmussen@hlkn.tamu.edu.

Whom do I contact about my rights as a research participant?
This research study has been reviewed by the Human Subjects' Protection Program and/or the Institutional Review Board at Texas A&M University. For research-related problems or questions regarding your rights as a research participant, you can contact these offices at (979)458-4067 or irb@tamu.edu.

Signature
Please be sure you have read the above information, asked questions and received answers to your satisfaction. You will be given a copy of the consent form for your records. By signing this document, you consent to participate in this study.

Signature of Participant: _____ Date: _____

Printed Name: _____

Signature of Person Obtaining Consent: _____Date: _____

Printed Name: _____

IRB-Protocol number **2010-0813**

TEXAS A&M UNIVERSITY
DIVISION OF RESEARCH AND GRADUATE STUDIES - OFFICE OF RESEARCH COMPLIANCE

979.458.1467
FAX 979.862.3176
http://researchcompliance.tamu.edu

Human Subjects Protection Program | Institutional Review Board

DATE: 29-Apr-2011

MEMORANDUM

TO: KREIDER, RICHARD B.
77843-4243

FROM: Office of Research Compliance
Institutional Review Board

SUBJECT: Amendment

Protocol Number:	2010-0813
Title:	The effects of the Curves 90-Day Fitness Challenge on health outcomes in women
Review Category:	Full Board
Approval Period:	29-Apr-2011 **To** 08-Feb-2012

Approval determination was based on the following Code of Federal Regulations:
45 CFR 46 Subpart A.

Provisions: Added 5 ml to blood draw for additional cytokine measures during scheduled blood draws.

This research project has been approved for one (1) year. As principal investigator,you assume the following responsibilities

1. **Continuing Review:** The protocol must be renewed each year in order to continue with the research project. A Continuing Review along with required documents must be submitted 30 days before the end of the approval period. Failure to do so may result in processing delays and/or non-renewal.
2. **Completion Report:** Upon completion of the research project (including data analysis and final written papers), a Completion Report must be submitted to the IRB Office.
3. **Adverse Events:** Adverse events must be reported to the IRB Office immediately.
4. **Amendments:** Changes to the protocol must be requested by submitting an Amendment to the IRB Office for review. The Amendment must be approved by the IRB before being implemented.
5. **Informed Consent:** Information must be presented to enable persons to voluntarily decide whether or not to participate in the research project.

This electronic document provides notification of the review results by the Institutional Review Board.

APPENDIX B

MEDICAL HISTORY QUESTIONNAIRE

Texas A&M UNIVERSITY

EXERCISE & SPORT NUTRITION LABORATORY

Medical History Inventory

Directions. The purpose of this questionnaire is to enable the staff of the Exercise and Sport Sciences Laboratory to evaluate your health and fitness status. Please answer the following questions to the best of your knowledge. All information given is **CONFIDENTIAL** as described in the **Informed Consent Statement.**

Name:_____ Age _____
Date of Birth_____

Name and Address of Your Physician:_____
_____

MEDICAL HISTORY

Do you have or have you ever had any of the following conditions? (Please write the date when you had the condition in the blank).

____ Heart murmur, clicks, other cardiac findings?
____ Frequent extra, skipped, or rapid heartbeats?
____ Chest Pain of Angina (with or without exertion)?
____ High cholesterol
____ Heart attack or any cardiac surgery?
____ Leg cramps (during exercise)?
____ Chronic swollen ankles?
____ Varicose veins?
____ Frequent dizziness/fainting?
____ Muscle or joint problems?
____ High blood sugar/diabetes?
____ Thyroid Disease?
____ Low testosterone/hypogonadism?
____ Glaucoma?
____ Asthma/breathing difficulty?
____ Bronchitis/Chest Cold?
____ Cancer, Melanoma, or Suspected Skin Lesions
____ Emphysema/lung disease
____ Epilepsy/seizures?
___ Rheumatic fever?
____ Scarlet fever?
____ Ulcers?
____ Pneumonia?
____ Anemias?
____ Liver or kidney disease
____ Autoimmune disease?
____ Nerve disease?
____ Psychological Disorders?

Do you have or have you been diagnosed with any other medical condition not listed?
_____
_____

Please provide any additional comments/explanations of your current or past medical history.
_____
_____

Please list any recent surgery (i.e., type, dates etc.).

_____
_____

List all prescribed/non-prescription medications and nutritional supplements you have taken in the last 3 months.

_____
_____

What was the date of your last complete medical exam?

_____
_____

Do you know of any medical problem that might make it dangerous or unwise for you to participate in this study? (including strength and maximal exercise tests) ____ If yes, please expain._____
_____

Recommendation for Participation (for ESNL use only):

____ No exclusion criteria presented. Subject is ***cleared*** to participate in the study.
____ Exclusion criteria is/are present. Subject is ***not cleared*** to participate in the study.

Signed: _____ Date: _____

APPENDIX C

PERSONAL INFORMATION WORKSHEET

Texas A&M University

EXERCISE & SPORT NUTRITION LABORATORY

Personal Information

Name: _____

Address: _____

City: _____ State: _____ Zip Code _____

Home Phone: (___) _____ Work Phone: (___) _____

Beeper: (___) _____ Cell Phone: (___) _____

Fax: (___) _____ E-mail address: _____

Birth date: _____ / _____ / _____ Age: _____ Height: _____Weight: _____

Exercise History/Activity Questionnaire

1. Describe your typical occupational activities.

2. Describe your typical recreational activities

3. Describe any exercise training that you routinely participate.

4. How many days per week do you exercise/participate in these activities?

5. How many hours per week do you train?

6. How long (years/months) have you been consistently training?

APPENDIX D

DATA COLLECTION FORM

Texas A&M University: Exercise & Sport Nutrition Laboratory

Trial: Effects of exercise and diet-induced weight loss on overweight women on characterization of serum/plasma microRNAs and cytokine gene transcription

Demographics ESNL Staff Initials: _____

Name: _____ Testing Session: _____ Group: _____
Date: _____ D.O.B.: _____ Age: _____

Resting Measures ESNL Staff Initials: _____

Psychological Questionnaires/Informed Consent:

HLKN Informed consent: _____
Radiation consent: _____
Activity Log: _____
Food Log: _____
Post-Study Questionnaire (T4 only): _____

Physiological Parameters: ESNL Staff Initials: _____

Height: _____ in.
Weight: _____ lb.
REE #1: _____
Time: _____am
Last Meal: _____am/pm
Hrs Fasted: _____hr.
Last Wkout: _____ DEXA #2: _____
Lab (EBNL): _____ (3) SST Tubes/ (1) EDTA ECG (Rest): _____ #1 or #2
Time: _____am Max Test: _____ #1 or #2
Yr. /Menopause _____ Notes: _____

Exercise Measures: Strength Testing: ESNL Staff Initials: ____

Leg Press: Foot Position: _____ Sled Position: _____

Preceding Weights/Reps: _____x_____: _____x_____: _____x_____: _____x_____: _____x_____
1 RM: _____ 80% 1RM: _____ 80% 1RM repetitions: _____

Bench Press: Hand Position: _____

Preceding Weights/Reps: _____x_____: _____x_____: _____x_____: _____x_____: _____x_____
1 RM: _____ 80% 1RM: _____ 80% 1RM repetitions: ____