EFFECTS OF EXERCISE AND DIET-INDUCED WEIGHT LOSS IN SEDENTARY OBESE WOMEN ON INFLAMMATORY MARKERS, RESISTIN AND VISFATIN

A Dissertation

by

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ABSTRACT

Resistin and visfatin are secreted by adipose tissue and are potential regulators of inflammation and insulin sensitivity. This study examined the effects of exercise and diet-induced weight loss on resistin and visfatin. Twenty six sedentary obese women were randomly assigned into control group (C) or an exercise plus diet group (DE) that involved circuit resistance-exercise (4 d/week) with walking (10,000 steps/d, 3 d/week) while consuming 1,200 kcal/day for 1 week and 1,500 kcal/d diet for 11 weeks consisting of 45:30 % Protein:Carbohydrate. Body composition and fasting blood samples were obtained and analyzed by MANOVA and Pearson correlation analysis. Data are presented as mean±SD changes from baseline. Participants in the DE group lost more weight (DE: -5.9±4.0; C: 0.64±1.4 kg, $p<0.001$) and fat (DE: -5.1±4.5; C: 0.4±1.5 kg, $p=0.001$). Significant differences were seen between groups in leptin (DE: -17.8±21.9; C: 4.5±16.0 ng/ml, $p=0.003$), IL-6 (DE: -1.9±4.2; C: 2.7±1.2 ng/ml, $p=0.001$) and TNF-α (DE: -.2±2.1; C: 1.7±1.5 ng/ml, $p=0.013$) while visfatin (DE: 0.85±14.9; C: 20.2±37.1 ng/ml, $p=0.10$) and insulin (DE: -8.5±15.0; C: 0.12±7.7 IU/ml, $p=0.07$) tended to differ between groups. No significant differences were seen in changes in resistin (DE: 18.6±100; C: 59.9±162.2 ng/ml, $p=0.452$) or glucose (DE: -3.8±19.8; C:-2.8±6.9 %, $p=0.87$). Significant correlations were seen between changes in IL-6 and resistin ($r=0.430$, $p=0.028$) and changes in resistin and visfatin ($r=0.417$, $p=0.034$). These findings indicate that exercise and diet-induced weight loss have an effect on resistin and visfatin.

DEDICATION

To my parents Mrs. Santosh Khanna and Mr. Prakash Chand Khanna who instilled in me the virtues of perseverance and hard work and relentlessly encouraged me to strive for excellence. Throughout my life, they have actively supported me in my determination to find and realize my potential. I just wanted to say thank you for all the sacrifices you have made, all the support you have given me, all the guidance you gave when I needed it most and for shaping me into the person I have become today. I also dedicate my dissertation to my newborn bhanja “Toshu”.

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NOMENCLATURE

DE	Diet & Exercise Group
C	Control Group
ASCVD	Atherosclerotic Cardiovascular Disease
CRP	C-reactive protein
IL-6	Interleukin-6
IL-8	Interleukin-8
IL-10	Interleukin-10
TNF-α	Tumor Necrosis Factor- Alpha
MCP-1	Monocyte Chemoattractant Protein-1
IRS-1	Insulin Receptor Substrate-1
IRS-2	Insulin Receptor Substrate-2
MAPK	Mitogen Activated Protein Kinase
HOMA	Homeostasis Model Assessment
HOMA-IR	Homeostasis Model Assessment of Insulin Resistance
HDL	High Density Lipoprotein
BMI	Body Mass Index
ESNL	Exercise and Sport Nutrition Laboratory
CVD	Cardiovascular Disease
TGF-β	Tumor Growth Factor-Beta
IL-1Ra	Interleukin-1 receptor antagonist
NFκB	nuclear factor kappa-light-chain-enhancer of activated B cells
IL-1 β	Interleukin-1 Beta
NEFA	Non-esterified Fatty Acid
PPAR	Peroxisome proliferator-activated receptor
PAI-1	Plasminogen activator inhibitor-1

ICAM1	Intercellular Adhesion Molecule 1
TNF-R type 2	Tumor necrosis factor receptor 2
LPS	Lipopolyssacharide
Retn	Resistin
CAD	Coronary Artery Disease
NAD	Nicotinamide adenine dinucleotide
WAT	White Adipose Tissue
CD14+	Cluster of Differentiation 14
MEK1	Mitogen-activated Protein Kinase Kinase 1
Nampt	Nicotinamide Phosphoribosyltransferase
PBEF	Pre-B cell colony-enhancing factor
GH	Growth Factor
HDLc	High Density Lipoprotein Cholesterol
sTNFR1	Soluble Tumor Necrosis Factor Receptors 1
SDS-BMI	Standard Deviation Score -Body Mass Index (BMI)
A1C	Glycated Hemoglobin
HsCRP	High-sensitivity C-reactive protein
VO_{2max}	Maximum Rate of Oxygen Consumption
NO	Nitric Oxide
HMB	β-Hydroxy β-Methylbutyrate
DHEA	Dehydroepiandrosteron
DEXA	Dual Energy X-ray Absorptiometry
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme-linked Immunosorbent Assay
SA-HRP	Streptavidin-horseradish peroxidase
FFM	Fat Free Mass
VAT	Visceral Adipose Tissue

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CHAPTER I

INTRODUCTION

Obesity has become a public health crisis in United States. Nearly two thirds of adult Americans are either overweight or obese [1, 2]. Despite the emphasis on the benefit of healthier diets and increased physical activity by health professional, media, public and mass educational campaigns, the prevalence of obesity in United States has more than doubled over the past four decades. There exist large disparities between population groups and trends in the associated patterns [1-3]. In 2009-2010, the prevalence of obesity was 35.5% among adult men and 35.8% among adult women [4]. There are two major factors that contributed to the increase in obesity prevalence, plentiful supplies of inexpensive foods and sedentary jobs [5]. Obesity has many health, social, psychological, and economic consequences for both the individual being affected and the society [6]. According to one study, medical expenditures attributed to overweight and obesity in 2008 was $209.7 billion [7].

Obesity is associated with atherosclerotic cardiovascular disease (ASCVD) and can also be an underlying risk factor for cardiovascular diseases [8]. Obesity is also accompanied by other medical complications such as diabetes, fatty liver, cholesterol gallstones, sleep apnea, osteoarthritis, and polycystic ovary disease. These disorders are commonly found in individuals who have metabolic syndrome [9]. Metabolic syndrome is the collection of risk factors those of which contribute to ASCVD. It includes major risk factors (hypercholesterolemia, hypertension, and hyperglycemia) and emerging risk factors (atherogenic dyslipidemia, insulin resistance, proinflammatory state,

prothrombotic state). Obesity is associated with a chronic inflammatory response, characterized by abnormal adipokine production, and the activation of some pro-inflammatory signaling pathways, resulting in the alteration of several biological markers of inflammation such as C-reactive protein (CRP), IL-6, TNFα, adiponectin, leptin, resistin, visfatin etc. [10-14]. Conversely, a reduction in body weight is accompanied by a decrease or even a normalization of these biological parameters [15-21]. The exact mechanism of inflammation is unclear but several inflammatory factors have been proposed as stimulators such as infectious agent leading to endothelial injury [22]. Both central adiposity and obesity have also been associated with abnormal lipid and lipoprotein levels [23, 24].

Exercise and diet play an important role in weight loss in overweight and obese people. Many research studies have shown that diet and exercise are highly effective in increasing weight loss that leads to improve inflammatory markers of obesity and enhance health and overall fitness [25-28]. Most research have shown that programs including both diet and exercise produce greater weight loss than diet alone in overweight and obese individuals soon after intervention period and after 1 year of follow up [29-31]. 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 [32, 33]. Nevertheless, the main pathogenic mechanisms of obesity still remain unclear and more research needs to be done.

Several inflammatory markers related to obesity have been studied in the last few years. Among them, two recently discovered inflammatory markers, resistin and visfatin

have gained more attention due to their contradictory behavior and potentially contributing factors to insulin resistant which leads to diabetes. In 2001, resistin (or 'resistance to insulin') was originally discovered in mice. It was named due to its ability to resist (interfere with) insulin action [34]; which was initially proposed as a link between obesity and diabetes. Several studies have reported positive correlations between resistin levels and insulin resistance in vivo and in vitro [34]. Recently, resistin has been associated with metabolic syndrome components and on the other hand, it has been linked with early atherosclerosis in obese children [35]. Finally, resistin has been demonstrated to stimulate the secretion of several inflammatory factors like TNF-α, IL-6, IL-8, and MCP-1, known to play a role in the mechanism of insulin resistance [36]. Therefore, resistin may have an indirect effect on insulin resistance in humans through exacerbating inflammation, which has been shown to disturb insulin sensitivity.

Fukuhara et al. [37] recently identified a new adipokine mainly synthesized and secreted in visceral fat called 'visfatin'. Plasma visfatin levels were strongly correlated with total fat mass but not so much with subcutaneous fat mass. However, these results remain controversial [38-41]. According to both genetic and nutritional obesity models, visfatin expression is induced only in visceral adipose tissue. In vitro, just like insulin, visfatin enhances glucose uptake by myocyte and adipocyte and inhibits hepatocyte glucose release [37] . Visfatin and insulin have the same affinity for the insulin receptor, with visfatin physically interacting with the receptor, but at a different site. Its insulin-like effects are also observed in the insulin-transduction pathway. Just like insulin, visfatin induces tyrosine phosphorylation of insulin receptors IRS-1 and -2, and

activation of phosphatidylinositol-3-kinase, protein kinase B and MAP kinase. Regardless of these similarities between visfatin and insulin, there are also marked differences between the two [37]. The most important difference is that visfatin levels do not significantly change in fed or fasting states. The plasma levels of visfatin are 10% lower in a fasting state and 3% in a fed state when compared to insulin levels. These differences in plasma concentrations could account for the mild effect of visfatin in glycaemia. However, it is too early to consider this adipokine in the development of hypoglycemic drugs, recent research has shown that serum visfatin increases with progressive beta-cell deterioration in type 2 diabetic patients [42] . The above mentioned studies strongly suggest that visfatin could be primarily regarded as an inflammatory mediator involved in several pathological processes.

Additional research is warranted to understand the effects of exercise and diet-induced weight loss on resistin and visfatin. Moreover, ways in which exercise and diet-induced changes in resistin and visfatin may affect secondary responses such as insulin sensitivity, insulin resistant and their link to diabetes, triglycerides, HOMA, HDL etc. Thus, the main foci of this study were to examine whether obesity is associated with increase in inflammation; whether exercise and diet-induced weight loss reduces inflammatory markers- resistin and visfatin; how these inflammatory markers correlate with each other; how resistin and visfatin correlate with anthropometric indices and other inflammatory markers such as IL-6, TNF-α, and, whether changes in levels of markers explain how exercise and diet-induced weight loss can be beneficial in

improving markers of health, fitness and inflammation that may potentially lead to understanding the mechanism of certain diseases or disorders.

Statement of problem

Does exercise and diet-induced weight loss affect the inflammatory markers such as resistin and visfatin associated with obesity and how these markers correlated with anthropometric indices, blood markers and other inflammatory markers such as IL-6 and TNF-α?

Purpose of the study

The purpose of the study was to examine how exercise and diet-induced weight loss affect the markers of inflammation, resistin and visfatin and how these markers correlate with anthropometric indices, blood cholesterol indices and other inflammatory markers.

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 eligibility criteria were randomly assigned to a 12-week exercise and diet-induced weight loss group designed to improve fitness and promote fat loss and reduce inflammatory markers or a non-diet, non-exercise control group. The independent variables were exercise and diet intervention. The dependent variables were body composition (weight, fat mass, fat free mass, percent body fat); markers of fitness; inflammatory markers such as resistin, visfatin, IL-6, TNF-α, insulin, leptin, HDL, triglyceride and HOMA.

Hypotheses

H_1-The exercise and diet intervention will promote significantly greater weight loss and changes in body composition compared to controls.

H_2-The exercise and diet intervention will promote significant decrease in resistin levels compared to the controls

H_3-The exercise and diet intervention will promote significant decrease in visfatin levels compared to the control

H_4-There will be significant correlation between changes in resistin and changes in visfatin between the groups across the intervention period

H_5- There will be significant correlation between changes in resistin and changes in leptin between the groups across the intervention period

H_6- There will be significant correlation between changes in visfatin and changes in leptin between the groups across the intervention period

H_7- There will be significant correlation between changes in resistin and changes in TNF-α between the groups across the intervention period

H_8- There will be significant correlation between changes in visfatin and changes in TNF-α between the groups across the intervention period

H_9- There will be significant correlation between changes in visfatin and changes in triglyceride level between the groups across the intervention period

H_{10}- There will be significant correlation between changes in visfatin and changes in waist-to-hip ratio level between the groups across the intervention period

H_{11}- There will be significant correlation between changes in resistin and changes in waist-to-hip ratio level between the groups across the intervention period

H_{12}- There will be significant correlation between changes in resistin and changes in triglyceride level between the groups across the intervention period

H_{13}- There will be significant correlation between changes in resistin and changes in REE between the groups across the intervention period

H_{14}- There will be significant correlation between changes in visfatin and changes in REE between the groups across the intervention period

H_{15}- There will be significant correlation between changes in resistin and changes in IL-6 between the groups across the intervention period

H_{16}- There will be significant correlation between changes in visfatin and changes in IL-6 between the groups across the intervention period

Delimitations

The research study followed the guidelines listed below:

1. There were approximately 50 sedentary overweight female participants (BMI > 27) ages 18-65 who 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

Participants who were sedentary and obese females (BMI > 27) between the ages of 18-65 years, had to meet medical clearance and qualifying criteria. 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

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

CHAPTER II

LITERATURE REVIEW

Background

Obesity is a major public health concern worldwide, and is one of the most prevalent non-communicable diseases [43]. Obesity can be measured in different ways. The assessment methods measures different aspects of obesity such as the total or regional obesity. Each method's appropriateness and scientific acceptability depends on the situation since each method has its own advantages and disadvantages. Each method also produces different results when it is used to estimate morbidity and mortality. When there is increased body fat, there will also be necessary increases in some lean tissue including the fibrous and vascular tissues in adipose tissue, heart muscle, bone mass, and truncal or postural musculature. All these non-fat tissues have a higher density (1.0 g/ml) than fat (0.7 g/ml). The density of non-fat tissues is also increased by physical activity, which of course tends to reduce body fat. While body mass index (BMI) has traditionally been used to identify overweight and obese individuals, waist circumference was developed initially as a simpler and potentially a better indicator of health risk than BMI in health promotion. Waist circumference is the best anthropometric predictor of visceral fat and at least as good an indicator of total body fat as BMI or skinfold thicknesses [44]. Waist circumference alone is also a better predictor of visceral and total fat as compared to waist to hip ratio. Waist circumference is minimally related to height, so correction for height (as in waist to height ratio) does not improve its relation with intra-abdominal fat or ill health. People with a large waist are many times more at risk of ill health,

including features of metabolic syndrome (such as diabetes, hypertension, and dyslipidemia) as well as shortness of breath and poor quality of life. These increased risks also apply to people whose BMI is normal but who have a large waist. However, BMI and waist circumference are collinear, so combining the two measures adds relatively little to no risk prediction. Waist to hip ratio was introduced—mainly as a result of Swedish research—on the assumption that it would predict fat distribution better than waist circumference alone. Subsequent research, however, showed that it did not. Hip circumference does have a relation to health and disease, but in an inverse way, such that a relatively large hip circumference is associated with lower risks of diabetes and coronary heart disease. This is probably because hip circumference reflects muscle mass, which is reduced in type 2 diabetes and inactivity [44].

Obesity has inflammatory component which is in relationship with alterations such as insulin resistance, atherosclerosis, hypertension, and some types of cancer [45, 46]. Overweight and obese individuals have altered circulatory levels of inflammatory cytokines, such as TNFα, IL-6, C-reactive protein (CRP), IL-18, Resistin and Visfatin [47-50]. Measures of body fat have stronger correlation with inflammatory markers than BMI or total body fat [48, 50].

The application of lifestyle interventions, such as caloric restriction and exercise training, has been strongly advocated for the treatment of obesity and its related metabolic dysfunctions. Caloric restriction is a reasonable treatment for inflammation in obesity [51, 52]. However, dietary weight loss can become less effective as a long-term anti-inflammatory intervention [53]. Regular exercise can potentially modify metabolic

hormones [54] and may be important for the treatment of chronic inflammation and obesity-related conditions like the metabolic syndrome.

Brown and white adipose tissue

Both brown fat cells and muscle cells derived from the same stem cells in the embryo, having the same marker. The function of brown fat is to generate heat by burning calories. Brown fat is more commonly found in hibernating animals and newborns. The quantity of brown fat significantly decreases after life as an infant. Adults who have comparatively more brown fat tend to be younger and slender and have normal blood sugar levels. We can generate more brown fat by exercising, which can convert white-yellow fat to a more metabolically active brown fat; getting enough high-quality sleep, as proper melatonin production influences the production of brown fat; and exposing yourself to the cold regularly, such as exercising outdoors in the wintertime or in a cold room [55].

White fat is composed of a single lipid droplet and has far less mitochondria and blood vessels, thus resulting in its lighter white or yellow appearance. White fat is the predominant form of fat in the body, originating from connective tissue. It has many functions like providing largest energy reserve in the body, thermal insulator and cushion for our internal organs and protective cushions for our internal organs and during external interactions with our environment. It is a major endocrine organ, producing one form of estrogen as well as leptin, a hormone that helps regulate appetite and hunger. It’s also got receptors for insulin, growth hormone, adrenaline, and cortisol (stress hormone). Abdominal obesity (visceral fat) is associated with metabolic

syndrome—a group of symptoms that signal an increased risk for heart disease, diabetes, and cancer. Excess white fat throughout the body is associated with an increased risk of breast, colon, esophageal, gall bladder, and pancreatic cancer. It's also associated with sleep apnea, and physical disabilities such as knee arthritis. White adipose tissue is no longer considered an inert tissue mainly devoted to energy storage but is emerging as an active participant in regulating physiologic and pathologic processes, including immunity and inflammation. Macrophages are components of adipose tissue and actively participate in its activities [56]. Furthermore, cross-talk between lymphocytes and adipocytes can lead to immune regulation. Adipose tissue produces and releases a variety of proinflammatory and anti-inflammatory factors, including the adipokines leptin, adiponectin, resistin, and visfatin, as well as cytokines and chemokines, such as TNF-α, IL-6, monocyte chemoattractant protein 1, and others [57, 58]. Proinflammatory molecules produced by adipose tissue have been implicated as active participants in the development of insulin resistance and the increased risk of cardiovascular disease associated with obesity [59]. In contrast, reduced leptin levels might predispose to increased susceptibility to infection caused by reduced T-cell responses in malnourished individuals [60]. Altered adipokine levels have been observed in a variety of inflammatory conditions, although their pathogenic role has not been completely clarified.

Obesity: an immune disease?

Obesity, state of malnutrition by excess, leads to defective immune function. Excess body fat is associated with changes in leukocyte count such as neutrophils,

monocyte and lymphocyte counts but lower T- and B-cell mitogen-induced proliferation [61]. Could altered immune system underlie the onset of obesity? This conclusion would be too speculative or not sufficiently founded at the moment, but we can say, at least immune dysfunction is a major contributor to obesity associated alterations like inflammation and insulin resistance. Macrophages aggregates grew larger with increasing degree of obesity, similar to those observed in other inflammatory conditions, led to the idea that macrophages aggregate could partially explain the obesity-related inflammatory state [56]. There are two types of phenotypic macrophages described that have been related to the obesity: one known as M1 (classically activated) acts as pro-inflammatory and another is M2 (alternatively activated) acts as anti-inflammatory. In obesity, there is a switch from M2 to M1 phenotype which is pro-inflammatory [62]. Furthermore, most of the research findings showed absence of M2 phenotype associated with a higher susceptibility to obesity, inflammation and insulin resistance [63].

Prevalence of obesity

The prevalence of obesity in United States has more than doubled over the past four decades although there are large disparities between population groups and continuing changes in the associated patterns [1-3]. The prevalence of obesity for adults ages 20 to 74 years increased by 7.9 percentage points for men and by 8.9 percentage points for women between 1976-1980 and 1988-1994, and subsequently by 7.1 percentage points for men and by 8.1 percentage points for women between 1988-1994 and 1999-2000 [64]. In 2007-2008, the prevalence of obesity was 32.2% among adult

men and 35.5% among adult women [65] which became 35.5% in men and 35.8% in women respectively in 2009-2010 [4]. Women have experienced a particularly sharp increase in the prevalence of obesity from 40.2% to 63.9% in the last few years. According to the recent published data, 39.96% (weighted n = 36325297) of men and 29.74% (weighted n = 28894630) of women were overweight and 35.04% (weighted n=31847198) of men and 36.84% (weighted n = 35792733) of women were obese. The weight status distribution was similar for both sexes across racial groups, except for non-Hispanic white women, who had higher normal weight than the overweight category. Over 20 years, the greatest increase in the proportion of patients in the obesity class 3 category was among non-Hispanic black women [66].

Inflammation and its markers

Inflammation is a protective response to extreme challenges to homeostasis, such as infection, tissue stress, and injury. Inflammatory signals including cytokines, chemokines, biogenic amines, and eicosanoids participate in different biological processes ranging from local vascular responses to alterations of body temperature. Regardless of this complexity and diversity of functions, all the activities of inflammatory signals can be described in terms of their effects as a part of homeostasis. Inflammatory signals can directly stimulate or inhibit the flows of various homeostatic systems such as TNF-α and IL-1b activate lipolysis, inhibit gluconeogenesis, and increase vascular permeability to fluids and solutes, while IL-6 changes hepatic protein synthesis [67]. Inflammation increases the production of procoagulant factors, down-regulates anticoagulant mechanisms, and promotes tissue factor (TF) expression on

white blood cells and endothelial cells [68]. Cytokines, chemokines and adhesion molecules facilitate inflammation [69]. Endothelial cells also secrete growth factors and chemokines such as interleukin (IL)-6, IL-8, and IL-10 [70]. The process of thrombus resolution and inflammation culminates in vein wall thickening and fibrosis [71].Overall, inflammation is a very important process within homeostatic circuit and its markers could provide more detailed insight about the mechanism or pathophysiology of various diseases or disorders.

Obesity and inflammatory markers

The obesity associated state of chronic low-grade systemic inflammation, termed "metabolic inflammation", is considered a focal point in the pathogenesis of multiple disorders/diseases like insulin resistance, atherosclerotic and coronary diseases etc. [46, 72, 73]. Adipose tissue is a complex and active secretory organ that both sends and receives signals modulating energy expenditure, appetite, insulin sensitivity, endocrine and reproductive functions, bone metabolism, inflammation, and immunity. It also acts as a reservoir to store excess nutrients as triacylglycerol [12] and to release free fatty acids during fasting. Visceral adiposity, rather than simply a high body mass index (BMI), correlates well with increased risk of CVD and diabetes. However, the biochemical and physiologic reasons for this are still unclear. One possible explanation is that visceral fat has direct access to the portal circulation as compared to subcutaneous white adipose tissue that leads to the substances produced by visceral fat directly affecting the liver.

Adipocytes synthesize and secrete the proinflammatory cytokines like tumor necrosis factor alpha (TNF-α) and the hormone leptin which regulates appetite and energy balance [74]. Evidence shows that the adipose tissue secretes more than 50 hormones and signaling molecules, collectively called adipokines. These adipokines play a role in an autocrine, paracrine, or systemic manner and influence several physiological processes concerning energy, glucose metabolism, and immunity [75]. Adipose tissue from lean individuals preferentially secretes anti-inflammatory adipokines such as adiponectin, transforming growth factor beta (TGFβ), interleukin (IL)-10, IL-4, IL-13, IL-1 receptor antagonist (IL-1Ra), and apelin. In contrast, obese adipose tissue mainly releases proinflammatory cytokines among which are TNF-α, IL-6, leptin, visfatin, resistin, angiotensin II, and plasminogen activator inhibitor 1 [73]. We still don't know the role of increased cytokine production in obesity. We can only speculate that the answer can be searched in the enlarged, lipid loaded adipocytes. There must be mechanisms operating within and from the adipose cell in order to maintain or restore energy homeostasis in a situation of excessive nutrient inflow and storage. There should be a regulatory mechanism constituted by local production of these cytokines to stop the hypertrophied adipocyte from storing lipids. The problem arises when this becomes a systemic chronic state from a local reaction when the inflammatory response cannot be resolved due to sustained obesity. The mechanism between obesity and chronic low grade inflammation are yet not entirely understood, but different likely explanations have been proposed.

TNF-α is a potent proinflammatory cytokine, primarily secreted from myeloid cells via activation of MAPK and NFκB signaling pathways, resulting in the release of other inflammatory cytokines, such as IL-1β and IL-6 [76]. Leptin was one of the first proteins shown to be secreted from adipose tissue [74]. Leptin is primarily secreted by adipocytes proportionally to fat cell mass and is well known for its key contribution to energy metabolism [77]. Leptin exerts its effect on energy balance mainly by acting on the brain, either directly or indirectly by activating specific centers in the hypothalamus to decrease food intake, to increase energy expenditure, to influence glucose and lipid metabolism, or to alter neuroendocrine function. However, leptin levels are increased in obese subjects, with little or no impact to regulate energy homeostasis, which coined the well-established phrase “leptin resistance” in obesity. Certainly, preclinical and clinical experiments showed that obese rodents and humans displayed leptin resistance that may directly contribute to the reduction of lipid oxidation in insulin-sensitive organs, leading to accumulation of lipids and insulin resistance [78, 79]. The role of leptin on insulin resistance is still not fully understood. Leptin is decreased in low insulin states, such as experimentally induced diabetes, and increases after insulin treatment [80]. In humans, insulin resistance is associated with elevated plasma leptin levels independently of body fat mass [81]. However, in patients with lipodystrophy, a condition characterized by almost complete lack of adipose tissue [82], leptin levels are very low and correlate significantly with markers of insulin resistance [83]. Leptin therapy in lipodystrophic patients improves their metabolic state with remarkable improvements in insulin sensitivity, suggesting that leptin acts as a signal that contributes to regulation of total

body sensitivity to insulin [84]. Unlike leptin, the circulating levels of adiponectin, a hormone produced predominantly by adipocytes, are decreased in obesity [85]. Adiponectin has important insulin sensitizing effect: adiponectin-deficient transgenic mouse showed improved insulin sensitivity [86] and association studies have consistently linked plasma adiponectin levels to insulin sensitivity in rodent models and in humans [87].

Adipokines enlisted in regulation of insulin resistance are adiponectin, leptin, resistin, visfatin, chemerin, TNF-α, IL-1, IL-6, IL-8, IL-10, plasminogen activator inhibitor 1, monocyte chemoattractant protein-1, and retinol binding protein-4.

Fatty acids can induce inflammation

Abnormally increased levels of blood lipid, including NEFA, are a common feature in obesity [88]. The chemical nature of fatty acids is relevant to triggering the inflammatory response. According to this hypothesis, the adipose tissue as a whole has limited capacity to expend and to store energy. Exceeding this limit leads to enhanced lipolysis within the adipocyte and the subsequent release of Non-esterified fatty acid (NEFA) into the blood stream. NEFA reach other tissues and organs, and exert toxic effects on them, e.g. resistance to insulin action; a phenomenon known as lipotoxicity. According to some studies on weight discordant twins, obese individuals showed signs of insulin resistance and stimulated inflammatory and immune response pathways in adipose tissue [89]. How can fatty acids elicit an inflammatory response? It has been suggested that fatty acids modulate adipokine production or secretion [90, 91]. Alternatively, NEFA may directly induce inflammatory pathways through activation of

cell receptors. For instance, they are natural ligands for the PPAR. These transcription factors regulate cell metabolism and adipocyte differentiation, and can also suppress the activity of the NF-kB, another transcription factor crucial in the initiation of the inflammatory response. In addition, PPAR seem to be involved in the aforementioned phenotypic switch of adipose tissue macrophages (from M2 or anti-inflammatory to M1 or pro-inflammatory) [92].

Resistin and its association with obesity

Steppan et al [93] described resistin as an antagonist to insulin in both in vitro and in vivo environments. They also showed that resistin has higher concentration in obese and diabetic mice. Exogenous administration of resistin increases plasma glucose levels and its endogenous production in rodents [34, 94]. Resistin is produced in humans by mononuclear cells such as macrophages and by both adipocytes and macrophages in rodents [34, 95] while some other chemokines like TNF-α, IL-6, MCP-1, visfatin, and PAI-1 are expressed in adipocytes as well as activated macrophages and/or other immune cells. The relative amounts of each produced by the adipocyte versus the macrophages present in the adipose tissue are still unclear. The potential role of resistin as mediator linking adipose tissue, inflammation, and immunity has been recently reviewed [95]. Resistin has also been shown as a pro-inflammatory adipocytokine which enhances during marathon race and associates with markers of cartilage degradation [96].

It is suggested that resistin is engaged in inflammatory conditions in humans by means of its secretion in substantial quantities by mononuclear cells. Also, resistin levels

are mutually correlated with those of cell adhesion molecules such as ICAM1 in patients with obstructive sleep apnea, and in atherosclerotic patients, they are positively associated with other markers of inflammation, such as soluble TNF-R type II and lipoprotein-associated phospholipase A2 [97, 98]. Furthermore, LPS has been reported to induce resistin gene expression in primary human and murine macrophages via a cascade involving the secretion of pro-inflammatory cytokines, and in human peripheral blood mononuclear cells, resistin seems to induce and be induced by IL-6 and TNF (induction of these cytokines by resistin occurring via the NFκB pathway) [36, 99].

Human and rodent resistin gene and protein sequences share about 60% identity, less than most hormones conserved across species [100], yet, the genes are located on the same chromosome, and with the gene encoding resistin (Retn) on mouse chromosome 8 being located a similar distance from the insulin receptor gene as is the Retn gene on human chromosome 19. In addition, as in rodents, resistin levels decrease with thiazolidinedione (anti-diabetic) treatment in humans [101]. The lack of human resistin expression in adipocytes appears to be attributable to loss of a genomic binding site for the nuclear receptor peroxisome proliferator-activated receptor g (PPARg), which controls the adipocyte-specific expression of the mouse Retn gene [102]. The expression and secretion of resistin by human mononuclear cells are dramatically induced by inflammatory stimuli, [103-105], which also increase circulating resistin levels [98, 99]. Inflammation is increasingly recognized to play a pathogenic role in obesity and type 2 diabetes, as well as cardiovascular disease [46, 106]. As discussed below, recent genetic and epidemiologic data connect resistin to these human diseases.

Recently, in a study conducted by Silha et al on plasma resistin, adiponectin and leptin levels in lean and obese subjects. There was 17 lean subjects with a mean body mass index (BMI) of 23 and 34 non-diabetic obese individuals with a mean BMI of nearly 33. Insulin resistance was assessed using the homeostasis model assessment ratio (HOMA-Insulin Resistance) formula derived from fasting insulin and glucose levels. Results showed that resistin levels were not significantly different between the two groups but were significantly higher in women compared with men, 35.4±6.5 (S.E.) vs 15.4±2.9 mg/l, $P < 0.01$. Resistin did not correlate with BMI but did significantly correlate with HOMA-Insulin Resistance, $P<0.01$, and this correlation remained significant after adjustment for gender and BMI. Leptin levels were significantly higher in obese subjects and women and correlated with HOMA-Insulin Resistance and resistin, whereas there was no significant correlation between adiponectin and either leptin or resistin.

Epidemiologic evidence for resistin in human insulin resistance and diabetes

A mouse model in which human resistin is expressed primarily by monocytes and macrophages, as is the case in humans, is predisposed to developing insulin resistance, suggesting that the metabolic effects of resistin might be translatable from rodents to humans [107]. Given the association of resistin and insulin resistance in rodents, many studies have investigated the correlation between circulating resistin levels and type-2 diabetes. The results of initial efforts were conflicting, with some, but not all, studies identifying a significant correlation between serum resistin level and insulin resistance or type 2 diabetes [101].

Higher levels of resistin were observed in subjects with diabetes versus controls [108]. At least some of the association between resistin and insulin resistance in humans seems attributable to other factors known to affect insulin sensitivity, such as obesity and inflammation. However, although most studies have analyzed whether the relative risk associated with increased resistin levels is independent of previously established risk factors, the converse analysis has rarely been performed, leaving open the possibility that the biological effect of these other risk factors is mediated by resistin. Whether resistin is an active player or merely a responder in metabolic dysfunction cannot be fully determined without an understanding of the regulation of resistin itself. Genetic determinants of resistin expression might provide additional clues about the role of resistin in human susceptibility to disease.

Evidence for resistin in human atherosclerosis and coronary artery disease

Elevated resistin predicted the presence and severity of CAD, correlating with coronary artery calcium score in asymptomatic healthy subjects [98], and in patients undergoing angiography for chest pain [109, 110]. A European case–control study of 26,490 healthy individuals found a relative risk of 2.09 for the development of myocardial infarction in those in the highest quartile of resistin, adjusted for CRP [111]. Moreover, a Chinese study of 225 cases and controls revealed a markedly increased risk of CAD (OR = 3.01) in individuals with the G/G genotype, associated with increased resistin levels [112]. Several studies have not detected a correlation between resistin and either prevalence or outcome of coronary disease [113-115]. This could be a result of ethnic variation or differences in study design and patient selection.

Visfatin and its relationship with obesity

Fukuhara et al [37] identified a new novel adipose tissue cytokine called visfatin, a protein mediator secreted by fat cells (high levels of expression in visceral fat cells) which acts like an enzyme (Nicotinamide phosphoribosyl transferase) Nampt, which is involved in NAD+ salvage pathway. Initially it was identified as Pre B cell Colony Enhancing Factor (PBEF), secreted by human peripheral blood lymphocytes [116]. Visfatin has insulin-mimetic effect that was originally discovered in liver, skeletal muscle and bone marrow as a growth factor for B lymphocyte precursors [37, 117]. Circulating visfatin levels are closely correlated with WAT accumulation, visfatin mRNA levels increase in the course of adipocyte differentiation, and visfatin synthesis is regulated by several factors, including glucocorticoids, TNF, IL-6 and growth hormone. Visfatin is not only produced by WAT, but also by endotoxin challenged neutrophils, in which it prevents apoptosis through a mechanism mediated by caspases 3 and 8 [117]. Patients with inflammatory bowel diseases have elevated circulating visfatin levels and higher levels of visfatin mRNA in their intestinal epithelium. Visfatin has been shown to induce chemotaxis and the production of IL-1β, TNF, IL-6 and costimulatory molecules by CD14+ monocytes, and to increase their ability to induce alloproliferative responses in lymphocytes, effects which are mediated intracellularly by p38 and MEK1 [118]. High circulating visfatin levels have been observed in rheumatoid arthritis [119] and acute lung injury [120]. Significantly higher visfatin mRNA expression was found in inflamed Inflammatory Bowel Disease [118]. The binding affinity of visfatin/PBEF/Nampt to the IR (insulin receptor) was found to be similar compared with

that of insulin [37]. Many studies have demonstrated an increased level of visfatin in diabetes mellitus [42, 121, 122]. However, in an experiment conducted on cohort of obese patients, no correlation was found between PBEF/visfatin to glucose infusion [123]. Whether visfatin binds to the IR remains controversial. Whether visfatin binds to insulin receptors and exerts its insulin mimetic activity is still a controversy, but recent research has shown that Nampt/visfatin-mediated systemic NAD+ biosynthesis is necessary for β cell function, suggesting that visfatin helps in regulation of glucose homeostasis [124]. Circulating visfatin levels are closely correlated with WAT (White Adipose Tissue) accumulation, visfatin mRNA levels increase in the course of adipocyte differentiation, and visfatin synthesis is regulated by several factors, including glucocorticoids, TNF-α, IL 6, and GH [117]. Visfatin levels have been shown to be increased in children of more BMI indicating important implication of this new adipokine in inflammatory mechanisms of obesity starting already in childhood [125]. Visfatin were shown to be increased in females with obesity (visceral obesity) [37]. Decrease in circulating visfatin was found in morbidly obese women who lost more than 20% of their BMI, also increased plasma visfatin concentrations in morbidly obese subjects are reduced after gastric banding [126]. These studies show that more the BMI (obesity), more the visfatin levels and visfatin levels decrease after weight loss.

Interaction among inflammatory markers in obesity

The incidence of obesity has dramatically increased and has become epidemic in United States. Obesity has multifactorial etiology which includes genetic, environmental, socioeconomic, and behavioral or psychological influences. Obesity is

the result of a chronic positive energy balance which is regulated by a complex interaction between endocrine tissues and the central nervous system [127, 128]. Adipocytes produce and secrete several proteins, collectively called adipokines which play important roles in the inflammation. These adipokines include TNF-α, leptin, IL-6, adiponectin, resistin and visfatin. Vendrell et al [129] conducted a study on fifty-seven morbidly obese white subjects with a mean age of 42.2±9.2 years (8 men and 49 women) and nonmorbidly obese subjects (117; mean age 49.2±12.4 years; 28 men and 89 women). Descriptive statistics showed that morbidly obese subjects had higher values in all anthropometric measurements as compared to nonmorbidly obese patients which include BMI, waist to hip ratio and body fat. In the nonmorbidly obese group, significant differences were observed for leptin (13.3±7.0 vs. 34.2±13.4 ng/mL, $p<0.001$) and adiponectin (11.3±4.5 vs. 18.0±6.7 g/mL, $p<0.001$) in between men and women. There was significant gender difference for resistin.

In nonmorbidly obese subjects, leptin correlated positively with BMI ($r=0.390$, $p<0.01$) while Adiponectin showed a strong negative correlation with weight ($r=0.33$, $p=0.001$), triglycerides ($r=0.22$, $p=0.006$), and fasting insulin ($r=0.28$, $p=0.05$) but a positive correlation with HDLc ($r=0.36$, $p=0.001$). Serum resistin was positively related to sTNFR1 ($r=0.31$, $p=0.01$) and triglycerides ($r=0.24$, $p=0.01$). In the morbidly obese group, leptin, adiponectin, and ghrelin did not correlate with any measurements of body composition. Resistin was found to correlate positively and significantly with weight ($r=0.48$, $p<0.001$), BMI ($r=0.39$, $p<0.005$) and fat-free mass ($r=0.39$, $p<0.01$). The findings for the bivariate correlation analyses were further explored using multivariate

analysis to control for potential confounders. After adjusting for age, gender, and BMI in nonmorbidly obese patients, leptin was positively related to gender and BMI (β=0.52, $p<0.001$ and β=0.24, p=0.009 respectively). Adiponectin was positively related with HDLc and gender (β=0.31, p=0.007 and β=0.22, p=0.05, respectively) and negatively associated with weight (β= -0.38, $p<0.001$). Resistin circulating levels were found to be positively associated with sTNFR1 after adjusting for the above-mentioned variables (β=0.28, p=0.007), losing the correlation with triglycerides observed in the bivariate analysis. None of these adipokines was found to be associated with clinical or metabolic variables in morbidly obese subjects except for the resistin and ghrelin levels that were positively correlated with sTNFR2 after adjusting for age, gender, and BMI (β=0.41, p=0.008 and β=0.33, p=0.04 respectively).

Effects of diet-induced weight loss on resistin and visfatin

Diet, exercise and diet plus exercise are different ways to lose weight. Weight loss also changes the perceptions of oneself and improves quality of life [130]. Weight loss and caloric restriction have been shown to improve insulin resistance and lipid profiles [131, 132]. Two longitudinal analyses revealed changes in serum resistin to be positively correlated with changes in fat mass or weight status [133, 134] and mean insulin [133], while other studies reported no changes of resistin in weight loss [129, 135, 136]. Varady et al [137] has shown that decrease in body weight, as small as 5% from baseline, can improve circulating adipokines profiles and decrease adipocyte size in severely obese women. They reported an increase in the beneficial adipokines like adiponectin, and decreases in the less favorable adipokines, leptin and resistin, in women

who lost at least 5% of their initial body weight. This degree of weight loss also resulted in significant reductions in both subcutaneous and visceral fat cell size. Improvements in adipocyte physiology were associated with reductions in glucose and insulin, which may indicate protection from metabolic disease in this high risk population.

Koebnick et al [138] conducted a 4 month diet-induced weight loss intervention in overweight individuals to investigate changes in resistin concentration and markers of glucose and lipid metabolism Results showed that there was a mean weight reduction of 4.5 kg and significant increase in serum resistin level in overweight individuals. However, there was no direct association between resistin and insulin resistance in humans. Increase in serum resistin level contradicts the physiological understanding of resistin action based on previous mice experiments, where a strong relationship among adiposity, insulin resistance and resistin was observed [34, 93, 139]. Reinehr et al [140] conducted a study to assess changes in serum resistin levels before and after weight loss in obese children. Intervention period was for one year. Results showed no significant difference in serum resistin levels between obese and lean children. There was no significant correlation between resistin and insulin resistance index. Even though, obese children demonstrated significantly higher insulin resistance index ($P<0.001$) and insulin concentrations compared to the lean children; age, gender, pubertal stage, and resistin levels did not significantly differ between obese and lean children. At baseline, resistin serum concentrations did not significantly correlate to SDS-BMI, waist-to-hip ratio, percentage body fat, age, and HOMA. After the intervention period, there was a significant decrease in SDS-BMI by at least 0.5 (decrease in median 0.7, IQR 0.6– 0.9)

in 16 children out of the total participants. This degree of weight loss led to a significant decrease in insulin levels and HOMA but there were no significant changes in resistin levels. In the remaining 22 obese children with weight loss <0.5 SDS-BMI, there were no significant changes in insulin resistance index (HOMA), insulin, and resistin concentrations.

A study conducted by Ho and colleagues [141] on 28 overweight and obese nondiabetic subjects (F: 15, M: 13, age 39±5 years, BMI 33.2±4.6 kg/m^2) without any diseases where in participants underwent a one year calorie restricted weight loss intervention. After the intervention, participants achieved 9.4±6.9% weight loss, predominantly fat mass (7.7±5.6 kg, $p<0.0001$). Dietary intervention led to significant decrease in leptin, leptin-to-adiponectin ratio, and IL-6 (all $p<0.02$), and improvement in HOMA-IR and Insulin Sensitivity Index (SI) (both $p<0.001$). In response to weight loss IL-1β, IL-2, leptin, and resistin were significantly associated with insulin sensitivity. Table 2.1 provides a summary of some other studies that have done to look at the effects of exercise and/or dietary intervention on inflammatory marker, resistin.

De Luis et al [142] conducted a two month study on 41 morbidly obese patients (mean age= 49.9±15.7 years and mean BMI=44.6±5.6 with 8 males (19.5%) and 33 females (80.5%)) on a hypocaloric diet. Participants were on a mean caloric intake of 1741.9±812.7 kcal/day (49% of carbohydrates, 28% of lipid and 23% of proteins). Results have shown that there was a weight loss of average 4.41% in morbidly obese patients leads to decrease in BMI, weight, fat mass, fat free mass, waist circumference, systolic blood pressure, serum glucose, total cholesterol, insulin and HOMA. However,

no significant decrease in visfatin concentration (43.5+30.8 vs. 47.1+38.1 ng/ml) was seen. In the multivariate analysis, visfatin as a dependent variable in the model before diet, and C reactive protein (CRP) as an independent predictor, it was found that there was an increase of 1.82 ng/ml (CI95%:0.02-3.61) basal visfatin concentrations with each increase of 1 mg/dl of CRP. HOMA was the only independent predictor in the model after diet. There was an increase of 11.4 ng/ml (CI95%:1.76-21.11) post treatment visfatin concentrations with each increase of 1 unit HOMA. There was no significant change in circulating visfatin concentration in morbidly obese patients after 2 months with weight reduction due to hypocaloric diet.

De Luis and colleagues [155] have also conducted another 3 month study on 80 obese non-diabetic patients, with 20 men and 60 women with BMI 34.1±4.8 kg/m^2. After 3 months on a hypocaloric diet, they found that were decrease in BMI, fat mass, waist circumference, systolic blood pressure, fasting serum glucose, total cholesterol and low-density lipoprotein cholesterol. There was a significant change in visfatin level after weight loss in women (105±81 versus 90.9±40.1 ng/mL, $P<0.05$). In women, the association was observed with age ($r = -0.2$, $P<0.05$) and triacylglycerol concentration ($r = -0.54$, $P<0.05$). They did not find a significant correlation between visfatin concentration and anthropometric parameters, such as waist circumference, fat mass or lean mass. Agueda et al [156] has shown that changes in circulating serum visfatin levels were significantly and inversely associated with HOMA-IR ($P < 0.01$) after energy restricted diet intervention, regardless of achieved body weight loss. After energy restricted diet-induced weight loss, they did not find any significant association between

Table 2.1 Some published data on the effects of exercise and/or dietary intervention on resistin

Study, year	No. of participants	Duration	General Findings	Effects on Resistin
Kadolgou et al [143]	60 overweight/obese patients, BMI> 25 kg/m^2	16 week, Aerobic exercise training versus control	↓ A1C, ↓ Fasting plasma glucose, improvement in HOMA-IR, fasting insulin levels, systolic and diastolic blood pressure and lipid profile	↓ resistin correlated with HsCRP, IL-18 and VO_2max
Aghapour et al [144]	20 postmenopausal women	6 week aerobic training		↓ Resistin
Jamurtas et al [145]	9 young overweight males, BMI 27-32 kg/m^2	Acute exercise, 65% of their maximal oxygen consumption for 45 min		No change in resistin up to 48 h post-exercise
Jones et al [146]	12 overweight/obese subjects, BMI 31.8±5.2	32 weeks, exercise training	Significant ↓ in fat mass and percent body fat although weight, waist circumference, and BMI did not change	8% decrease in post training resistin level. Significant correlation between resistin and triglyceride level.

Table 2.1 continued

Study, year	No. of participants	Duration	General Findings	Effects on Resistin
Ozcan et al [147]	40 overweight/obese women, BMI (Aerobic group = 29.6 ± 0.79 Core group = 29.3 ± 0.49)	16 weeks, Aerobic & core exercise	significant decrease in fat mass and body weight ($p<0.001$)	Significant decrease in resistin (21% in aerobic group & 26.6% in core exercise group)
Zhang et al [79]	9 men & 13 women overweight/obese Chinese women	8 weeks, Slim exercise prescription	Decrease in weight, BMI, waist & hip circumference, blood sugar	↓ resistin
Prestes et al [148]	35 sedentary post-menopausal women, Mean body mass 57.84 kg	16 weeks, periodized resistance training	Reduce systemic inflammation	↓ resistin
Botero et al [149]	23 post-menopausal overweight/obese women, Mean body mass 67.56±2.26 kg	12 month periodized resistance training	Decrease in body mass, body fat mass	↓ 44.9% in resistin

Table 2.1 continued

Study, year	No. of participants	Duration	General Findings	Effects on Resistin
Bjersing et al [150]	48 fibromyalgic overweight/obese patients. 9 patients were lean (BMI 18.5 to 24.9), 26 overweight (BMI 25 to 29.9) and 13 obese	15 weeks, exercise outdoor twice a week		Increase in resistin after exercise period correlated with decreased fatigue
Many et al [151]	11 obese adolescents (BMI 41.4 ± 1.8 kg/m2)	8 weeks, aerobic exercise	Reduction in BMI and percent total body fat	14.5 ± 4.5% ↓ in resistin
Buyukyazi et al [152]	37 pre-menopausal women, BMI>27 for all groups	High and moderate intensity walking and control groups, 12 weeks for 5 days per week from 30-0 min/day	Decrease in body weight, percent body fat, BMI and waist circumference	23.5% ↓ in resistin in high intensity walking group
Izadpanah et al [153]	21 overweight/obese children, BMI 33.0 ± 1.8 kg/m^2	2 week, ad libitum, high-fiber, low-fat diet and daily exercise regimen	No significant change in weight	↓ resistin

Table 2.1 continued

Study, year	No. of participants	Duration	General Findings	Effects on Resistin
Ounis et al [154]	27 female adolescent, BMI>97th percentile	8 weeks, 3 groups: diet, individualize training and training combined with diet	BMI by 6.1, 1.5, 11.5%, and %BF by 7.3, 3.6, 15.1% in diet, training and diet/training groups respectively	20.3% ↑ in resistin in diet/exercise group
Azuma et al [133]	Sixty-four young (age 32±10 years), obese (BMI 32.9±5.6), nondiabetic subjects taking no medication, and 15 lean (BMI 21.1±1.3	1.5 years, Weight reduction program entailing dieting and exercise	Mean reduction in body weight of 4%	Significant ↑ in resistin level in obese subjects than in lean volunteers (24.58±12.93 ng/mL; n=64 vs. 12.83±8.30 ng/mL; n=15; $p<0.01$ positively correlated with BMI
Monzillo et al [136]	24 obese BMI 36.7 ± 0.9	26 weeks, 500 kcal/day deficit energy--restricted diet & moderate--intensity exercise	7.0% of weight	No change in resistin

↓ = significant reduction; BMI=Body Mass Index; A1C= glycosylated hemoglobin; HOMA-IR= Homeostasis Model Assessment of Insulin Resistance; HsCRP= Highly Sensitive C Reactive Protein; IL-6= Interleukin-6; VO_2max= Maximal Oxygen Consumption; %BF = Body fat Percentage

restricted diet-induced weight loss, they did not find any significant association between changes in visfatin levels and IL-1b, IL-6, IL-8, TNF-α and CRP levels (all $P > 0.2$). However, circulating visfatin concentration is associated with sensitivity improvement after the intervention. Furthermore, lean mass changes could be an influencing factor on visfatin concentrations and consequently, on the improvement of insulin sensitivity after weight loss in obese non-diabetic women. They did not provide any evidence for a role of visfatin increase on low-grade inflammation after weight loss.

Kovacikova et al [157] conducted a dietary intervention study on 47 pre-menopausal women (age 38·7 ± 1·7 years, range 25–57 years, BMI 27·9 ± 1·4 kg m^{-2}, range 17·3–50·5 kg m^{-2}). They divided the participants randomly into three different subgroups, lean (n =15), overweight (n = 16) and obese (n =16). Results showed that dietary intervention leads to decrease in body weight and BMI by 8%, body fat mass by 16% and waist circumference by 9% in the entire group of 32 overweight/obese women. They also established that Visfatin mRNA expression in subcutaneous adipose tissue is associated with TNF-α expression, and BMI in pre-menopausal women. The 12 week hypocaloric weight reducing diet caused an increase of visfatin mRNA levels ($P < 0·05$) and tended to decrease visfatin plasma levels in the entire overweight/obese group ($P < 0·1$). It is however notable that increase in visfatin gene expression did not reach significance in the subgroups of overweight or obese women, respectively. The relationship between the weight loss and the diet induced increase of visfatin mRNA expression was further supported by the finding of a positive correlation between these two variables in the whole overweight/obese group ($r = 0·427$, $P < 0·05$) and the

overweight subgroup. There was positive correlation between the diet induced changes of the expression of TNF-α and visfatin mRNA levels in subcutaneous adipose tissue in the whole overweight/obese group. Furthermore, there was a clear positive relationship between the magnitude of weight loss and the increase of visfatin mRNA levels. However, Visfatin expression is negatively related to body mass index and the diet induced weight reduction results in an increase of the visfatin expression in subcutaneous adipose tissue. Table 2.2 provides a summary of studies on the effects of dietary intervention on inflammatory marker, visfatin.

Effects of exercise induced weight loss on inflammation and its markers

Exercise improves many components of the cardiovascular risk factor profile especially endothelial function [158-162] which affects NO bioavailability. However not as well researched, it has been suggested that exercise may also improve levels of adipokines and oxidative stress. A large body of evidence exists suggesting that individuals who are either more physically active or more aerobically fit tend to have more favorable adipokine profiles and lower levels of oxidative stress [163-168]. However, most of these data are based on cross-sectional studies, and few studies have assessed the direct effects of exercise training on these variables. Many of the controlled intervention studies addressing this issue have shown that exercise improves adipokine and oxidative stress levels; however, most of these trials have reported concomitant improvements in body weight and/or composition that occurred during the exercise training period [27, 136, 169, 170] or did not include measures of body fat markers [171, 172]. Because adipocytes are the main mediators of these hormones, changes in body

weight/composition confound the data concerning the direct effects of exercise on these variables. Recent studies have challenged the notion that exercise directly stimulates improvements in adipokines and inflammatory markers independent of weight loss [173-175]. Although these studies are at odds with a large body of previous work, they provide a rationale for additional studies to be performed to further address this question.

Effects of exercise induced weight loss on resistin

Previous studies have shown mixed results on the effect of exercise induced weight loss on serum resistin level [136, 144, 145]. Aghapour et al [144] evaluated the effect of endurance exercise on resistin in old women aged 50-55 years, who performed 6 weeks aerobic exercise, three times a week for 45 minutes each session at 50% maximum heart rate reserve during the first week and 60 minutes of exercise at the sixth week with 60% of maximum heart rate. There was 5% intensity of aerobic activity added every two weeks. They showed that regular aerobic activity can reduce levels of resistin in human indicating the decline of inflammation even though there was no significant difference in resistin level before and after 6 weeks of aerobic exercise period.

Jamurtas and their colleagues [145] conducted study on nine young overweight males exercised for 45 minute at an intensity corresponding to 65% of VO_{2max}. Subjects were instructed to follow their normal eating pattern. They reported relatively stable

Table 2.2 Some published data on the effects of dietary intervention on visfatin

Study, Year	Number of Participants	Duration	General Findings	Effects on Visfatin
Agueda et al [156]	78 obese (BMI 34.0 ± 2.8 kg/m^2) women	12 weeks of energy restricted diet intervention	Mean weight loss 7.7 ± 3.0 kg and HOMA-IR decreased	Significant 11.9% ↑ in visfatin level and inversely associated with HOMA-IR ($P < 0.01$)
De Luis et al [142]	41 obese patients, BMI>40 kg/m^2	2 months Hypocaloric diet	Weight loss (average 4.41%)	No change in visfatin,
De Luis et al [155]	80 obese men (20)/women (60), BMI 34.1 ± 4.8 kg/m^2	3 month Hypocaloric diet	↓ in body mass index, fat mass, waist circumference	Significant 11.4 % ↓ in visfatin
Kovacikova et al [157]	47 overweight/obese women, BMI 27·9 ± 1·4 kg m^{-2}	12 week Hypocaloric diet	↓ in body weight	Significant 60% ↓ in visfatin in overweight group only

BMI=Body Mass Index; HOMA-IR= Homeostasis Model Assessment of Insulin Resistance

levels of resistin after acute exercise. This finding is in accordance with the available chronic exercise studies that did not find alterations in the levels of the hormone after aerobic training for several months [136, 176].

Jones and colleagues [146] conducted a 32 weeks study on sedentary overweight adolescents. Participants underwent for an aerobic exercise for 45 minutes session, 3 times per week at an intensity of 60-85% of measured $_{VO2\ max}$. Results showed that there was significant decrease in fat mass and percent body fat although weight, waist circumference, and BMI did not change. There was 8% decrease in post training resistin level and significant decrease in triglyceride concentration (23%). There was a significant correlation between resistin and triglyceride level. Ozcan et al [147] conducted a study on 40 sedentary middle aged women, randomly assigned into two groups, aerobic and core exercise. The exercise programs were performed 4 days a week for 16 weeks. Results showed that 16 weeks of exercise leads to significant decrease in fat mass and body weight ($p<0.001$) and resistin levels (21% in aerobic exercise group and 26.6% in core exercise group, $p<0.05$).

Another group of researchers [177] looked at the effect of exercise for 8 weeks on body fat mass, blood sugar and plasma resistin on overweight and obese individuals in Chinese population. Subjects consist of 9 men and 13 women, trained at the intensity of 60%-70% of functional capacity, 5 times per week for 60 minutes per session. Results showed that weight, BMI, body fay %, waist and hip circumference at indexes were significantly decreased ($P<0.01$). Plasms resistin level was significantly different for

men ($P<0.01$) and for women ($P<0.05$) as well. Furthermore, plasma resistin and BMI, waist and hip circumference have significant correlation for men but not for women.

Prestes et al [148] conducted a study on 35 sedentary women post-menopausal (mean age 63.18 years, s=4.8; height 1.64 m, s=0.07; body mass 57.84 kg, s=7.70). Subjects underwent 16 weeks of periodized resistance training consisted of two weekly sessions of three sets of 6–14 repetition maximum. There was a decrease in resistin after 24 and 48 h compared with baseline and a decline in baseline and immediately after levels compared with pre-training. Above mentioned results could be explained by the lower production of pro-inflammatory cytokines by the innate immune system. Periodized resistance training seems to be an important intervention to reduce systemic inflammation in sedentary women.

Botero and coworkers [149] demonstrated an effects of long term periodized resistance training on body composition, muscle strength and resistin in elderly post-menopausal women. 23 post-menopausal women (age= 63.02±4.42 years; height 1.55±0.06 m; body mass 67.56±2.26 kg) underwent periodized resistance training twice a week for 12 months. The training protocol consisted of 3 sets of 6-14 repetitions maximal (RM). Results showed that there was a significant increase in muscle strength and lean body mass and significant decrease in body mass, body fat percentage, fat mass and plasma resistin level after resistance training period. Bjersing et al [150] showed that in fibromyalgic patients, increase in resistin led to decreased fatigue which showed the beneficial effect of resistin. Another study demonstrated the benefit of moderate intensity aerobic exercise training for 8 weeks at an intensity of 40-55% nearly 180

minutes per week improved insulin sensitivity and led to decrease in resistin level [151]. Table 2.3 provides a summary of studies on the effects of exercise on inflammatory marker, resistin.

Effects of exercise induced weight loss on visfatin

Visfatin is an adipokine being mainly produced in visceral adipose tissue, also by cells, neutrophils and macrophages, and, furthermore, its plasma level correlates with the quantity of visceral fat in humans [37, 179]; however, the studies examining the effect of exercise on circulating visfatin levels are limited and often ambiguous. On the other hand, Buyukyazı et al [152] have found no significant change in the visfatin concentration in obese women (30–49 years) in moderate-intensity walking program. Pagano et al. [40] have reported that visfatin levels were lower in subcutaneous fat locations and higher in visceral adipose tissue of the obese subjects compared to lean individuals.

Berndt et al [123] have not reported any significant relationships between visfatin levels and the amounts of visceral adipose tissue as determined by CT measurements. A positive correlation between visceral adipose tissue visfatin gene expression and body mass index (BMI) had been found, but the relationship between subcutaneous fat visfatin and BMI was negative suggesting that visfatin regulation may differ depending on different fat patterns [41]. It could be concluded that changes in circulating visfatin levels are involved in the improvement of metabolic status by exercise and may be useful markers for exercise evaluation and prescription.

Table 2.3 Some published data on the effects of exercise on resistin

Study, year	No. of participants	Duration	General findings	Effects on Resistin
Valsamakis et al [134]	41 overweight/obese women, BMI 46 ± 8.6 kg/m^2	6 months, Treated with Sibutramine	Mean weight loss of 5.4%, & waist circumference was reduced by 4.5 ± 1.4 cm	Significant 16.8% ↓ in resistin; Change in waist was associated with change in resistin.
Ho et al [141]	28 overweight/obese men (15)/women (13) BMI 33.2±4.6 kg/m^2	12 months, calorie restriction with behavioral support	9.4±6.9% weight loss, mostly fat mass	resistin were significantly associated with insulin, sensitivity
Varady et al [137]	13 obese women, body mass index, 50 ± 3 kg/m^2	3 weeks, low-calorie diet	Decrease in body weight	Non-significant 11.6% ↓ in resistin in <5% weight loss group & significant 27.4% ↓ in resistin in 5%-10% weight loss group
Koebnick et al [138]	50 overweight/obese women BMI >25 kg/m2	4 month, Dietary intervention	Overweight subjects lost 4.570.6 kg body weight and 3.370.6% body fat	19.4% ↑ in resistin & inversely related to changes in waist-to-hip ratio and positively to serum apolipoprotein B, HOMA-IR, NEFA

Table 2.3 continued

Study, year	No. of participants	Duration	General findings	Effects on Resistin
Lopez et al [178]	70 obese healthy subjects, BMI >27 kg/ m^2	8 weeks, Supplementation with multi-ingredient weight loss product	Decrease in body weight, fat mass, lean mass, waist girth, hip girth	Non-significant 15.8% ↓ in resistin in METABO group
Reinehr et al [140]	63 obese children vs 36 lean children	1 year, Weight reduction through "Obedicks' intervention"	↓ body weight in obese children	No change in resistin, No significant correlations between changes of resistin and changes of SDS-BMI, changes of % body fat, changes of waist-to-hip ratio and changes of insulin resistance index

BMI= Body Mass Index; HOMA-IR= HOMA-IR= Homeostasis Model Assessment of Insulin Resistance; NEFA= Non-esterified Fatty Acid; METABO= multi-ingredient supplement containing primarily raspberry ketone, caffeine, capsaicin, garlic, ginger and Citrus aurantium

Walhin and cowokers [180] investigated the effects of short term overfeeding and decreased physical activity independent of energy imbalance in healthy young men. 26 active young men age 25±7 years were randomly assigned into two groups; one who consumed 50% more calorie diet an restricting their physical activity, another without any intervention. Result has shown that there was a significant down-regulation of visfatin in the group with intervention as compared to the control. Vigorous intense activity counteracted this effect even the participants has standardized energy surplus. Another study conducted by Rudwill et al [181] demonstrated a negative association of visfatin with activity energy expenditure and no relationship with insulin sensitivity. Another group of researchers compared the effect of aerobic exercise and aerobic combined with resistance exercise. They found that both aerobic exercise and aerobic combined with resistance exercise decrease visfatin level but there was no significant difference in the magnitude within and between group comparisons [182]. Table 2.4 provides a summary of studies on the effects of exercise training and/or dietary intervention on inflammatory marker, visfatin.

Effects of diet and exercise induced weight loss on inflammatory markers

A study conducted by Lopez and coworkers [178] on 70 obese healthy subjects divided participants into two groups, placebo and METABO. Subjects in METABO group underwent 8 weeks of daily supplementation, a calorie restricted diet and exercise training. Significant differences were observed in body weight (METABO -2.0% vs. placebo -0.5%, $P < 0.01$), fat mass (METABO -7.8 vs. placebo -2.8%, $P < 0.001$), lean mass (METABO +3.4% vs. placebo +0.8%, $P < 0.03$), waist girth (METABO -2.0% vs.

placebo -0.2%, $P < 0.0007$) and hip girth (METABO -1.7% vs. placebo -0.4%, $P < 0.003$). There was a decrease in serum resistin ($P < 0.08$). All these above mentioned significant results could be due to the effect of diet, exercise or both.

Another study conducted by Elloumi et al [17] on 21 obese adolescent boys (BMI = 30.8 ± 3.2 kg/m^2), participated in one of the weight loss program, energy restricted, individualized exercise training or energy restricted and training for 2 months. After the training period completion, combined group showed a significant improvement in body composition ($p<0.01$). Both, exercise and combined group showed a significant increase in resistin level ($p<0.05$). There was also a significant correlation between an increased ratio of adiponectin/leptin and resistin level. They concluded that energy restriction improved the ability to better oxidize lipids which was associated with normalization of inflammatory markers such as adiponectin, leptin and resistin.

Izadpanah and colleagues [153] examined the effect of short term diet and exercise intervention on inflammation and markers of metabolic syndrome. They had 21 overweight/obese children (9 boys/12 girls, age 13.0 ± 0.5 yr, BMI 33.0 ± 1.8 kg/m^2) were placed on ad libitum, high-fiber, low-fat diet and daily exercise regimen for 2 wk. They found that there was a decrease in resistin level along with IL-6, leptin, insulin and TNF-α even though there was no significant weight loss. They were among the few researchers who showed the correlations of inflammatory makers with fatty acid. They also revealed that there was negative correlation of inflammatory markers with a cluster of polyunsaturated fatty acids and positive correlation with saturated fatty acids.

Table 2.4 Some published data on the effects of exercise training and/or dietary intervention with exercise on visfatin

Study, year	No. of participants	Duration	General Findings	Effects on Visfatin
Rudwill et al [181]	33 healthy subjects, BMI 23.4 ± 0.6 $kg \cdot m^{-2}$	3 months long, Induction of physical inactivity	Decrease in fat free mass and body mass	54.9% & 49.7% ↑ in visfatin in 10 days confinement and 3 months bed rest groups respectively
Frydelund et al [179]	15 healthy young men, BMI 24.9 ± 2 kg/m^2	Acute exercise, 3 h f cycling at 60% VO_2max followed by 6 h of recovery		No change in visfatin level
Haider et al [183]	18 patients with type 1 diabetes, BMI 28.5 ± 5.2 kg/m^2	4 months aerobic exercise		Significant 98.4% ↓ in visfatin after 4 months of training
Buyukyazi et al [152]	37 pre-menopausal women, BMI (High intensity group-28.2±4.4, Moderate intensity group-29.3±2.5, Control group-27.3±4.9)	High and moderate intensity walking and control groups, 12 weeks for 5 days per week from 30-0 min/day	Decrease in body weight, percent body fat, BMI and waist circumference	↓ visfatin in high intensity walking group

Table 2.4 continued

Study, year	No. of participants	Duration	General Findings	Effects on Visfatin
Kadoglou et al [182]	100 patient with type-2 diabetes, BMI>25 kg/m2	4 weeks, aerobic and aerobic + resistance exercise	No significant changes in anthropometric measures	↓ visfatin
Choi et al [184]	48 overweight/obese women, BMI≥25 kg/m^2	12 week exercise, aerobic and muscle strength training	Visfatin levels were associated with BMI (R^2 = 0.255) and eotaxin levels were associated with WC and body weight (R^2 = 0.307)	43.4% ↓ in visfatin levels (13.6 ± 12.0 to 7.7 ± 7.9 ng/ml)
Walhin et al [180]	26 healthy young men, BMI 23.8 ± 2.5 kg m^{-2}	Two groups, Energy surplus and surplus +exercise. one had 45 min of daily treadmill running at 70% of maximum oxygen uptake and another group restricting their physical activity	Body mass, waist and hip circumference and lean mass were significantly increased at follow-up in both groups	Visfatin was down regulated in surplus group but no change in exercise and surplus

Table 2.4 continued

Study, year	No. of participants	Duration	General Findings	Effects on Visfatin
Sheu et al [185]	21 obese women with BMI 32.5 ± 1.2 kg/m^2 Versus 10 lean women served as controls	12-week caloric restriction and light exercise-based weight loss program	Significant reductions in BMI, fasting glucose and insulin concentrations, mean serum high-sensitivity C-reactive protein (hs-CRP), migration inhibitor factor (MIF), leptin and visfatin levels decreased by 49.0, 66.6, 17.2, and 50.2%, respectively	Significant 50.2% ↓ in visfatin after weight loss

BMI= Body mass Index; VO2max= Maximal Oxygen Consumption

Ounis et al [154] conducted a study on twenty seven 13 year old female adolescents with BMI greater than 97th percentile. They were divided into 3 groups: diet, individualize training and training combined with diet. After the 8 week intervention, there was decrease in BMI by 6.1, 1.5, 11.5%, and %BF by 7.3, 3.6, 15.1% in diet, training and diet/training groups respectively. Diet/training group had a significant decrease in leptin, TNF-α and IL-6, and a significant increase in adiponectin and resistin. The post program usual index of insulin resistance (HOMA-IR) was significantly improved in training ($p < 0.05$) and diet/training ($p < 0.01$) groups. Diet group did not present a significant reduction in HOMA-IR. The most important effect of these interventions of food intake and exercise was weight loss which led to improvement of body image. In this study, changes in resistin level were significantly correlated with changes in insulin sensitivity. The data supported the hypothesis that resistin opposes the insulin action and decrease of resistin is associated with improvement of insulin sensitivity.

Based on the numerous studies conducted in the past on overweight/obese women, combination of diet and exercise always results in the best reduction of body weight, probably with a greater negative energetic balance as compared to just diet or exercise intervention.

Conclusion

Metabolic homeostasis is maintained by multiple organ systems. Adipose tissue and muscles are a few of them. Adipocytes secrete hormones/chemicals known as adipokines which act on multiple cells or organs to regulate metabolism. The

understanding of the concentrations of these hormones in children, adolescents and adults including elderly and overweight/obese people as well as the role they may play in obesity, metabolic syndrome, diabetes, coronary, atherosclerotic diseases, hypertension etc. is imperative. It is also important to understand how lifestyle choices such as dietary intervention, regular exercise (aerobic or resistance), supplementation or combination of any of these affect adipokines/hormones concentrations so there is a better insight into their regulation and pathophysiology. Roles of resistin and visfatin in metabolism/homeostasis are not completely understood; given the interest in these hormones remain high. There are a very few studies published so far that have investigated the impact of weight loss on resistin and visfatin expression in a longitudinal study on sedentary obese healthy women. The purpose of this study is to examine the combined effects of exercise and diet-induced weight loss on markers of health and inflammation such as resistin and visfatin and their interactions among each other. The results of this study will provide additional information and data evaluating whether exercise and diet-induced weight loss affects inflammatory markers such as resistin and visfatin and their association with other anthropometric and inflammatory markers that will eventually provide an insight to better understand the inflammatory mechanism related to obesity and metabolic syndrome. We believe that this exercise and diet-induced weight loss program would alter these markers. This study will compare the results of overweight/obese population to control group.

CHAPTER III

METHODS AND MATERIALS

Study design and site

Data for this study were obtained from a large comparative effectiveness study evaluating different weight loss program approaches. Subjects were randomly assigned into non-exercise and non-diet control group (C) or an exercise and weight loss group (DE). This study was conducted for 90-days. In this study, all the subjects had a baseline testing session and post-training 12 week testing session (0 & 12 wk). Both the sessions included exercise testing. Exercise and diet-induced weight loss was the independent variable while body composition (body weight, fat mass, fat free mass and body fat percent), resistin, visfatin, glucose, insulin, leptin, TNF-α, IL-6, triglyceride and total cholesterol; maximum repetition 1(RM) and 80% 1RM isotonic strength were the dependent variables. Table 3.1 below shows the overall research design, time course for assessments, summary of the research design and the testing schedule for our 12 wk study program. Exercise & Sport Nutrition Laboratory (ESNL) in the Department of Health, and Kinesiology at Texas A&M University was the study site where all familiarization, testing, exercise training and assays were conducted.

Participants and familiarization sessions

Twenty six (26) overweight/obese sedentary women subjects (BMI > 27) between the ages of 18-65 were recruited to participate in this portion of the study. Participants with a history of electrolyte abnormalities, uncontrolled metabolic disorder,

arrhythmias, heart disease, diabetes, or thyroid disease; a history of hypertension, musculoskeletal, autoimmune, hepatorenal, or neurological disease were excluded from participation in the study. Other exclusion criteria included use of any hypoglycemic, or androgenic medications; and/or, 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 who met eligibility criteria of our study program were informed of the requirements of the study and requested to sign consent statements in compliance with the Human Participants Guidelines of Texas A&M University and the American College of Sports Medicine.

Individuals showing interest in the study were interviewed by phone to determine whether or not they were 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 information about testing procedures, blood collection procedures and were introduced to the equipment that would be used for the 12 wk study period.

Randomization

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

Table 3.1 Overview of research design and testing schedule

Familiarization and Entry	0 week (Baseline)	12 weeks
Phone interview	High Protein Diet Review	High Protein Diet Review
Familiarization session	Body Weight	Body Weight
Complete of paper work	Blood collection (white blood cells/serum)	Blood collection (white blood cells/serum)
Review medical history	Dual Energy X-ray	Dual Energy X-ray
Randomization into exercise and diet-induced weight loss and control programs	Absorptiometry (DEXA)	Absorptiometry (DEXA)
	Graded Exercise Testing (GXT)	Graded Exercise Testing (GXT)
	1RM at 80% 1RM Isotonic Bench and Isotonic Leg Press	1RM at80% 1RM Isotonic Bench and Isotonic Leg Press
	30 minutes Resistance Exercise/Zumba 4 times/week	30 minutes Resistance Exercise/Zumba 4 times/week

DEXA= Dual energy X-ray absorptiometry

Diet intervention

In the diet intervention program, subjects were instructed to follow Curves International (*Waco, TX*) Curves Complete high protein diet plan. The diet was implemented in two phases. First phase of the study includes higher protein weight loss diet (1,200 kcals/day) for one week and the second phase includes higher protein weight

loss diet (1,500 kcals/day) for 11 weeks. There were same micronutrient content: 45% protein, 30% carbohydrate, 25% fat in both phases of the study. Participants were encouraged to take multivitamins and minerals containing 800 mg of calcium and 520 mg of Omega-3 fatty acids everyday with food. A registered dietitian also met with subjects once a week throughout the study to discuss any dietary challenges or concerns and reviewed the higher protein diet (HPD), exercise and fitness and weight management plan provided to the participants along with food & exercise diary and Essentials-2-go dietary supplements. The Fitness and Weight Management Plan dietary intervention protocol is summarized in Table 3.2.

Training protocol

Subjects were randomized into the exercise and diet-induced weight loss group (DE) and control group (C). Subjects in DE group followed the Curves™ training program while subjects in the C group followed their normal eating and daily activities with no exercise. Subjects in DE group who underwent three regular resistance workouts each week for 12 weeks and one Zumba dance workout maintaining a greater than 90% compliance record (43 out of 48 workouts) were included in the physical training program. The circuit training platform utilized the computerized Curves Smart system. The smart system used the software designed by MYTRAK (*version 4.2.1, copyright 2004-2011, MYTRAK Health System Inc, Mississauga, Ontario, Canada*). The circuit training equipment was set in the Exercise and Sports Nutrition Laboratory at Texas A&M University. The circuit based exercise training comprising of 13 bi-directional hydraulic resistance exercise machines 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 training period, subjects were coached to perform as many repetitions as possible within 30-second time period on each resistance machine. Between machines, subjects performed floor-based aerobic exercises or stepping exercise between machines. It was done to maintain an increased heart rate. Subjects completed the entire circuit twice during the 26 minute regular circuit workout. During the Curves Zumba workout, subjects performed 1 minute of Zumba dance moves as taught by a certified Zumba instructor. The circuit training sessions were supervised by trained fitness instructors. The instructors aided the subjects with appropriate exercise technique and self-monitoring of heart rate so as to maintain heart rate between 60-80% of target heart rate using age-predicted maximal heart rate (220-age). Subjects also completed 4 minutes of whole body stretching following circuit workouts and were also encouraged to walk for 30 min at a brisk pace at 60-80% of target heart rate on one of the days when they were not using the curves equipment.

Testing methods

Participants were instructed to refrain from exercising for 48 hours and fasting for 12 hours prior to baseline testing. Participants reported to the ESNL for assessment of body composition and clinical assessments followed by completing a radiation questionnaire. Participants were measured for weight, assessed for body composition by using Dual Energy X-ray Absorptiometry (DEXA) and their blood samples were

collected. In addition, they were taught to perform cardiopulmonary symptom-limited maximal exercise stress test; and, perform 1 repetition maximum (RM) and 80% of 1RM on the isotonic bench press and leg press.

Table 3.2 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	Diet Intervention + Exercise	PRO CHO FAT	135 90 33	540 360 300	45 30 25
Phase II (11 weeks)	1,500 kcals/d	Diet Intervention + 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

PRO=Protein; CHO= Carbohydrate

Body composition

Standard anthropometry was used to measure height. Healthometer (*Bridgeview, IL, USA*) self-calibrating digital scale with a precision of +/-0.02 kg was used to determine total body weight. Hologic Discovery W QDR series DEXA (*Hologic Inc, Waltham, MA, USA*) equipped with APEX software (*APEX Corporation Software,*

Pittsburgh, PA, USA) was used to measure body composition. Participants were informed of any or all inherent hazards that could result from the radiation exposure and completed a radiation exposure questionnaire prior to all scans. Before each testing session quality control (QC) calibration procedures were performed on a spine phantom (*Discovery W-CALIBER Model DPA/QDR-1 anthropometric spine phantom*). The DEXA has been validated as an accurate method for assessing body composition [186, 187]. The mean test-retest reliability of studies performed on a few 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 [186].

Cardiopulmonary exercise tests

Cardiopulmonary exercise tests were performed at baseline and at 12 weeks using the standard procedures described by the American College of Sports Medicine's (ACSM) *Guidelines for Exercise Testing and Prescription,* [188]. Heart function was evaluated by the Nasiff Cardio Card electrocardiograph (*Nasiff Associates, Inc, Central Square, NY, USA*) using a standard 12-lead arrangement. Parvo Medics TrueMax 2400 Metabolic Measurement System (*ParvoMedics, Inc., Sandy, UT*) was used to assess cardiopulmonary measurements.

Following the speeds and inclination grades of the Parvo Medics TrueMax 2400 Metabolic Measurement System (*ParvoMedics, Inc., Sandy, UT*), the participants completed the Bruce or modified Bruce treadmill protocol [189]. Continuous monitoring of heart rate (HR), electrocardiographic tracings, and expired gases were carried out

throughout the exercise test. At the end of each stage, blood pressure (BP) and ratings of perceived exertion (RPE) were measured. The participants were instructed to exercise to their maximum unless they experienced clinical signs requiring test termination As stated by the ACSM's Guidelines for Exercise Testing and Prescription, participants were advised to exercise to their maximum except in events of experiencing clinical signs that required test termination [190]. Applying the Bruce or modified Bruce protocol, the mean coefficient of variation for assessing peak Vo_2 was found to be 6.5% (range, 2.0-14%). Determination of maximal aerobic capacity and anaerobic threshold, and thus measurement the effects of exercise and diet-induced weight loss training on markers of health and fitness on exercise capacity was the purpose of this test.

Isotonic strength tests

In the exercise and diet-induced weight loss program, participants' in DE group 1RM was determined using an isotonic Olympic bench press (*Nebula Fitness, Versailles, OH, USA)* and a standard hip sled/leg press (*Nebula Fitness, Versailles, OH, USA*). This was to observe changes in maximal strength according to standardized procedures. Standardization between trails was ensured by hand positioning on the bench press and foot and seat position on the hip sled/leg press. Using standard lifting techniques and testing criteria, muscular endurance was assessed by participants performing as many repetitions as possible at 80% of their predetermined 1RM on the bench press and leg press.

Using standard procedures and under the supervision of certified lab assistants experienced in conducting strength exercise testing, all strength/exercise tests were

executed. Participants were assessed for upper body strength and endurance. They did one repetition maximum (1RM) test on the isotonic bench press and the Nebula Fitness (*Versailles, OH, USA*) Olympic Power Station (*#1005*), and a warm-up (2 sets of 10 repetitions at approximately 50% of anticipated 1RM). The bench press and the warm-up were followed by progressive lifts starting at about 70% of anticipated 1RM and increasing by 5–10 lbs. until 1 RM was reached. Once the 1RM was achieved, participants performed as many repetitions as possible with 80% of their 1 RM efforts. Participants relaxed for 4 minutes, and then underwent a warm up 8-10 times at approximately 50% of anticipated maximum on the Nebula 45° Leg press. Starting at about 70% of anticipated 1RM and increasing by 10–25 lbs. until 1RM was achieved, participants performed successive lifts on the leg press. Once the 1RM was reached, subjects performed the lifts repeatedly and as many as possible with 80% of their 1 RM efforts. Test-retest reliability of carrying out these strength tests on resistance-trained subjects in our lab has shown 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 $r = 0.91$).

Blood collection and analysis

From each participant, approximately 2 teaspoons of fasting venous blood (10 milliliters) were obtained. Adhering to standard phlebotomy procedures, blood samples were collected using standard sterile venipuncture of an antecubital vein by ESNL laboratory technicians or graduate research assistants proficient in phlebotomy. Blood collection procedures were in compliance with guidelines laid down by the Texas

Department of Health and Human Services. While collecting and handling blood samples, the phlebotomists and lab technicians wore personal protective clothing (gloves, lab coats, etc.). From each participant, about 10 ml of whole blood were collected in EDTA tubes. The EDTA tube contains a gel that helps in formation of physical barrier between serum, white blood cells (buffy coat) and red blood cells during centrifugation. To spin down the blood cells, BD Vacutainer® SST™ Serum Separation Tubes were centrifuged immediately at 1,200g for 15min at 4^{o}C after blood collection. The supernatant (serum) were then shifted 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 labeled 1.5 ml Eppendorf tubes. Serum blood samples were sent to Quest Diagnostics (*Houston, TX*). Using an Olympus AAU 5400 Chemistry Immuno Analyzer (*Olympus America Inc., Center Valley, PA*), the blood samples were analyzed for comprehensive metabolic panel analysis. Using an Abbott Cell Dyn 3500 automated hematology analyzer (*Abbott Laboratories, Abbott Park, IL*) whole blood samples were examined for complete blood counts with platelet differentials. Reported test-retest reliability of carrying out these assays generally range from 2 to 6% for individual assays. Samples were run in duplicate to ascertain if the observed values were above control values and/or clinical norms in accordance 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) and inflammatory markers- resistin and visfatin.

Hormone analysis

Blood samples were analyzed to detect human resistin by using enzyme-linked immunosorbent assay for quantitative detention. Human resistin platinum ELISA kits (BMS2040) were purchased from Affymetrix Ebiosciences (Santa Clara, California, USA). Kits were stored at temperature 80^{o}C. Human Visfatin ELISA kits (RAB0377) were purchased from Sigma-Aldrich (St. Louis, Montana, USA). Resistin and visfatin kits were stored at temperature 80^{o}C. The resistin and visfatin Enzyme Immunoassay (EIA) Kits are an in vitro quantitative assay for detecting resistin and visfatin peptides respectively based on the principle of competitive enzyme immunoassay. The microplate in the kit is pre-coated with anti-rabbit secondary antibody. After a blocking step and incubation of the plate with anti-peptide antibody, both biotinylated resistin/visfatin peptide, and peptide standard or targeted peptide in samples interacts competitively with the resistin/visfatin antibody. Uncompeted (bound) biotinylated resistin/visfatin peptide then interacts with Streptavidin-horseradish peroxidase (SA-HRP), which catalyzes a color development reaction. The intensity of colorimetric signal is directly proportional to the amount of biotinylated peptide SA-HRP complex and inversely proportional to the amount of resistin/visfatin peptide in the standard or samples. This is due to the competitive binding to resistin/visfatin antibody between biotinylated resistin/visfatin peptide and peptides in standard or samples. A standard curve of known concentration of resistin/visfatin peptide was established and the concentration of resistin/visfatin peptide in the samples was calculated accordingly. After the analysis, microplates were read by microplate reader capable of measuring the hormone level at 450 nm absorbance.

Statistical analysis

Analysis of Variance (ANOVA) was performed on baseline variables (PASW Statistics version 22, 2013, SPSS Inc, Chicago, IL.) followed by Multivariate Analysis of Variance (MANOVA) with repeated measures were used to analyze the related data. Wilks' Lamda time and group x time p-levels as well as MANOVA univariate ANOVA group effects were used to study the overall MANOVA effects. Within the MANOVA model, Greenhouse-Geisser univariate tests of within-subjects time and group x time effects and between-subjects univariate group effects were reported for each variable analyzed. ANOVA for repeated measures were used to calculate and analyze delta values or percent difference on selected variables to examine variations from baseline values. Delta values were calculated by subtracting the baseline values from the 12^{th} week values. To calculate the percent change, previously calculated delta values were divided by T1 followed by multiplying by 100 [(T4-T1)/T1·100] when the probability of type I error was 0.05 or less then the data were considered statistically significant and statistical trends were considered when the probability error ranged between $p > 0.05$ to $p < 0.10$. All data are presented as means ± standard deviation for baseline to 12 wk data analyses. Finally, the relationship of changes in body composition to markers of inflammation were obtained by Pearson product correlation.

CHAPTER IV

RESULTS

Figure 4.1 presents a Consort Diagram for participant recruitment, entry, and completion observed in this portion of the study. Twenty six (26) sedentary obese women participated in this portion of study. All 26 obese sedentary women (Age 46.7±11.1 years, Body Fat 38.6±7.4, BMI 34.8±5.4 kg/m^2) completed baseline testing and were cleared to participate in the study. Some of the participants dropped out of the study. The primary reasons for participants to drop out were due to time constraints, work, moving, falling sick, complaints about the exercise program (that it was too intense) and they did not want to participate in the control group once they found out they were in the control group.

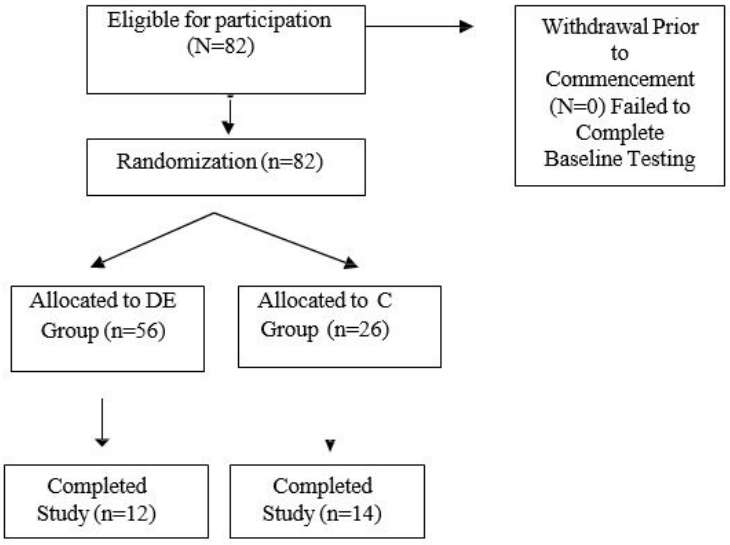

Figure 4.1: Consort diagram for participation.

Table 4.1 presents the participant demographics data. Twenty-Six obese women (age 46.7±11.1 years; weight 90.2±12.4 kg; BMI 34.8±5.4 kg/m^2; Body fat 38.6±7.4; Fat free mass 45.3±6.5; REE 0.90±0.05; body fat % 45.8±4.2;) completed this portion of the 12-week study (DE group n=12; C group n=14). One-way ANOVA no significant differences between groups in baseline age, weight, BMI, body fat, fat free mass, resting energy expenditure (REE) and percent body fat.

Table 4.1 Baseline demographics for the exercise and diet-induced weight loss (DE) and control (C) groups

	Exercise Group (N=12)	**Control Group (N=14)**	**P-value**
Age (years)	43.5±11.2	49.4±10.7	0.18
Weight (kg)	90.9±14.8	89.6±10.4	0.80
BMI[a] (kg/m²)	34.9±6.4	34.7±4.6	0.92
Body Fat (kg)	39.3±9.1	38.0±5.9	0.68
Fat Free Mass (kg)	45.3±5.6	45.4±7.5	0.96
REE[b]	0.90±0.04	0.90±0.06	0.78
Fat (%[c])	46.1±3.4	45.7±4.9	0.81
Waist/hip ratio	0.78±0.03	0.79±0.06	0.47
VAT[d] (kg)	0.90±0.43	1.04±0.48	0.45

Note: All data is presented as means ± SD at baseline.
[a] Body mass Index
[b] Resting Energy Expenditure
[c] Dual Energy X-ray Absorptiometry
[d] Visceral Adipose Tissue

Exercise and diet intervention effects

Table 4.2 Changes in protein intake values obtained at 0 week (baseline) and 12 weeks of program participation

	Group	0 weeks	12 weeks	Group	p-value (GG)
Total Calories (kcal)	DE	1696±395	1431±280*†	1563±112	G=0.014
	Control	2142±748	1793±508	1967±103†	T=0.037
	Time	1936±641	1626±450*		I=0.764
Carbohydrate (gm)	DE	182.7±85.0	119.6±28.6*†	151.1±16.4	G=0.014
	Control	236.6±107.4	183.8±58.3	210.2±15.2†	T=0.009
	Time	211.7±99.7	154.2±56.5*		I=0.802
Protein (gm)	DE	76.0±11.2	84.1±21.4	80.1±4.8	G=0.915
	Control	85.8±31.6	75.8±22.5	80.8±4.5	T=0.886
	Time	81.3±24.5	79.7±22.0		I=0.800
Fat (gm)	DE	66.9±16.5	60.2±19.9	63.5±5.6	G=0.075
	Control	82.4±31.3	73.1±22.5	77.7±5.2	T=0.144
	Time	75.2±26.3	67.2±21.9		I=0.169

Data are from 12 participants in the DE group and 14 participants in the C group (n=26) who completed the 12-week study. Data are presented for the Diet & Exercise Group (DE); Total calories; Carbohydrate;Protein and Fat. Data are individual group (G) and time (T) data expressed as means ± SD, while group effects are presented as means ± SEM. MANOVA analysis revealed overall Wilks' Lambda time (p=0.089) and group x time (p=0.564) effects. Univariate ANOVA p-levels from MANOVA analysis are presented for each variable. Greenhouse-Geisser (GG) univariate ANOVA p-levels are listed for G, T and GxT interactions effects. * indicates $p<0.05$ p-level significance from baseline. † represents $p<0.05$ difference between groups.

Table 4.2 represents the changes from baseline to 12-week between exercise and diet-induced weight loss and control group on total calories, carbohydrate, protein and fat intake in obese women. MANOVA analysis of all these above mentioned variables revealed overall time (Wilks' Lamda p=0.089) and time by diet effects (Wilks' Lamda p=0.564) which are not significant. Univariate ANOVA p-levels from MANOVA analysis are presented for each variable. Univariate analysis revealed significant time effects for total calories (p=0.037) and fat mass (p=0.009). Greenhouse-Geisser analysis showed significant time but not group x time interaction for total calories (T: p=0.037, I:

p=0.764) and carbohydrate intake (T: p=0.009, I: p=0.802). Greenhouse-Geisser analysis showed no significant time and group x time interaction for protein (T: p=0.886, I: p=0.800) and fat intake (T: p=0.144, I: p=0.169).

Table 4.3 and figure 4.2 represent the changes from baseline to 12-week between exercise and diet-induced weight loss and control group on body composition and anthropometric measurements in obese women respectively. MANOVA analysis of body composition data revealed overall time (Wilks' Lamda p=0.004) and time by diet effects (Wilks' Lamda p<0.001). Univariate analysis revealed significant time effects for body weight (p<0.001), fat mass (p<0.001), and percent body fat (p=0.005). Univariate ANOVA p-levels from MANOVA analysis are presented for each variable. Greenhouse-Geisser analysis showed significant time and group x time interaction for weight (T: p<0.001, I: p<0.001), fat mass (T: p=0.001, I: p<0.001), body fat percentage (T: p=0.005, I: p=0.002) and significant group x time interaction for visceral adipose tissue (T: p=0.041). Least significant difference post-hoc analysis revealed that participants in the DE group lost significantly more weight, fat mass, and body fat percent than the C group whose body composition values did not significantly change from baseline. Delta analysis from baseline values showed participants in the diet & exercise group lost more weight (DE: -5.9±4.0; C: 0.64±1.4 kg, p<0.001), fat mass (DE: -5.1±4.5; C: 0.4±1.5 kg, p=0.001) and body fat percentage (DE: -3.4±3.3; C: 0.18±1.9 kg, p=0.005). No differences between groups were observed changes in FFM (DE: -0.40±1.4; C: 0.13±2.0 kg, p=0.695), changes in waist to hip ratio (DE: 0.001±0.02; C: 0.01±0.06 kg, p=0.570) and changes in visceral adipose tissue (DE: -180.7±203.5; C: -62.1±339.9 kg, p=0.302).

Based on these findings, hypothesis H_1 indicating that there would be significant differences between the DE and C groups in body composition variables was accepted. The exercise and diet intervention promoted statistically significant differences in body composition measures compared to controls.

The significant changes in body composition observed in response to the DE intervention leads us to further evaluate the effect of diet and exercise on blood markers such as glucose, triglyceride and insulin, inflammatory markers like IL-6 and TNF-α and adipose hormones such as leptin, visfatin and resistin and their correlations among each other to better understand their physiologic roles.

Blood and inflammatory markers and hormone levels

Table 4.4 Changes in blood markers, inflammatory markers and hormones values obtained at 0 week (baseline) and 12 weeks of program participation in obese women. MANOVA analysis of glucose and insulin revealed overall Wilks' Lambda time ($p=0.149$) and group x time ($p=0.199$) effects, IL-6 and TNF- α revealed overall Wilks' Lambda time ($p=0.097$) and group x time ($p<0.001$) effects and leptin, resistin and visfatin revealed overall Wilks' Lambda time ($p=0.053$) and group x time ($p=0.028$) effects. Univariate analysis revealed significant time effects for TNF- α ($p=0.039$) and tend to be significant for insulin ($p=0.077$), leptin ($p=0.052$), and visfatin ($p=0.078$). Univariate ANOVA p-levels from MANOVA analysis are presented for each variable. Univariate ANOVA p-levels are listed by the Greenhouse-Geisser (GG)

Table 4.3 Changes in anthropometric measures values obtained at 0 week (baseline) and 12 weeks of program participation

Variable	Group	0 weeks	12 weeks	Group	p-value (GG)
Weight (kg)	DE	90.9±14.8	85.0±14.1*†	87.9±3.6	G<0.001
	Control	89.6±10.4	90.2±10.7	89.9±3.3†	T<0.001
	Time	90.2±12.4	87.8±12.4*		I<0.001
FM (kg)	DE	39.3±9.1	34.1±8.8*†	36.7±2.1	G<0.001
	Control	38.0±5.9	38.5±5.5	38.3±1.9†	T<0.001
	Time	38.6±7.4	36.5±7.4*		I<0.001
FFM (kg)	DE	45.3±5.6	44.9±5.8	45.1±1.9	G=0.437
	Control	45.4±7.5	45.5±6.6	45.5±17	T=0.695
	Time	45.3±6.5	45.2±6.14.90		I=0.437
BF (%)	DE	46.1±3.4	42.7±5.0*†	45.8±0.8	G=0.002
	Control	45.7±4.9	45.8±3.7	44.4±0.9†	T=0.005
	Time	45.8±4.2	44.4±4.6*		I=0.002
W/H Ratio	DE	0.78±0.03	0.78±0.03	0.78±0.01	G=0.631
	Control	0.79±0.06	0.80±0.06	0.80±0.01	T=0.570
	Time	0.79±0.05	0.79±0.05		I=0.631
VAT (kg)	DE	0.90±0.43	0.72±0.32*	0.81±0.11	G=0.293
	Control	1.04±0.48	0.98±0.30	1.01±0.01	T=0.041
	Time	0.97±0.46	0.86±0.33		I=0.302

Data are from 12 participants in the DE group and 14 participants in the C group (n=26) who completed the 12-week study. Data are presented for the Diet & Exercise Group (DE); fat mass (FM) Fat Free Mass (FFM); percent body fat percentage (BF), and waist to hip (W/H) ratio. Data are individual group (G) and time (T) data expressed as means ± SD, while group effects are presented as means ± SEM. MANOVA analysis revealed overall Wilks' Lambda time ($p=0.004$) and group x time ($p<0.001$) effects. Univariate ANOVA p-levels from MANOVA analysis are presented for each variable. Greenhouse-Geisser (GG) univariate ANOVA p-levels are listed for G, T and GxT interactions effects. * indicates $p<0.05$ p-level significance from baseline. † represents $p<0.05$ difference between groups.

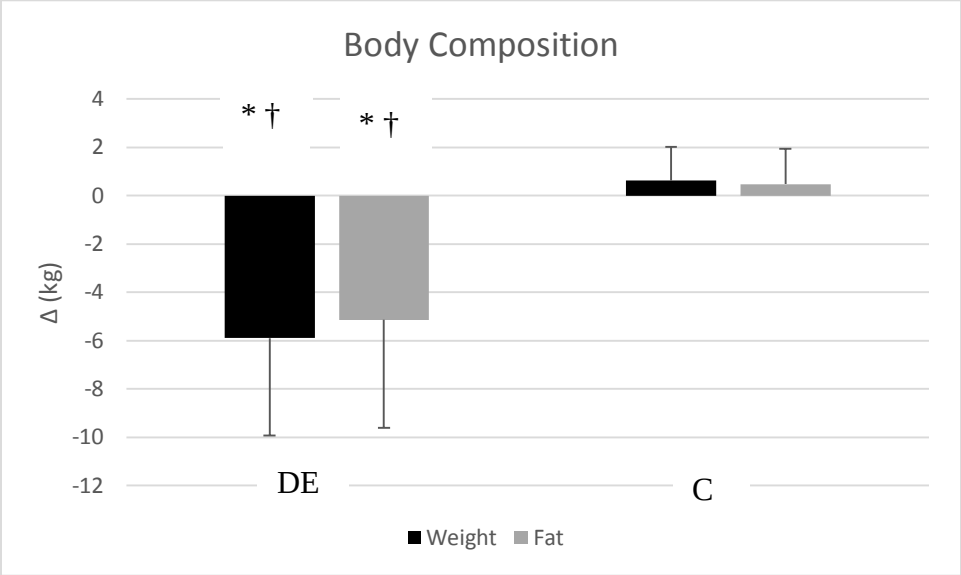

Figure 4.2. Changes in body composition from baseline to 12-wk between exercise and diet-induced weight loss and control groups Data are presented as means ±SD of markers of fitness. *P<0.05 significant differences from baseline to 12-wk between the exercise and diet-induced weight loss and control groups on body composition. † represents p<0.05 difference between groups

analysis to demonstrate the potential shape of the time or group x time interaction. Greenhouse-Geisser analysis showed significant time and group x time interaction for TNF- α (T: p=0.039, I: p==0.013), significant group x time interaction for IL-6 (I: p= 0.001) and leptin (I: p=0.003) and tend to be significant time and group x time interaction for insulin (T: p=0.077, I: p==0.070), time for leptin (T: p=0.052) and time for visfatin (T; p=0.078). Figure 4.3, 4.4, 4.5, 4.6 & 4.7 represent changes from baseline to 12 –wk between our exercise and diet-induced weight loss and control group on leptin, IL-6, TNF-α resistin and visfatin.

Figures 4.3 through 4.7 present changes in hormones evaluated for the DE and C groups. Furthermore, participants in the diet & exercise group showed more decrease in glucose (DE: -3.8±19.8; C: -2.8±6.9 mg/dl, p=0.865), insulin (DE: -8.5±15.0; C:

0.12±7.7 IU/ml, p=0.070), IL-6 (DE: -1.9±4.2; C: 2.7±1.2 kg, p<0.001), TNF- α (DE: -0.2±2.1; C: 1.7±1.5 kg, p<0.001) and leptin (DE: -19.8±21.9; C: 4.5±16.0 kg, p=0.003). Based on the MANOVA analysis, we can say that exercise and diet-induced weight loss has a significant effect on insulin, leptin, IL-6 and TNF-α during the period of 12 week intervention.

H_2 hypothesis stated exercise and diet intervention would promote statistical significant differences in resistin level compared to controls. Based on resistin (P=0.452) data observed, we therefore rejected our H_2 hypothesis. We also hypothesized ($H_{3)}$ that exercise and diet intervention promoted significant differences in visfatin expression compared to the control. Based on our results, we found visfatin was not statistically significantly different (P=0.104) but they tended to differ. Therefore, we rejected our hypothesis H_3.

We also analyzed the relationship among the anthropometric measures, inflammatory and blood health markers at baseline and 12 week level. Table 4.4 and 4.5 provide overview of correlations among different markers. We found a significant negative relationship between resistin and TNF-α (r=0.401: p=0.042) at baseline level and significant positive correlation between resistin with IL-6 (r=0.430: p=0.028) and visfatin (r=0.417: p=0.034) at 12 week level. We did not find any other significant correlation of resistin and visfatin with other anthropometric measures, inflammatory and blood health markers at baseline and 12 week level

Table 4.4 Changes in blood markers, inflammatory markers and hormones values obtained at 0 week (baseline) and 12 weeks of program participation

Variable	Group	0 week	12 weeks	Group Mean	p-value (GG)
Glucose (mg/dl)	DE	99.6±21.5	95.8±13.4	97.7±3.5	G=0.865
	Control	96.0±10.9	93.2±9.2	94.6±3.3	T=0.257
	Time	97.7±16.4	94.4±11.2		I=0.865
Insulin (IU/ml)	DE	27.3±45.7	18.8±31.5	23.1±7.6	G=0.070
	Control	11.1±8.1	11.2±5.4	11.2±7.1	T=0.077
	Time	18.6±31.9	14.7±21.6		I=0.07
IL-6 (ng/ml)	DE	7.2±1.5†	5.3±1.7*	6.21±0.4	G<0.001
	Control	3.4±1.3	6.2±1.5*	4.81±0.4†	T=0.484
	Time	5.2±3.2	5.8±1.6		I=0.001
TNF-α (ng/ml)	DE	7.2±1.5†	7.0±1.4	7.07±0.3	G<0.001
	Control	5.6±1.6	7.3±.6	6.43±0.3	T=0.039
	Time	6.3±1.8	7.2±1.0		I=0.013
Leptin (ng/ml)	DE	56.2±26.9	36.4±23.1*	46.3±6.6	G=0.003
	Control	54.0±19.5	58.5±28.9	56.2±6.1†	T=0.052
	Time	55.0±22.8	48.3±28.2†		I=0.003
Resistin (ng/ml)	DE	223.8±108.2	242.4±127	233.1±35.7	G=0.452
	Control	233.3±136.2	293.1±178.4	263.2±33.1	T=0.159
	Time	228.9±121.8	269.7±156		I=0.452
Visfatin (ng/ml)	DE	24.1±13.2	25.0±11.4	24.5±14.1	G=0.104
	Control	46.2±51.1	66.4±82.1	56.3±13.1	T=0.078
	Time	36.0±39.5	47.3±63.3		I=0.104

Data are from 12 participants in the DE group and 14 participants in the C group (n=26) who completed the 12-week study. Data are presented for the Diet & Exercise Group (DE); Interleukin-6 (IL-6) and Tumor Necrosis Factor- Alpha (TNF- α). Data are individual group (G) and time (T) data expressed as means ± SD, while group effects are presented as means ± SEM. MANOVA analysis of glucose and insulin revealed overall Wilks' Lambda time (p=0.149) and group x time (p=0.199) effects, IL-6 and TNF- α revealed overall Wilks' Lambda time (p=0.097) and group x time (p<0.001) effects and leptin, resistin and visfatin revealed overall Wilks' Lambda time (p=0.053) and group x time (p=0.028) effects. Univariate ANOVA p-levels from MANOVA analysis are presented for each variable. Greenhouse-Geisser (GG) univariate ANOVA p-levels are listed for G, T and GxT interactions effects. * indicates p<0.05 p-level significance from baseline. † represents p<0.05 difference between groups.

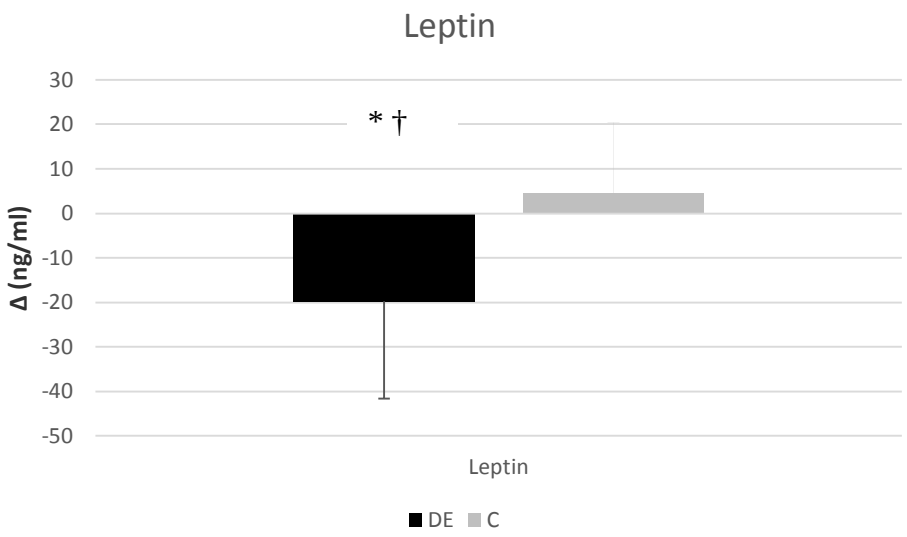

Figure 4.3. Changes in leptin from baseline to 12-wk between exercise and diet-induced weight loss and control groups Data are presented as means ±SD of markers of fitness. *P<0.05 significant differences from baseline to 12-wk between the exercise and diet-induced weight loss and control groups on leptin. † represents p<0.05 difference between groups.

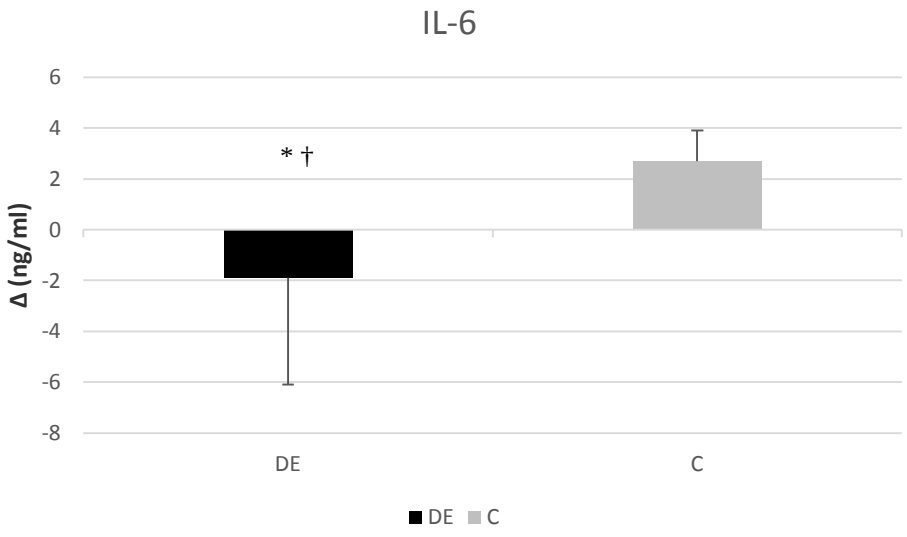

Figure 4.4. Changes in IL-6 from baseline to 12-wk between exercise and diet-induced weight loss and control groups Data are presented as means ±SD of markers of fitness. *P<0.05 significant differences from baseline to 12-wk between the exercise and diet-induced weight loss and control groups on IL-6. † represents p<0.05 difference between groups.

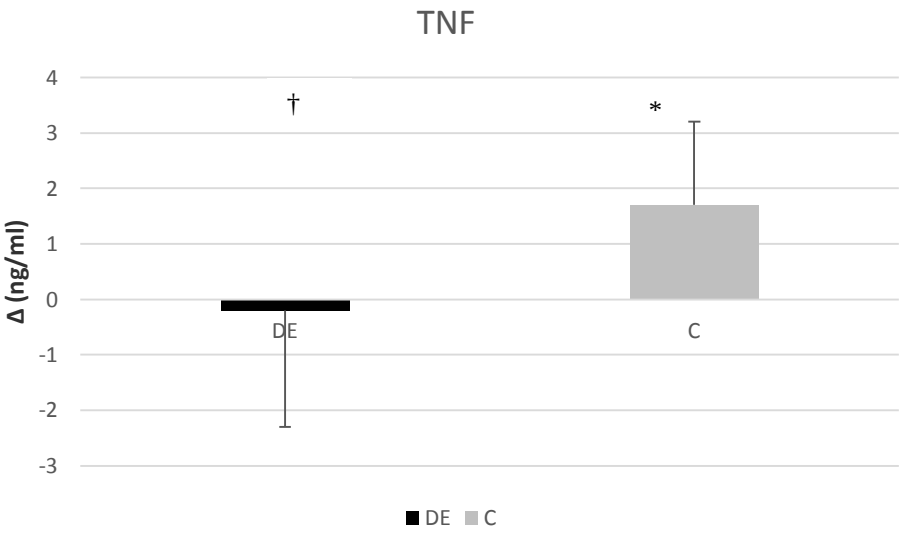

Figure 4.5. Changes in TNF-α from baseline to 12-wk between exercise and diet-induced weight loss and control groups Data are presented as means ±SD of markers of fitness. *P<0.05 significant differences from baseline to 12-wk between the exercise and diet-induced weight loss and control groups on TNF-α. † represents p<0.05 difference between groups.

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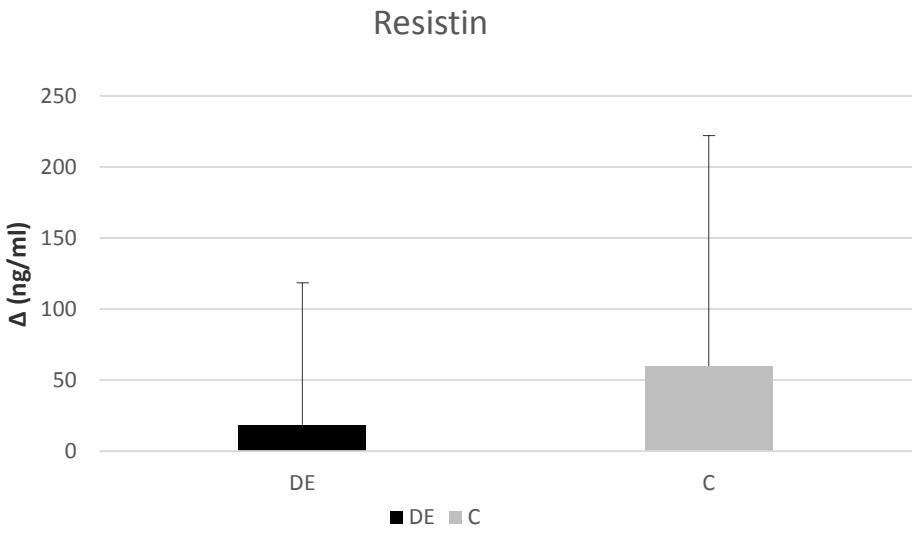

Figure 4.6. Changes in resistin from baseline to 12-wk between exercise and diet-induced weight loss and control groups Data are presented as means ±SD of markers of fitness. *P<0.05 significant differences from baseline to 12-wk between the exercise and diet-induced weight loss and control groups on resistin. † represents p<0.05 difference between groups.

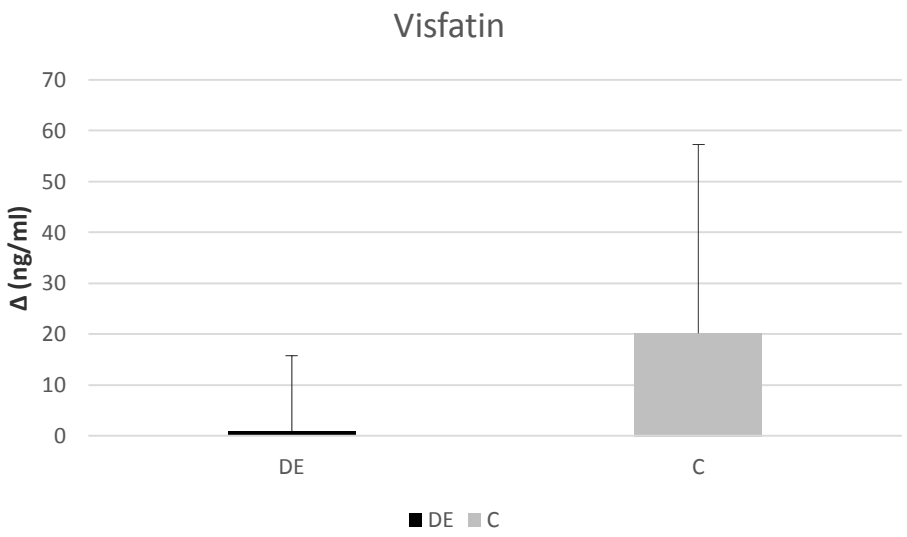

Figure 4.7. Changes in visfatin from baseline to 12-wk between exercise and diet-induced weight loss and control groups Data are presented as means ±SD of markers of fitness. *P<0.05 significant differences from baseline to 12-wk between the exercise and diet-induced weight loss and control groups on visfatin. † represents p<0.05 difference between groups.

Furthermore, we analyzed the relationship among changes in anthropometric measures, blood health markers, inflammatory markers and adipose hormones level. Table 4.6 provides overview of correlations among different inflammatory markers. We found significant positive correlations between Δ weight and Δ fat (r= 0.919, p<0.001), Δ weight and Δ triglyceride (r= 0.397, p=0.045), Δ weight and Δ insulin (r= 0.397, p=0.045), Δ weight and Δ leptin (r= 0.785, p<0.001), Δ fat and Δ leptin (r= 0.681, p<0.001), Δ FFM and Δ insulin (r=0.501, p=0.009), Δ REE and Δ Waist/hip ratio (r=0.391, p=0.049), Δ REE and Δ FFM (r= 0.624, p=0.001), Δ triglyceride and Δ leptin (r=0.421, p=0.032), Δ insulin and Δ leptin (r=0.425, p=0.03), Δ IL-6 and Δ resistin (r=0.430, p=0.028), Δ resistin and Δ visfatin (r=0.417, p=0.034).

Table 4.5 Overview of correlations among different inflammatory markers at baseline level

	Weight	Fat	FFM	Waist/hip ratio	REE	VAT	Carbohydrate	Fat	Protein	Triglyceride	Glucose	Insulin	IL-6	TNF- α	Leptin	Resistin	Visfatin
Weight		0.870 p<0.001	0.829 p<0.001	0.343 p=0.086	-0.020 p=0.924	0.636 P<0.001	-0.128 p=0.535	-0.168 p=0.413	0.001 p=0.997	0.019 p=0.928	0.235 p=0.247	0.178 p=0.385	0.007 p=0.973	-0.174 p=0.395	0.640 p<0.001	0.072 p=0.726	-0.341 p=0.088
Fat			0.449 p=0.021	0.068 p=0.741	-0.120 p=0.558	0.734 p<0.001	-0.062 p=0.763	-0.051 p=0.804	0.061 p=0.768	0.-0.183 p=0.372	0.269 p=0.185	0.094 p=0.648	0.003 p=0.989	-0.299 p=0.138	0.718 p<0.001	0.058 p=0.777	-0.341 p=0.088
FFM				0.550 p=0.004	0.100 p=0.628	0.348 p=0.081	-0.139 p=0.497	-0.241 p=0.235	-0.080 p=0.699	0.256 p=0.206	0.144 p=0.484	0.200 p=0.328	-0.003 p=0.988	0.018 p=0.932	0.345 p=0.084	0.064 p=0.757	-0.236 p=0.246
Waist/hip ratio					0.069 p=0.737	0.209 p=0.305	0.091 p=0.091	-0.164 p=0.424	-0.075 p=0.718	0.265 p=0.191	0.147 p=0.473	-0.021 p=0.918	0.061 p=0.767	0.206 p=0.313	0.068 p=0.742	-0.138 p=0.501	-0.265 p=0.191
REE						-0.098 p=0.634	0.014 p=0.944	0.041 p=0.844	-0.189 p=0.354	0.139 p=0.500	0.269 p=0.184	0.238 p=0.242	-0.024 p=0.906	0.347 p=0.082	0.183 p=0.370	0.016 p=0.938	0.137 p=0.505
VAT							0.062 p=0.765	-0.121 p=0.556	-0.257 p=0.205	0.035 p=0.865	0.311 p=0.122	0.143 p=0.485	-0.162 p=0.428	-0.209 p=0.306	0.507 p=0.008	-0.086 p=0.675	-0.213 p=0.296
Carbohydrate								-0.062 p=0.763	0.493 p=0.011	-0.129 p=0.531	-0.031 p=0.882	-0.017 p=0.936	-0.075 p=0.716	-0.374 p=0.060	-0.066 P=0.748	0.472 p=0.015	-0.085 p=0.679
Fat									0.340 p=0.089	-0.146 p=0.475	0.354 p=0.076	-0.028 p=0.892	-0.080 p=0.696	-0.484 p=0.012	0.087 p=0.673	0.272 p=0.179	-0.100 p=0.626
Protein										-0.314 p=0.118	-0.041 p=0.843	-0.004 p=0.986	-0.139 p=0.500	-0.451 p=0.021	-0.011 p=0.956	0.365 p=0.067	0.010 p=0.962
Triglyceride											0.269 p=0.184	-0.095 p=0.644	-0.139 p=0.497	0.156 p=0.445	0.093 p=0.650	-0.371 p=0.062	0.184 p=0.368
Glucose												0.302 p=0.133	0.036 p=0.863	-0.041 p=0.842	0.237 p=0.243	-0.365 p=0.067	-0.113 p=0.582
Insulin													0.050 p=0.808	0.086 p=0.676	0.111 p=0.590	0.093 p=0.652	-0.080 p=0.699
IL-6														0.234 p=0.249	-0.207 p=0.310	0.195 p=0.340	-0.352 P=0.078
TNF-α															-0.042 p=0.840	-0.401 P=0.042	0.082 P=0.690
Leptin																0.102 p=0.621	-0.168 p=0.412
Resistin																	-0.156 p=0.447
Visfatin																	

p<0.05

p>0.05 – p<0.10

*P<0.05 significant correlation.

Table 4.6 Overview of correlations among different inflammatory markers at 12 week level

	Weight	Fat	FFM	Waist/hip ration	REE	VAT	Carbohydrate	Fat	Protein	Triglyceride	Glucose	Insulin	IL-6	TNF- α	Leptin	Resistin	Visfatin
Weight		0.919 P<0.001	0.288 p=0.153	0.106 p=0.607	0.137 p=0.504	0.792 p<0.001	0.122 p=0.554	0.256 p=0.207	0.273 p=0.178	0.397 p=0.045	-0.108 p=0.601	0.397 p=0.045	0.284 p=0.160	0.401 p=0.042	0.785 p<0.001	0.054 p=0.793	0.327 p=0.103
Fat			-0.077 p=0.708	0.016 p=0.938	-0.084 p=0.682	0.760 p<0.001	0.234 p=0.251	0.300 p=0.137	0.760 p<0.001	0.194 p=0.342	-0.152 p=0.459	0.181 p=0.375	0.295 p=0.143	0.344 p=0.085	0.681 p<0.001	0.112 p=0.587	0.348 p=0.081
FFM				0.310 p=0.123	0.624 p=0.001	0.627 p<0.001	-0.060 p=0.771	0.144 p=0.482	0.410 p=0.037	0.509 p=0.008	0.112 p=0.586	0.501 p=0.009	-0.084 p=0.685	0.238 p=0.241	0.332 p=0.098	-0.121 p=0.555	-0.026 p=0.900
Waist/hip ratio					0.391 p=0.049	0.482 P=0.013	-0.109 p=0.596	-0.166 p=0.418	-0.189 p=0.356	0.056 p=0.786	0.075 p=0.717	0.111 p=0.590	-0.044 p=0.830	0.219 p=0.282	0.377 p=0.058	-0.051 p=0.806	0.125 p=0.542
REE						0.414 p=0.035	0.408 p=0.039	0.312 p=0.121	-0.163 p=0.426	0.245 p=0.228	-0.002 p=0.993	0.446 p=0.022	-0.064 p=0.755	0.312 p=0.121	0.218 p=0.285	-0.188 p=0.357	0.108 p=0.412
VAT							0.282 p=0.162	0.205 p=0.316	0.162 p=0.428	0.458 p=0.019	0.175 p=0.392	0.002 p=0.992	-0.275 p=0.173	0.018 p=0.931	0.452 P=0.020	-0.165 p=0.420	0.018 p=0.931
Carbohydrate								0.597 p<0.001	0.004 p=0.986	0.151 p=0.462	-0.045 p=0.827	-0.112 p=0.585	0.100 p=0.628	0.168 p=0.412	0.360 p=0.070	0.296 p=0.142	0.078 p=0.704
Fat									0.497 p=0.010	0.169 p=0.410	0.081 p=0.694	-0.116 p=0.574	-0.130 p=0.527	-0.246 p=0.227	0.271 p=0.181	0.126 p=0.538	0.175 p=0.393
Protein										0.324 p=0.106	0.305 p=0.129	-0.118 p=0.565	-0.297 p=0.141	-0.413 p=0.036	-0.126 p=0.540	-0.254 p=0.210	-0.123 p=0.548
Triglyceride											0.043 p=0.833	0.205 p=0.314	-0.043 p=0.835	0.275 p=0.174	0.421 p=0.032	-0.058 p=0.779	0.212 p=0.298
Glucose												0.122 p=0.552	0.073 p=0.724	0.174 p=0.395	-0.073 p=0.723	0.057 p=0.782	0.072 p=0.727
Insulin													0.174 p=0.395	0.183 p=0.370	0.425 p=0.030	0.137 p=0.505	0.044 p=0.832
IL-6														0.270 p=0.183	0.019 p=0.927	0.430 p=0.028	0.351 P=0.078
TNF-α															0.341 p=0.089	0.064 P=0.756	0.203 P=0.319
Leptin																0.064 p=0.755	0.342 p=0.087
Resistin																	0.417 p=0.034
Visfatin																	

p<0.05

p>0.05 – p<0.10

*P<0.05 significant correlation.

Table 4.7 Overview of correlations among different inflammatory markers

	Δ Weight	Δ Fat	Δ FFM	Δ Waist/hip ratio	Δ REE	Δ VAT	Δ Protein	Δ Carbohydrate	Δ Fat	Δ Triglyceride	Δ Glucose	Δ Insulin	Δ IL-6	Δ TNF- α	Δ Leptin	Δ Resistin	Δ Visfatin
Δ Weight		0.919 p<0.001	0.288 p=0.153	0.106 p=0.607	0.137 p=0.504	0.368 p=0.064	-0.252 p=0.215	0.206 p=0.312	0.019 p=0.926	0.397 p=0.045	-0.108 p=0.601	0.397 p=0.045	0.284 p=0.160	0.401 p=0.042	0.785 p<0.001	0.054 p=0.793	0.327 p=0.103
Δ Fat			-0.077 p=0.708	0.016 p=0.938	-0.084 p=0.682	0.427 p=0.029	-0.200 p=0.327	0.154 p=0.453	0.052 p=0.801	0.194 p=0.342	-0.152 p=0.459	0.181 p=0.375	0.295 p=0.143	0.344 p=0.085	0.681 p<0.001	0.112 p=0.587	0.348 p=0.081
Δ FFM				0.310 p=0.123	0.624 p<0.001	-0.203 p=0.321	-0.185 p=0.367	0.181 p=0.376	-0.016 p=0.937	0.509 p=0.008	0.112 p=0.586	0.501 p=0.009	-0.084 p=0.685	0.238 p=0.241	0.332 p=0.098	-0.121 p=0.555	-0.026 p=0.900
Δ Waist/hip ratio					0.391 p=0.049	-0.519 p=0.007	-0.094 p=0.647	-0.124 p=0.547	-0.190 p=0.353	0.056 p=0.786	0.075 p=0.717	0.111 p=0.590	-0.044 p=0.830	0.219 p=0.282	0.377 p=0.058	-0.051 p=806	0.125 p=0.542
Δ REE						-0.167 p=0.413	-0.105 p=0.610	-0.017 p=0.933	-0.114 p=0.580	0.245 p=0.228	-0.002 p=0.993	0.446 p=0.022	-0.064 p=0.755	0.312 p=0.121	0.218 p=0.285	-0.188 p=0.357	0.108 p=0.412
ΔVAT							-0.159 p=0.439	0.203 p=0.320	-0.059 p=0.776	0.014 p=0.946	-0.095 p=0.644	0.143 p=0.487	0.101 p=0.622	-0.007 p=0.974	0.091 p=0.657	0.136 p=0.508	0.200 p=0.328
Δ Protein								0.471 p=0.015	0.513 p=0.007	-0.220 p=0.280	-0.026 p=0.902	-0.074 p=0.719	-0.175 p=0.392	-0.527 p=0.006	-0.373 p=0.061	-0.157 p=0.444	-0.326 p=0.104
Δ Carbohydrate									0.519 p=0.007	0.052 p=0.800	-0.084 p=0.683	0.113 p=0.582	0.121 p=0.557	-0.201 p=0.326	-0.139 p=0.500	0.129 p=0.530	-0.034 p=0.868
Δ Fat										-0.050 p=0.809	0.176 p=0.388	-0.025 p=0.902	-0.105 p=0.609	-0.205 p=0.315	-0.244 p=0.229	0.010 p=0.960	0.223 p=0.274
Δ Triglyceride											0.043 p=0.833	0.205 p=0.314	-0.043 p=0.835	0.275 p=0.174	0.421 p=0.032	-0.058 p=0.779	0.212 p=0.298
Δ Glucose												0.122 p=0.552	0.073 p=0.724	0.174 p=0.395	-0.073 p=0.723	0.057 p=0.782	0.072 p=0.727
Δ Insulin													0.174 p=0.395	0.183 p=0.370	0.425 p=0.030	0.137 p=0.505	0.044 p=0.832
Δ IL-6														0.270 p=0.183	0.019 p=0.927	0.430 p=0.028	0.351 P=0.078
Δ TNF-α															0.341 p=0.089	0.064 P=0.756	0.203 P=0.319
Δ Leptin																0.064 p=0.755	0.342 p=0.087
Δ Resistin																	0.417 p=0.034
Δ Visfatin																	

p<0.05; p>0.05 – p<0.10

Δ = Post value (12 weeks) - Pre value (0 weeks). *P<0.05 significant correlation.

We hypothesized (H_4) that there will be significant correlation between Δ resistin and Δ visfatin. Based on our result, r=-0.417. p=0.034, we accepted our hypothesis H_4 but we did not find any significant correlation between Δ resistin and Δ leptin so we rejected hypothesis H_5, r=-0.064, p=0.755. Figure 8 shows correlation data between Δ resistin and Δ visfatin. We found that Δ visfatin were tended to correlate to Δ leptin but not statistically significant, r=0.342, p=0.087. Therefore, we rejected our hypothesis H_6.

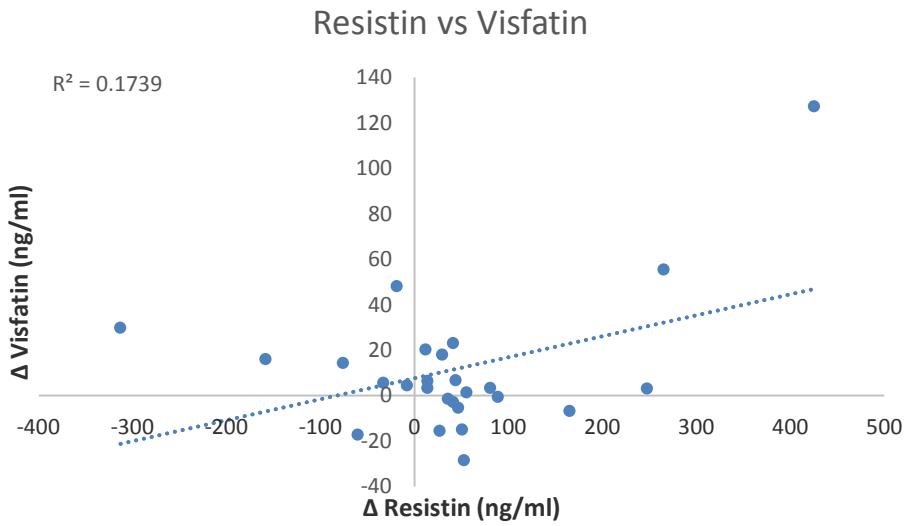

Figure 4.8. Significant correlation data between Δ resistin and Δ visfatin.

We did not find statistically significant correlation between Δ resistin and Δ TNF-α r=-0.064, p=0.756. So, we rejected hypothesis H_7. Correlation data revealed that Δ visfatin and Δ TNF-α (r=0.351; p=0.078), Δ visfatin and Δ triglyceride (r=0.212; p=0.298) and Δ visfatin and Δ waist/hip ratio were not correlated. Hence, we rejected hypotheses H_8, H_9 and H_{10} respectively. We also did not find significant correlation between Δ resistin and Δ waist/hip ratio (r=-0.051; p=0.806), Δ resistin and Δ

triglyceride (r=-0.058; p=0.779) and Δ resistin and Δ REE (r=-0.188; p=0.357). Therefore, we rejected the hypotheses H_{11}, H_{12} and H_{13} respectively.

We also looked at the correlations between Δ visfatin and Δ REE (r=-0.168; p=0.412), could not find any significant correlation so we rejected hypothesis H_{14}. We found a strong positive significant correlation between Δ resistin and Δ IL-6 (r=0.430; p=0.028). Hence, we accepted hypothesis H_{15}. Figure 9 shows correlation data between Δ resistin and Δ IL-6.

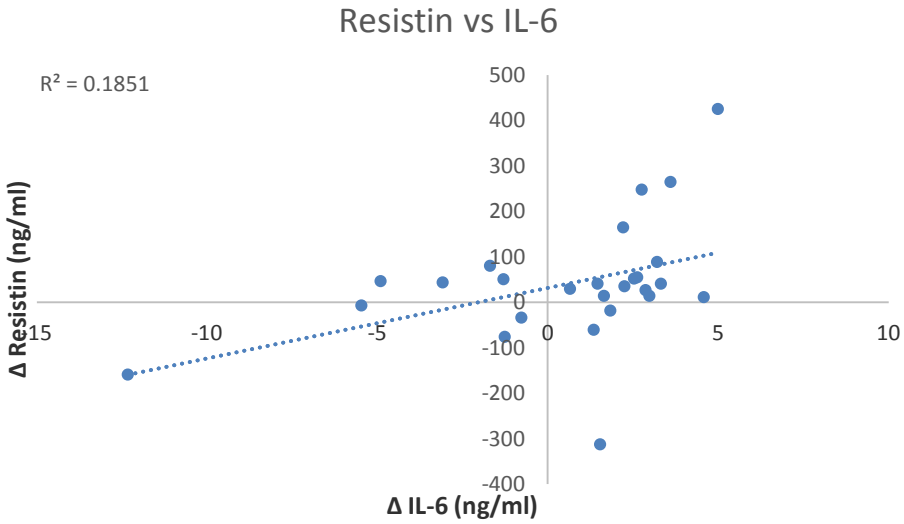

Figure 4.9. Significant correlation data between Δ IL-6 and Δ resistin.

Finally, we could not find significant correlation between Δ visfatin and Δ IL-6 (r=0.203; p=0.319). So, we rejected hypothesis H_{16}. In this study we analyzed the effect of exercise and diet-induced weight loss on inflammatory markers, resistin and visfatin in sedentary obese women. We found significant decrease in weight, fat mass and body fat % in DE group as compared to control group. We also found significant differences

between groups in leptin, IL-6 and TNF-α while visfatin and insulin tended to differ and no significant differences between groups in resistin and glucose. However, significant positive correlations were seen between changes in resistin with changes in IL-6 and changes in visfatin. These findings indicate that exercise and diet-induced weight loss has an effect on resistin and visfatin. We also looked at the correlation between visceral adipose tissue with anthropometric measures, inflammatory and other blood health markers at baseline, 12 week and changes between post and pre values. We found significant positive correlation between visceral adipose tissue with weight($r=0.636$: $p<0.001$), fat ($r=0.734$: $p<0.001$) and leptin ($r=0.507$: $p=0.008$) at baseline level and significant positive correlation with weight ($r=0.792$: $p<0.001$), fat ($r=0.760$: $p<0.001$), fat free mass ($r=0.627$: $p<0.001$), waist/hip ratio ($r=0.482$: $p=0.013$), REE ($r=0.414$: $p=0.035$), triglyceride ($r=0.458$: $r=0.019$) and leptin ($r=0.452$: $p=0.020$) at 12 week of intervention. We also found significant positive correlation between changes in visceral adipose tissue with changes in fat ($r=0.427$: $p=0.029$) and significant negative correlation between changes in waist/hip ratio ($r=-0.519$: $p=0.007$) after 12 week of exercise and dietary intervention.

Furthermore, we looked at the correlation between changes in protein intake with changes in anthropometric measures, inflammatory and other blood health markers between the groups. We found significant negative correlation between protein intake and TNF-α ($r=-0.451$: $p=0.021$) at baseline level and positive correlation between protein intake with fat ($r=0.760$: $p<0.001$), fat free mass ($r=0.410$: $p=0.037$) and negatively correlated with TNF-α ($r=-0.413$: $p=0.036$). Consequently, we also found

significant negative correlation between changes in protein intake with changes in TNF-α (r=-0.527: p=0.006). We did not find significant correlation between changes in protein intake with changes in visaftin (r=-0.326, p=0.104) and changes in resistin (r=-0.157, p=0.444.

CHAPTER V

DISCUSSION AND CONCLUSION

Obesity has been a major public health crisis from the last few decades due to mainly inexpensive food supplies and sedentary jobs [191]. Previous studies [2, 4, 5] have shown an association between central and total obesity with different inflammatory markers, but this association has not been fully comprehended. Obesity is a chronic low grade inflammation and associated with increase in inflammatory markers [16, 43, 45, 47, 127]. Correlating different inflammatory markers with each other, separately in men and women will help in understanding the basic mechanism or association between obesity and inflammation.

This is the first study that shows the effect of exercise and diet-induced weight loss on levels of resistin and visfatin and their correlations with other health, blood and inflammatory markers. In this study, twenty-six 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, 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 fat free mass (FFM) in comparison to controls. Participants also improved their fitness, some markers of health, and experienced a significant reduction in some markers of inflammation. Our investigation extended previous research by examining the effects of exercise and diet-induced weight loss on resistin and visfatin and their correlation with anthropometric markers, blood health markers and other

inflammatory markers [126, 137, 138, 181, 185]. 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. We found that diet and exercise group had significant weight loss but no significant change in resistin level. Resistin was also not correlated with weight ($r=0.054$, $p=0.793$), fat free mass ($r=0.112$, $p=0.587$) or visceral adipose tissue ($r=0.136$, $p=0.508$).

Previous studies [27, 140, 151, 168, 185] showed mixed results on effects of exercise and/or hypocaloric diet on resistin. For example, Reinehr and colleagues [140] studied the change in weight status and resistin level over a period of 1 year. 38 obese children had 1-year outpatient Obeldick's intervention program which was based on physical exercise, nutrition education (high carbohydrate, fat reduced diet), and behavior therapy including individual psychological care of the child and his or her family. They found significant weight change ($p<0.001$) but no significant change in resistin levels ($p=0.079$). Koebnick et al [138] also showed similar results on 50 overweight to obese individuals who underwent dietary and physical activity intervention as compared to controls ($n=20$). Nutritional consulting was provided for a time period of 16 weeks, weekly during the first 8 weeks and biweekly for another 8 weeks. Participants were instructed to increase physical activity level during their daily routine (taking stairs instead of escalators, standing during a telephone call instead of sitting). Walking, light jogging, and gymnastics were suggested as programmed sports activities. They had a mean weight reduction of 4.5 kg and significant increase in serum resistin level (3.6 ± 0.2 vs 4.3 ± 0.2, $p<0.001$). The increase in serum resistin level observed in this study

contradicted the physiological understanding of resistin action, where a strong relationship among adiposity, insulin resistance and resistin was observed.

Bai et al [177] reported the influence of exercise prescription on plasma resistin for overweight and obese students. In this study, participants exercised aerobically at an intensity of 60%-70% of functional capacity for a session of 60 mins each time, 5 times per week and 13-15 levels of rate of perceived exertion. Energy metabolism was 500-600 kcal at a time. The relative indexes were detected after 8 weeks. Their results showed that there was a significant decrease in weight, BMI, percentage body fat and blood sugar ($P<0.01$). Plasma resistin was significantly different in male students ($P<0.01$), however the female students were significantly different ($P<0.05$). Resistin also had significant correlation with BMI in male students but not in female students. Vendrell et al [129] found that among a surgically treated morbidly obese group, body weight decreased significantly and weight loss was best predicted by resistin concentration after weight loss. They found resistin was significantly positively correlated with weight ($r=0.48$, $p<0.001$) and fat-free mass ($r=0.39$, $p<0.01$). Their results do not agree with the results obtained during our exercise and diet-induced weight loss study.

We also did not find any significant correlation between change in resistin with change in insulin ($r= 0.137$, $p=0.505$). There are conflicting evidence existed about the association of resistin with insulin resistance, because several studies reported no association of resistin with insulin resistance or indicators of type-2 diabetes mellitus [192-194]. There was a study conducted by Mozillo et al [136] studying the effect of

weight loss in response of lifestyle modification program on adipose tissue derived cytokines in obese individual with insulin resistance. They found that participants had a 6.9 ± 0.1 kg average weight loss, with a significant improvement in insulin sensitivity index. They did not find any change in resistin levels in response to the intervention which was comparable with our results.

In our investigation, we found that IL-6 (7.2±1. vs 5.3±1.75, p= 0.484) and TNF-α (7.2±1.5 vs 7.0±1.4, p=0.039) tended to reduce through the exercise program in diet and exercise group as compared to control group. Similar results were also reported by Lambert and colleagues [195]. They conducted a study on obese elderly persons, evaluated 12 wk of exercise (aerobic and resistance) or 12 wk of weight loss (∼7% reduction) effect. Participants in the exercise group participated in exercise-training sessions (3 days/wk) followed by a physical therapist. The exercise program consisted of physical therapy, endurance, and resistance exercise. Each session lasted around 90 min included 15 min of flexibility exercises, 20–30 min of aerobic exercise, 30–40 min of progressive resistance training (PRT), and 15 min of balance exercises. Participants in the weight loss group received a combination of an energy-deficit diet and behavior therapy. They had a balanced diet to provide an energy deficit of 500–750 kcal/day with a goal of 1–2% loss of body weight per week. Participants lost significant weight in weight loss group (−7.5 ± 1.2 kg, p = 0.001) as compared to exercise group (−0.3 ± 0.8 kg, P = 0.74). They found significant 50% decrease in IL-6 and TNF-α (P<0.05). Geilen et al [196] also conducted a study on 20 male congestive heart stable patients, randomized to a training group (n=10) or a control group (n=10). Training group had

aerobic exercise at about 70% VO_{2max} for 20 min/d for 60 min/wk. Participants in training group showed a significant 42% reduction in IL-6 level. However, they did not find any significant change in level of resistin (p=0.159) across the group during an intervention period.

We also found a statistically significant positive correlation between changes in resistin and changes in IL-6 (r=0.430, p=0.028). Interestingly, a study conducted by Kaser et al [103] showed that human peripheral blood mononuclear cells seem to be a major source of resistin and is strongly increased 2.5-fold by the proinflammatory cytokines interleukin-6 (IL-6). They also showed that in human resistin may be a link in the well-known association between inflammation and insulin resistance. Another study conducted by Tuttolomondo et al [197] showed that higher plasma levels of IL-6 and resistin in diabetic subjects with foot ulcers. Subjects having diabetes with foot ulcers (n=34) and diabetes without foot ulcers (n=37) were recruited. They found subjects having diabetes with foot ulcer have higher median plasma levels of IL-6 [3.21 (1.23-5.34) pg/ml vs 2.73 (1.24-3.97 pg/ml)] and resistin [3.860 (2.96-6.29 ng/ml) vs 3.690 (2.37-6.5 ng/ml)]. Stejskal et al [198] conducted a study on patients with type 2 diabetes mellitus and patients with acute inflammatory disease. They divided patients into 3 groups: group A - with clinical signs of inflammatory disease of respiratory tract (n=35); group B - with well controlled type 2 DM treated by oral antidiabetic drugs, without clinical signs of inflammation and negative case history of acute disease (n=12); group C - without clinical signs of inflammation and negative case history of acute disease (n=77). They showed patients with clinical signs of severe inflammation had higher

concentrations of IL-6 and resistin. They also showed significant positive correlation between resistin and inflammatory markers (correlation coefficient 0.3-0.5). All the above mentioned studies agreed with our finding of a significant positive correlation between changes in resistin and changes in IL-6.

Based on the results of past research studies, we could say that circulating resistin level increased in obese people in correlation with inflammatory markers especially IL-6 and TNF-α. Increase in serum resistin was observed in obese people as compared to their lean counterparts. On the contrary, our study reported that exercise and diet-induced weight loss led to the reduction in IL-6 level but at the same time increased resistin level which normally as a pro-inflammatory marker, led to an increase in IL-6. Therefore, the decrease in inflammatory markers such as IL-6 seen in weight loss was masked by an increase in resistin level in our study. Another reason of this contradiction could be due to the degree of severity of the baseline inflammatory burden or some other inflammatory markers that could have influenced the level of resistin. We also showed a significant positive correlation between changes in resistin and changes in IL-6 which indicated that there were some other pro-inflammatory or inflammatory factors affecting the level of resistin. Further research is warranted to explore other factors, inflammatory or adipose cytokines, which affect the level of resistin in overweight or obese people.

In our study, we found that exercise and diet-induced weight loss led to slight increase in mean visfatin level in exercise group as compared to control group but it was not statistically significant (DE: 0.85±15.0; C: 20.2±37.1 ng/ml, p=0.078). Our result

was contradicted to the result reported by Seo et al [199] on obese middle-aged women for 12 weeks of combined resistance and aerobic exercise training program. They reported significant change in body composition and reduction in visfatin levels. Brema et al [200] also showed significant reduction in visfatin level (~80%) after 12 weeks of aerobic exercise training in severely obese young subjects with type 2 diabetes. Another study conducted by De Luis et al [142] on 41 morbidly obese patients, provided hypocaloric diet with mean caloric intake of 1741.9±812.7 kcal/day (49% of carbohydrates, 28% of lipid and 23% of proteins) for two months. Results have shown that there was weight loss of average 4.41% in morbidly obese patients leading to decrease in BMI, weight, fat mass, fat free mass, waist circumference, systolic blood pressure, serum glucose, total cholesterol, insulin and HOMA. However, no significant decrease in visfatin concentration (43.5+30.8 vs. 47.1+38.1 ng/ml) was seen which were consistent with our results. Another group of researchers [157] conducted a dietary intervention on 47 pre-menopausal women (age 38·7 ± 1·7 years, range 25–57 years, BMI 27·9 ± 1·4 kg/m^2, range 17·3–50·5 kg/m^2). The diet was designed to provide 25–30% of kcals from fat, 55–60% of kcals from carbohydrates, and 10–15% of kcals from proteins. Overall, participants had 600 kcal/day less than the individually calculated energy requirements. Results showed that dietary intervention led to decrease in body weight and BMI by 8%, body fat mass by 16% and waist circumference by 9% in the entire group of overweight/obese women. Twelve week hypocaloric weight reducing diet caused an increase of visfatin mRNA levels ($P < 0·05$) and tended to decrease

visfatin plasma levels in the entire overweight/obese group (P < 0·1). Their results also agreed with our findings.

We also found positive correlations between changes in visfatin and changes in resistin (r=0.417; p=0.034) but there were no significant correlations between changes in visfatin with changes in weight (r=0.327; p=0.103), changes in fat mass (r=0.348; p=0.081), or changes in FFM (r= -0.026; p=0.900). A study conducted by Berndt et al [123] showed that visfatin plasma concentration correlates positively with the BMI (r = 0.250; p = 0.004), percent body fat (r = 0.220; p = 0.01), and negatively with subcutaneous visfatin mRNA expression (r = -0.424; p< 0.0001) which contradicted our results. Interestingly, Novak and colleagues [201] showed negative correlation between visfatin and waist circumference (p=0.027). The same conclusion was also drawn by Botella-Carretero et al [15]. They also showed increase in serum visfatin level after bariatric surgery in relation to the amount of weight lost and to the changes in waist circumference, and this increase was higher in diabetic patients.

Furthermore, we did not find any significant correlation between changes in protein intake with changes in resistin (r=-0.157, p=0.444) and changes in visfatin (r=-0.326, p=0.104). This is the first study looked at the relationship between protein intake with changes in resistin and changes in visfatin after the significant weight loss in an intervention group. Most of the studies in the past looked at the effect of overall dietary intake or fat intake on resistin and visfatin [194, 202, 203].

According to our results, exercise and diet-induced weight loss led to a slight increase in visfatin level which was statistically insignificant as compared to control

group which has greater increase in visfatin level. However, Cohen's effect size value (d=0.68) suggested a moderate to high practical significance. This could be due to no change in fat free mass in both the groups during the intervention period or small sample size. We also found significant positive correlation of changes in resistin with changes in visfatin even though both resistin and visfatin tend to increase in both the groups during the intervention period. Diet and exercise group has slight increase as compared to control group which showed greater increase in both resistin and visfatin. Our study provided sufficient evidence to conclude that these above mentioned observations were due to exercise and diet-induced weight loss program and exercise training protected against low-grade systemic inflammation link to obesity.

Overall, our results indicated that exercise and diet-induced weight loss affected anthropometric measure, inflammatory markers such as IL-6, TNF-α suggesting an anti-inflammatory response compared to control group. Our result was in accordance with other research studies done on inflammatory markers, leptin, resistin and visfatin. These results suggested that resistin and visfatin expression was not only associated with weight and fat mass and inflammatory markers like TNF-α and IL-6 but they could be affected by fat free mass and other inflammatory markers or cytokines associated with obesity since the weight and fat mass loss did not result in significant reduction in resistin and visfatin.

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. Second, the study participants were continually monitored and encouraged to meet program requirements. Third, the results observed in our trial were limited to the population studied, sedentary and overweight/obese women, and might not be applicable to other populations. Although our finding on the effects of exercise and diet-induced weight loss on resistin and visfatin were novel, they should be considered preliminary as this was the first study to investigate the effects of exercise and diet-induced weight loss on resistin and visfatin and their correlation with anthropometric markers, blood markers like insulin, glucose and triglyceride and other inflammatory markers using blood samples collected from sedentary obese women compared to a control only group. Finally, study was limited due to small sample size. Additional research needs to be done with larger sample size to further assess the relationship.

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 slight increase in resistin and visfatin in diet and exercise group as compared to control group which had greater increase. We also showed statistically significant positive correlation between changes in resistin with changes in visfatin and changes in IL-6. 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 related to inflammation in obese and insulin resistance individuals as well as the effects of different types of diet and exercise on resistin and visfatin linked to inflammation, obesity, and weight loss.

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APPENDIX A

BAYLOR 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, or other cardiac findings?
____ Frequent extra, skipped, or rapid heartbeats?
____ Chest Pain of Angina (with or without exertion)?
____ High cholesterol?
____ Diagnosed high blood pressure?
____ 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?
____ Gluacoma?
____ Asthma/breathing difficulty?
____ Bronchitis/Chest Cold?
____ Cancer, Melanoma, or Suspected Skin Lesions?
____ Stroke or Blood Clots?
____ 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 explain:
_____
_____
_____

Recommendation for Participation

____ 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: _____

Baylor University
Exercise & Sport Nutrition Laboratory

Personal Information

Name:

Address:

City: _____ State: _____ Zip Code _____ SS# _____

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

Beeper: (___) _____ Cellular (___)

Fax: (___) _____ email 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?

Baylor University
Exercise & Sport Nutrition Laboratory

Radiation Exposure Questionnaire for Women of Child Bearing Age

Radiation exposure may affect fetal development. Although the DEXA test will only expose you to a small amount of radiation (1.5mR per scan), you should be aware that there is a possibility that if you become pregnant during the course of the study that the x-ray exposure may be harmful to the fetus. Therefore, it is important to conduct x-ray tests within 10-14 days of the start of a female's menstrual cycle if the she is of child bearing age, sexually active, and/or is not taking birth control pills. The following questionnaire must be completed so that we know when it is an appropriate time to conduct the DEXA body composition tests. Please be assured that this information will be kept confidential within the limits permitted by law.

Current Age? _____
Age of first period? _____
Date of last period? _____
Normal length of menstrual cycle? _____
Have you been sexually active within the last month? _____
Do you use birth control pills? _____
Are you pregnant or have a desire for pregnancy? _____

Note: If you happen to get pregnant during the course of this study, you must notify research assistants so that appropriate precautions can be made.

I confirm that I have completed this questionnaire honestly and agree to notify researchers within the ESNL of any change in the length of my menstrual cycle and/or pregnancy status.

_____ _____

Name Date

Baylor University
Exercise & Sport Nutrition Laboratory

NAME _____ Date _____

INSTRUCTIONS

1. Record everything you eat for 4 days (including one weekend day). If you eat pretzels, record how many. If you eat a bag of chips, record the number of ounces. For drinks, record the number of cups or ounces. Record everything you drink except water.
2. Record the Food, Amount, Brand Name, and Preparation Methods. For example: baked vs. fried chicken; 1 cup of rice; 2 teaspoons of margarine; 1 cup of 2% milk; McDonald's, Healthy Choice, or Frosted Flakes.
3. Record immediately after eating. Waiting until that night may make it difficult to remember all foods and quantities.

Food (include brand) Method of Preparation Quantity (cups, oz., no.)

BREAKFAST:

_____ _____
_____ _____
_____ _____
_____ _____
_____ _____

LUNCH:

_____ _____
_____ _____
_____ _____
_____ _____
_____ _____

DINNER:

SNACKS:

Name _____ **Baylor University**
Date _____ **Exercise & Sport Nutrition Laboratory**

PSYCHOLOGICAL SELF REPORT INVENTORY

Instructions

The following items contain a series of statements that involve how people might think, feel, or behave. You will be asked to indicate the extent to which each statement pertains to you personally. But first, using a #2 pencil, we ask that you provide some important information on the left side of the computerized answer sheet (Please do not make any marks on the actual questionnaires themselves).

This information will ONLY be used to cross-validate the questionnaire. It will not be used to identify you or your answers. In the section labeled NAME, mark your last name and fist name and mark your group assignment (i.e., A, B, or C) in column 10. In the section labeled IDENTIFICATION NUMBER, mark the test session and fill in the circle (i.e., test session 2 = 2).

Your answers to the items in the questionnaire are for verifying the make-up of the questionnaire and for research purposes. In order to complete the questionnaire you should read each statement carefully and decide how you feel about it as it pertains to you personally. Using a scale like the one below, indicate on the computerized answer sheet how much you agree or disagree with each statement by blackening the appropriate circle to the right of the number of the statement. Note: Several scales are printed at the top of each section for easy reference since parts of the questionnaire require different reference points. Please work quickly through the questionnaire and do not ponder too long through the assessment. Finally, be frank and honest with your answers.

Part I

Below is a list of words that describe feelings people have. Please read each one carefully, then circle the appropriate letter to the right which best describes HOW YOU HAVE BEEN FEELING DURING THE PAST WEEK AND TODAY.

A Not At All B A Little C Moderately D Quite A Bit E Extremely

1.	Friendly	5.	Unhappy	9.	Sorry for things done
2.	Tense	6.	Clear-headed		
3.	Angry	7.	Lively	10.	Shaky
4.	Worn Out	8.	Confused	11.	Listless

12. Peeved
13. Considerate
14. Sad
15. Active
16. On edge
17. Grouchy
18. Blue
19. Energetic
20. Panicky
21. Hopeless
22. Relaxed
23. Unworthy
24. Spiteful
25. Sympathetic
26. Uneasy
27. Restless
28. Unable to Concentrate
29. Fatigued
30. Helpful
31. Annoyed
32. Discouraged
33. Resentful
34. Nervous
35. Lonely
36. Miserable
37. Muddled
38. Cheerful
39. Bitter
40. Exhausted
41. Anxious
42. Ready to fight
43. Good natured
44. Gloomy
45. Desperate
46. Sluggish
47. Rebellious
48. Helpless
49. Weary
50. Bewildered
51. Alert
52. Deceived
53. Furious
54. Efficient
55. Trusting
56. Full of Pep
57. Bad-Tempered
58. Worthless
59. Forgetful
60. Carefree
61. Terrified
62. Guilty
63. Vigorous
64. Uncertain about things
65. Bushed

Part II

Now for a change of pace, please respond to the following statements. Read each of the statements and fill in the corresponding circle on your answer sheet that best describes your feelings right now. Be sure to blacken one letter choice for each group. If more than one statement applies, blacken the higher letter of the alphabet. (Example: d is higher than a).

66. a. I do not feel sad.
    b. I feel sad.
    c. I am sad all the time, and I can't snap out of it.
    d. I am so sad or unhappy that I can't stand it.

67. a. I am not particularly discouraged about the future.
    b. I feel discouraged about the future.
    c. I feel I have nothing to look forward to.
    d. I feel that the future is hopeless and that things cannot improve.

68. a. I do not feel like a failure.
    b. I feel I have failed more than the average person.
    c. As I look back on my life, all I can see is a lot of failures.
    d. I feel I am a complete failure as a person.

69. a. I get as much satisfaction out of things as I used to.
    b. I don't enjoy things the way I used to.
    c. I don't get real satisfaction out of anything anymore.
    d. I am dissatisfied or bored with everything.

70. a. I don't feel particularly guilty.
    b. I feel guilty a good part of the time.
    c. I feel quite guilty most of the time.
    d. I feel guilty all of the time.

71. a. I don't feel I am being punished.
    b. I feel I may be punished.
    c. I expect to be punished.
    d. I feel I am being punished.

72. a. I don't feel disappointed in myself.
    b. I am disappointed in myself.
    c. I am disgusted with myself.
    d. I hate myself.

73. a. I don't feel I am any worse than anybody else.

b. I am critical of myself for my weaknesses or mistakes.
c. I blame myself all the time for my faults.
d. I blame myself for everything bad that happens.

74. a. I don't have any thoughts of killing myself.
b. I have thoughts of killing myself, but I would not carry them out.
c. I would like to kill myself.
d. I would kill myself if I had the chance.

75. a. I don't cry any more than usual.
b. I cry more now than I used to.
c. I cry all the time now.
d. I used to be able to cry, but now I can't cry even though I want to.

76. a. I am no more irritated by things than I ever was.
b. I am slightly more irritated now than usual.
c. I am quite annoyed or irritated a good deal of the time.
d. I feel irritated all the time now.

77. a. I have not lost interest in other people.
b. I am less interested in other people than I used to be.
c. I have lost most of my interest in other people.
d. I have lost all of my interest in other people.

78. a. I make decisions about as well as I ever could.
b. I put off making decisions more than I used to.
c. I have greater difficulty in making decisions than before.
d. I can't make decisions at all anymore.

79. a. I don't feel that I look any worse than I used to.
b. I am worried that I am looking old or unattractive.
c. I feel that there are permanent changes in my appearance that make me look unattractive.
d. I believe that I look ugly.

80. a. I can work about as well as before.
b. It takes an extra effort to get started at doing something.
c. I have to push myself very hard to do anything.
d. I can't do any work at all.

81. a. I can sleep as well as usual.
b. I don't sleep as well as I used to.
c. I wake up one to two hours earlier than usual and find it hard to get back to sleep.
d. I wake up several hours earlier than I used to and cannot get back to sleep.

82. a. I don't get more tired than usual.
b. I get tired more easily than I used to.
c. I get tired from doing almost anything.
d. I am too tired to do anything.

83. a. My appetite is no worse than usual.
b. my appetite is not as good as it used to be.
c. my appetite is much worse now.
d. I have no appetite at all anymore.

84. a. I haven't lost much weight, if any, lately.

b. I have lost more than five pounds.
c. I have lost more than ten pounds.
d. I have lost more than fifteen pounds.

85. a. I am no more worried about my health than usual.
b. I am worried about physical problems such as aches and pains, or upset stomach, or constipation.
c. I am very worried about physical problems, and it's hard to think of much else.
d. I am so worried about my physical problems that I cannot think about anything else.

86. a. I have not notices any recent change in my interest in sex.
b. I am less interested in sex than I used to be.
c. I am much less interested in sex now.
d. I have lost interest in sex completely.

Part III

Directions: Read each statement carefully. For each statement, fill in the circle with the letter which fits you best.

A	B	C	D	E
Rarely or Never True	Occasionally True	Often True	Usually True	True Most of the Time

87. I don't seem to be able to get much done at school.
88. I dread going to school lately.
89. I am bored with my work.
90. I find myself getting behind in my work lately.
91. I have accidents on the job as of late.
92. The quality of my school work is good.
93. Recently, I have been absent from work.
94. I find my school work interesting and/or exciting.
95. I can concentrate on the things I need to at school.
96. I make errors or mistakes in my work.
97. Lately, I am easily irritated.
98. Lately, I have been depressed.
99. Lately, I have been feeling anxious.
100. I have been happy lately.
101. So many thoughts run through my head at night that I have trouble falling asleep.
102. Lately, I respond badly in situations that normally wouldn't bother me.
103. I find myself complaining about little things.
104. Lately, I have been worrying.
105. I have a good sense of humor.
106. Things are going about as they should.
107. I wish I had more time to spend with close friends.
108. I quarrel with my spouse/girlfriend/boyfriend.
109. I quarrel with friends.
110. My spouse/girlfriend/boyfriend and I are happy together.
111. Lately, I do things by myself instead of with other people.
112. I quarrel with members of my family.
113. Lately, my relationships with people are good.
114. I find that I need time to myself to work out my problems.
115. I wish I had more time to spend by myself.
116. I have been withdrawing from people lately.
117. I have unplanned weight gains.
118. My eating habits are erratic.

119. I find myself drinking a lot lately.
120. Lately, I have been tired.
121. I have been feeling tense.
122. I have trouble falling and staying asleep.
123. I have aches and pains I cannot explain.
124. I eat the wrong foods.
125. I feel apathetic.
126. I feel lethargic.

THAT'S IT!!

Thank you for your time and effort in completing this form. If you had any questions concerning any questions on this assessment, please ask one of the assistants.

Baylor University
Exercise & Sport Nutrition Laboratory

NAME _____ Date _____

INSTRUCTIONS

Circle the number or dot between numbers that best indicates the degree you have felt the following symptoms during the last week:

Appetite

None Low Moderate High Severe
0 . . . . . 1 . . . . . 2 . . . . . 3. . . . . .4. . . . . .5. . . . . .6. . . . . .7. . . . . .8. . . . . .9. 10

Hunger

None Low Moderate High Severe
0 . . . . . 1 . . . . . 2 . . . . . 3. . . . . .4. . . . . .5. . . . . .6. . . . . .7. . . . . .8. . . . . .9. 10

Satisfaction from Food

None Low Moderate High Severe
0 . . . . . 1 . . . . . 2 . . . . . 3. . . . . .4. . . . . .5. . . . . .6. . . . . .7. . . . . .8. . . . . .9. 10

Feeling of Fullness

None Low Moderate High Severe
0 . . . . . 1 . . . . . 2 . . . . . 3. . . . . .4. . . . . .5. . . . . .6. . . . . .7. . . . . .8. . . . . .9. 10

Amount of Energy

None Low Moderate High Severe

0 . . . . . 1 . . . . . 2 . . . . . 3. . . . . .4. . . . . .5. . . . . .6. . . . . .7. . . . . .8. . . . . .9. 10

Overall Quality of Diet

None Low Moderate High Severe

0 . . . . . 1 . . . . . 2 . . . . . 3. . . . . .4. . . . . .5. . . . . .6. . . . . .7. . . . . .8. . . . . .9. 10

Texas A&M University
Exercise & Sport Nutrition Laboratory

Post Study Questionnaire

NAME _____ Date _____

INSTRUCTIONS

Circle the number or dot between numbers that best indicates the degree you have felt the following symptoms during the last week:

Overall Impressions of the Curves 30-Minute Fitness Program

None Low Moderate High Severe
0 . . . . . 1 . . . . . 2 . . . . . 3. . . . . .4. . . . . .5. . . . . .6. . . . . .7. . . . . .8. . . . . .9. 10

Overall Impressions of the Weight Loss Program

None Low Moderate High Severe
0 . . . . . 1 . . . . . 2 . . . . . 3. . . . . .4. . . . . .5. . . . . .6. . . . . .7. . . . . .8. . . . . .9. 10

Rate the Difficulty in Adhering to the Fitness Program

None Low Moderate High Severe
0 . . . . . 1 . . . . . 2 . . . . . 3. . . . . .4. . . . . .5. . . . . .6. . . . . .7. . . . . .8. . . . . .9. 10

Rate the Difficulty in Adhering to the Diet

None Low Moderate High Severe
0 . . . . . 1 . . . . . 2 . . . . . 3. . . . . .4. . . . . .5. . . . . .6. . . . . .7. . . . . .8. . . . . .9. 10

Rate Your Satisfaction with the Improvements in Fitness that You Made

None Low Moderate High Severe
0 . . . . . 1 . . . . . 2 . . . . . 3. . . . . .4. . . . . .5. . . . . .6. . . . . .7. . . . . .8. . . . . .9. 10

Rate the Satisfaction in the Changes in Body Composition that You Made

None Low Moderate High Severe
0 . . . . . 1 . . . . . 2 . . . . . 3. . . . . .4. . . . . .5. . . . . .6. . . . . .7. . . . . .8. . . . . .9. 10

Comments/Suggestions About the Curves Fitness & Weight Loss Program

Want to Get in Shape
and Lose Weight?

Women Needed for a
Fitness & Weight Loss Study

Researchers in the Exercise & Sport Nutrition Lab at Baylor University are recruiting 200 healthy, untrained, and moderately overweight female subjects between the ages of 18 and 50 to participate in a study to evaluate the effects of the Curves for Women® Fitness and Weight Loss Program Subjects will be required to follow an exercise program, one of several types of diets, and participate in five testing sessions during a 14-week period. Eligible subjects will receive free supervised training, body composition/bone density screening, exercise tests, nutritional counseling, and $125 for completing the study.

For more information call:

Exercise & Sport Nutrition Lab
Department of HHPR
MCL 122
254/710-7199

Weekly Follow-up Assessment

Effects of Calcium Supplementation on Body Composition, Metabolism, and Exercise Capacity in Post-Menopausal Women participating in the Curves for Women® Fitness and Weight Loss Program

Subject Name: _____ Subject #: _____ Date: _____

Week	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Are you training on schedule?														
Are you following the diet plan?														
Rate the ***frequency*** of the following symptoms according to the scale where: 0 = none 1 = minimal (1-2 per/wk) 2 = slight (3-4 per/wk) 3 = occasional (5-6 per/wk) 4 = frequent (7-8 per/wk) 5 = severe (9 or more per/wk)														
Dizziness?														
Headache?														
Fast or racing heart rate?														
Heart skipping or palpitations?														

Shortness of breath?														
Nervousness?														
Blurred Vision?														
Any other unusual or adverse effects?														
Rate the ***severity*** of the following symptoms according to the scale where: 0 = none 1 = minimal 2 = slight 3 = moderate 4 = severe 5 = very severe														
Dizziness?														
Headache?														
Fast or racing heart rate?														
Heart skipping or palpitations?														
Shortness of breath?														
Nervousness?														
Blurred Vision?														
Any other unusual or adverse effects?														
Nurse signature: Melyn Galbreath MSN, FNP-C														

PLEASE REMEMBER TO COMPLETE THIS QUESTIONNAIRE WEEKLY AT THE SLC

Further Questions: Contact the Research Nurse, Melyn Galbreath at 710-7199/3243 or by e-mail @ May_Galbreath@baylor.edu

Thank you for your participation!

APPENDIX B

Texas A&M University: Exercise & Sport Nutrition Laboratory
Trial: The effects of the Curves 90-Day Fitness Challenge on health outcomes in women.

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: _____ Body Image: _____
Radiation consent: _____ MOS SF-36: _____
Activity Log: _____ Eating Satisfaction: _____
Food Log: _____ Post-Study Questionnaire (T4 only): _____

Physiological Parameters: ESNL Staff Initials: _____

Height: _____ in. Waist: _____in.
Weight: _____ lb. Hip: _____in.
REE #1: _____ Resting H.R.: _____bpm.
Time: _____am Resting B.P.: _____/_____mmHg
Last Meal: _____am/pm BIA: _____
Hrs Fasted: _____hr. Handheld BIA: _____ #1 or #2
Last Wkout: _____ DEXA #2: _____
Lab (EBNL): _____ (2) SST Tubes/ (1) EDTA Tube 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_____:
_____x_____: ____x_____

1 RM: _____
80% 1RM: _____ 80% 1RM repetitions: _____

Bench Press: Hand Position: _____
Preceding Weights/Reps:
_____x_____: _____x_____: _____x_____: _____x_____: _____x_____:
_____x_____: _____x_____

1 RM: _____
80% 1RM: _____ 80% 1RM repetitions: _____

Updated 10/01/2010

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 about 20 milliliters (4 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. 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 microamps 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=60), a high carbohydrate/low fat diet group (45% CHO, 30% PRO, 25% FAT, N=60), a Weight Watchers diet group (N=60), a Jenny Craig diet group (N=60), a Nutrisystem diet group (N=60) and a control

group (N=60). In addition one additional group will follow the high protein/low fat diet group (30% CHO, 45% PRO, 25% FAT, N=60) and record diet and activity progress on-line.

If you are randomized into one of the first two groups (N=120), or the additional one group that will utilize the on-line recording system (N=60), 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, or the additional one group that will utilize the on-line recording system, 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=60) 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=120 total), you will follow the dietary guidelines set forth by those respective plans. The sixth group (N=60) 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=120 total), or the additional one group that will utilize the on-line recording system (N=60), 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 encouraged to walk for 30-minutes at a brisk pace (60 – 80% of heart rate reserve) on days you do use the Curves equipment. If you are randomized to participate in third group (N=60) 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 comfortable 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 training 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 4 teaspoons (20 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. The likelihood of any of these occurring is slim.

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. In the event you withdraw from the study prior to completion disbursement will occur on a pro-rated basis.

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 NUMBER: IRB2010-0813F

IRB APPROVAL DATE: 02/06/2013

IRB EXPIRATION DATE: 01/31/2014