EFFECT OF WEIGHT LOSS ON INFLAMMATORY MARKERS IN SEVERELY OBESE ADULTS

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EFFECT OF WEIGHT LOSS ON INFLAMMATORY MARKERS IN SEVERELY OBESE ADULTS

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Introduction: The role that inflammation plays in the atherosclerotic disease process is well established. Obesity is coupled with a state of chronic inflammation and is associated with increased circulating inflammatory markers including C-Reactive Protein. **Purpose:** The purpose of this study was to evaluate the additive effect of aerobic or resistance exercise training to caloric restriction for weight loss on high sensitivity C-Reactive Protein changes compared to dietary restriction alone in class II and class III obese individuals. **Methods:** 24 healthy, sedentary, obese women (BMI: 35.0 to <45 $kg/m^{2;}$ Age: 45.4 $\pm$ 6.9yrs.) underwent a 12-week diet and exercise intervention: Caloric restriction weight loss program with no exercise (DIET); Caloric restriction with aerobic endurance training (DIET+AT); and Caloric restriction with resistance training (DIET+RT). Blood was drawn at baseline and 12-weeks and assayed for hs-CRP. **Results:** Weight was decreased significantly in all groups ($p<0.001$) in the intervention. Hs-CRP was unchanged at 12-weeks ($p>0.05$). **Conclusions:** In conclusion, this investigation was successful in producing weight loss, BMI decreases, decreases in body fat percentage, and positive changes in fitness markers, though no changes in hs-CRP were associated with weight loss or weight loss with exercise. Further investigations into the influence of weight loss and exercise on CRP levels among Class II and Class III individuals should be completed to examine and expand upon the results observed in this study.

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PREFACE

Acknowledgements:

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1.0 INTRODUCTION

Excess weight is a major public health issue in most Western countries, and has seen a dramatic rise in developing nations (US Dept of Health and Human Services, 1996). The number of people in the United States who can be categorized as overweight or obese is exceedingly high according to recent reports (Flegal, 2010). In 2004, the Department of Health and Human Services elevated obesity to the level of "illness", fittingly recognizing the disease's emergence from a social stigma to an international epidemic (Esposito, 2006). In most age and sex groups obesity exceeds 30% (Flegal, 2010), indicating that among the U.S. population, approximately 100 million people are considered to be obese. Among adults 20–74 years of age, obesity rates have more than doubled since 1976–1980. From 1976–1980 to 2005–2006, the percentage of adults who were obese increased from 15% to 35% (age-adjusted) (Figure 1.1)

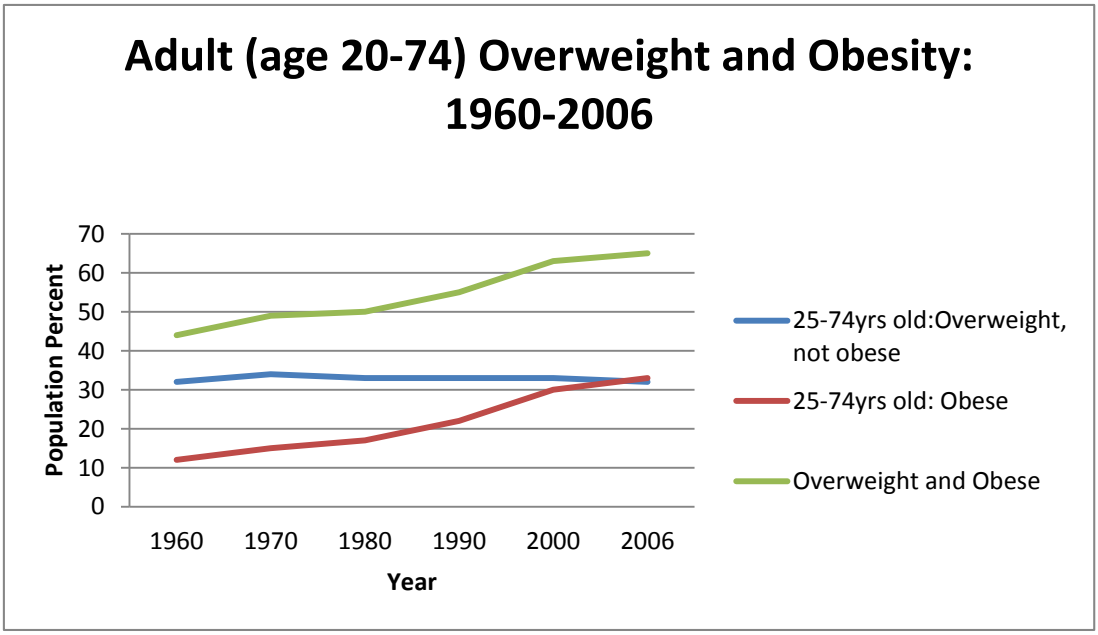


Figure 1.1. Change in Overweight and Obesity 1960-2006

(Adapted from http://www.cdc.gov/nchs/data/hus/hus09.pdf)

1.1 SIGNIFICANCE

Obesity has been correlated to increased morbidity and risk of chronic disease. Obesity is associated with abnormal cardiac structure and function, with the severity of the defects being linked to the severity and duration of the obesity (Klein 2004). Cardiovascular disease (CVD) death rates can also be directly related to BMI in both men and women (Klein 2004). In the mid-to-late 1990's, studies showed the risk of CVD mortality in obese persons with a BMI of greater

than 35kg/m^2 to be 2 to 3 times that of lean persons (BMI 18.5 to 24.9kg/m^2) (Calle 1999), with a 30% higher CHD mortality rate occurring for every 5-unit increase in BMI (Selmer 1995). Recent research shows age-adjusted relative risk of CHD increases linearly with increasing BMI; those with a BMI over 30 have more than double the relative risk of developing CHD than those in the low-normal (18.5-22.9kg/m^2) BMI category (Flint, 2010).

1.1.1 Theoretical Rationale

Obesity is coupled with a state of chronic inflammation. Obesity is associated with increased circulating inflammatory markers including C-Reactive Protein (CRP) and various pro-inflammatory cytokines, such as interleukin-6 [IL-6] (Klein 2004). C-reactive protein (CRP) is a plasma protein synthesized by hepatocytes in response to an inflammatory state (Pepys, 2003, Clyne 1999). CRP has been utilized for inflammation screening, as a marker of disease activity, and as a diagnostic tool in medicine and in research (Pepys, 1981). Inflammation plays a primary role in theories describing the atherosclerotic process (Tracy, 1998), and CRP has been established as a valid marker of inflammation with strong predictive significance for cardiovascular disease (Hawkins, 2004).

1.1.2 Inflammation

The role that inflammation plays in the atherosclerotic disease process is well established (Tracy, 1998; Ross, 1999). Chronic systemic inflammation is associated with the development of age- and inactivity-related diseases, such as diabetes and atherosclerosis (Argiles, 2003, Lechleitner, 2002, Smith, 2001). All stages of the pathological process of atherosclerosis – initiation, growth, and complication of the atherosclerotic plaque – can be considered to be an inflammatory response to injury, with cigarette smoking, hypertension, atherogenic lipoproteins, and hyperglycemia being the major injurious factors (AHA/CDC statement, 2003).

The large dysfunctional adipocytes that develop due to chronic positive energy balance (Mathieu, 2010) are biologically active cells that drive the formation of CRP in the liver (Abeywardena, 2009). The increased secretion of inflammatory mediators among the obese generates a chronic state of low-grade inflammation that appears to bring about many of the negative metabolic and cardiovascular outcomes frequently observed in this population (Ikeoka 2010). The detrimental outcomes attributed to inflammation may be decreased with weight loss, however, as weight reduction has been shown to improve the inflammatory profile.

1.1.3 Weight Loss and Inflammation

Lifestyle modification is the cornerstone of obesity management (Snow, 2005). The primary outcome of obesity therapy is weight reduction through the use of interventions that focus on increasing physical activity and reducing caloric intake (Esposito, 2006). A weight loss of 4.5 kg (9.9lbs) achieved with lifestyle changes is associated with a reduced incidence of coronary heart disease (CHD) (Eilat-Adar, 2005), and intentional weight loss, independent of total weight

change, is associated with lower all cause mortality (Gregg, 2003). Furthermore, it has been shown that adherence to lifestyle modification interventions is high, and that approximately 80% of participants will complete a 6-month weight-loss intervention (Wadden, 2005).

Weight reduction has been shown to improve inflammatory markers. Esposito and colleagues showed that the removal of fat via liposuction yielding an average decrease in body weight of 3.5kg caused a significant amelioration of inflammatory status (Esposito, 2006). Bariatric surgery has also been shown to effectively reduce CRP levels (Tziamalos, 2010), with decreases in CRP greater among those who lost more weight after surgery (Agrawal, 2009, Vasquez, 2005). In interventions utilizing diet with no other lifestyle modification, the reductions in CRP seem to be directly and linearly related to the amount of weight lost (O'Brien, 2005, Heilbronn, 2001) and the amount of fat mass lost (Klein, 2004). Tchernof and colleagues found that in 61 obese, postmenopausal women (age: 56.4$\pm$5.2y; BMI: 35.6$\pm$5) a 14.5 kg weight loss was accompanied by a significant 32% reduction in plasma CRP (Tchernof, 2002). Similarly, Hielbronn, Noakes, and Clifton found that among 83 obese, otherwise healthy women (age: 48.0+0.9, BMI: 33.8+0.4) a 7.9 kg weight loss resulted in a significant 26% reduction in CRP values (Heilbronn, 2001).

1.1.4 Exercise and Inflammation

Cross-sectional studies have shown that regular exercise may significantly lower CRP and inflammatory cytokine concentrations (Goldhammer, 2005, Okita, 2004, and Toft, 2002), and that there is a negative correlation between physical activity and CRP levels (Aronson, 2004;

Pischon, 2003). Interventions including both diet and exercise also tend to decrease inflammatory markers (Tsiomalos, 2010). Interventions that include exercise alone or in combination with caloric maintenance diets have a less clear impact on CRP, however (Tziomalos 2010).

A 2006 meta-analysis of randomized controlled trials concluded that aerobic exercise does not reduce CRP levels in adults (Kelley and Kelley, 2006). The STRRIDE study reported similar conclusions, with participants achieving significant decreases in fat mass (2.0-4.9kg) due to aerobic exercise, but no significant change in CRP or IL-6 attributed to aerobic exercise during the 8-month intervention (Slentz, 2004). Secondary analysis of the STRRIDE study showed that even with 9%-11% decreases in adipose tissue mass, exercise training did not significantly alter cytokine concentrations (Huffman, 2007). Stewart, et al found that despite increases in fitness, 6-months of aerobic exercise training did not improve CRP, though improvements in CRP were associated with reductions in weight (1.0-1.3kg) (Stewart, 2010). The work of Church (2010) and colleagues agreed with that of the previous studies, showing that exercise training without weight loss was not associated with a reduction in CRP, but that changes in weight, body fat, and abdominal body fat were associated with decreases in CRP. In contrast, Campbell, et al found that a year-long aerobic exercise intervention of a moderate intensity yielded a 10% reduction in CRP with a non-significant 1.8kg weight loss (Campbell, 2009).

Resistance exercise has been used in conjunction with aerobic exercise, and as an independent modality to study the effect of exercise on CRP. Stewart and colleagues found that the use of a combined resistance and aerobic training program significantly reduced serum CRP concentrations by as much as 58% in young and old physically inactive participants; at the end of

the training intervention, CRP levels of previously inactive participants were not significantly different from those of active participants (Stewart, 2007). It should be noted that the subjects in the Stewart study all had significant, though small (0.7-1.8kg) reductions in body weight during the 12-week intervention. (Stewart, 2007). Additionally, some research indicates that resistance training may be more beneficial than aerobic training in lowering CRP levels. Donges, et al found that when aerobic and resistance training protocols were compared over a 10-week intervention, only resistance training participants showed a reduction in CRP concentrations, with resistance training participants seeing a 32% decrease in CRP without associated losses of weight (Donges 2010). The results of studies utilizing resistance training alone show conflicting results as well, however. Some studies suggest that resistance training has no benefit over weight loss alone in decreasing markers of inflammation (Levinger, 2009, Brochu 2009) while the work of Olson, et al, indicates that resistance training alone, without accompanying weight loss, significantly reduces CRP by 9% (Olson, 2007).

Research comparing the effect of diet-alone versus diet-plus-exercise interventions is sparse and provides conflicting results. Some research shows that CRP is reduced with exercise only when used in conjunction with a weight loss regimen (Rokling-Anderson, 2007), or that insignificant reductions with exercise can be augmented considerably when weight loss is combined with exercise (Nicklas, 2004). Following a 1-year intervention, Rokling-Anderson, et al found an increase in CRP of 9% with aerobic exercise only, but decreases of 18% with diet-only and 21% with diet and exercise in conjunction (Rokling-Anderson, 2007). Niklas, et al showed that following an 18-month intervention using subjects with osteoarthritis, inconsequential (<1%) decreases in CRP with a training program consisting of 30-minutes of aerobic training and 15-minutes of resistance training, were greatly improved (5.8%) among

those who underwent diet-induced weight loss (Niklas, 2004). In contrast to the previous findings, Giannopoulou, et al utilized a 14-week intervention consisting of diet, aerobic exercise, or diet and aerobic exercise combined and found that all groups achieved significant reductions on CRP ($P<0.01$), and there were no differences between groups (Giannopoulou, 2005). You, et al found that following a 6-month intervention during which subjects underwent a dietary weight loss program, or a program that combined dietary weight loss with an aerobic exercise program only the participants participating in exercise showed a decrease in CRP (1.9µg/ml) (You, 2004).

1.1.5 Need for Additional Research

To date, research investigating obesity, inflammation, and cardiovascular disease has utilized lifestyle interventions focused on weight loss and exercise among groups with BMI classifications of normal, overweight and mildly obese. A limitation of recent investigations is the failure to utilize participants who are significantly overweight. Those whose BMI classification extends to Class II and Class III obesity (BMI>35.0, and BMI>40.0) have not yet been studied, yet these individuals have been shown to be able to substantially improve their cardiovascular risk profiles without attaining a BMI below 25 (Church, 2005; NIH Clinical Guidelines, 1998). Inactivity has been linked to increased adiposity (Hunter, 1997) and a trend toward higher levels of CRP has been seen in research subjects who are inactive (Stewart, 2007). It is necessary to study the relationships between weight loss and weight loss with added physical activity among Class II and Class III obese participants in order to gain a more thorough knowledge of the effects of BMI on circulating markers of inflammation and the changes

associated with weight loss and physical activity on inflammatory markers among this population. A second limitation of the current research is the use of strictly progressive resistance training protocols. The protocols used have increased constantly in intensity or volume, if not both. Constant increases in intensity tend to lead to overtraining among participants, highlighting a need for more variation of intensity and volume in the training protocol in order to avoid stagnation and overtraining. Finally, no research has studied the specific impact of diet and resistance training on CRP.

1.2 SPECIFIC AIMS

1) Evaluate the additive effect of aerobic and resistance exercise training on hs-C-Reactive Protein changes associated with weight loss in class II and class III obese individuals and compare changes in hs-CRP between intervention groups: Caloric restriction weight loss program with no exercise (DIET); Caloric restriction with aerobic endurance training (DIET+AT); and Caloric restriction with resistance training (DIET+RT).
2) Compare changes in anthropometric measures between DIET only, DIET +AT, and the DIET +RT.
3) Compare changes in body composition assessed via DXA between DIET only, DIET +AT, and the DIET +RT.

1.3 HYPOTHESES

1) All groups will experience significant decreases in C-Reactive Protein attributable to weight loss; the Diet +RT group will experience an additive decrease in CRP attributable to training; the Diet +AT group will not experience any additive decrease in CRP attributable to training
2) There will be no significant differences in anthropometric measures of waist, hip and waist-to-hip ratio between the Diet only, Diet +AT, and the Diet +RT.
3) There will be a significant difference in percent body fat change between Diet only, Diet +AT, and the Diet +RT.

2.0 REVIEW OF LITERATURE

2.1 INTRODUCTION

The United States Centers for Disease Control and Prevention define overweight and obesity as ranges of weight that are greater than what is generally considered healthy for a given height for adults, determined by using weight and height to calculate a number called the "body mass index" (BMI). (cdc.gov, 2010). The terms overweight and obese are also used to identify ranges of weight that have been shown to increase the likelihood of certain diseases and other health problems (cdc.gov, 2010). Recent evidence has revealed that 68% of adults in the United States are overweight or obese (Flegal, 2010). A full fifty percent of the overweight population can be categorized as obese, meaning that 33.8% of the entire adult population in the United States is obese (Flegal, 2010). Flegal and associates analyzed data obtained through the most recent [2007-2008] National Health and Nutrition Examination Survey (NHANES) for their results (Flegal, 2010). Data was analyzed for the sample of 5555 men and women over the age of 20 who were interviewed and examined. The data reveal that, as stated, 68% of American adults are overweight or obese, and that almost 34% are obese (Flegal, 2010). A recent article by Romero-Corral, et al suggests that a full 10% of U.S. adults with a normal BMI (<25) may have

normal weight obesity – high percentage body fat despite a normal BMI (Romero-Corral, 2010). If this is indeed the case, it is possible that, almost 80% of U. S. adults are carrying excess body fat and are predisposed to the chronic health issues associated with excess fatness (Main, 2010). Of tremendous interest is the large number of people who may be classified as Class II or Class III obese, according to Body Mass Index (BMI). More than 14% of adults may be classified as Class II obese, having a BMI > 35kg/m^2 (Flegal, 2010), and almost 6% [5.7%] can be categorized as Class III obese, having a BMI > 40 kg/m^2 (Flegal, 2010).

The Obesity issue facing the U.S. may be more completely understood by looking at epidemiologic mortality trends across time. The Epidemiologic Transition is a theory of the epidemiology of population change postulated by Omran in 1971 (Omran, 1971). This theory traces human history based on the primary causes of mortality. The first phase, constituting the largest part of all human history, is categorized by pestilence and famine; the second is termed the era of receding pandemics and is comprised of the latter part of the 19th and early years of the 20th centuries; in the third stage – the middle of the 20th century – an increase in mortality is characterized by cardiovascular disease and cancer, or degenerative diseases; the fourth stage, beginning in the mid 1960s, is termed the stage of delayed degenerative diseases in which cardiovascular degenerative disease declined based on advances in preventive medicine and public health strategies (Gazaino, 2010). The new stage of the epidemiologic transition is one in which obesity and inactivity have materialized to threaten the progress made in postponing morbidity and mortality (Gaziano, 2010). The gains made in quality of life and longevity that were buoyed by advances in the address of cardiovascular risk factors are threatened by the obesity epidemic/hyperendemic (Gaziano, 2010).

2.2 OBESITY AND CHRONIC DISEASE

2.2.1 Obesity and CVD/CHD

Over a decade ago, an estimated 97 million adults could be classified as either overweight or obese (NIH guidelines 1998), and that number may now approach 140 million. According to data from 2007-2008, the prevalence of overweight and obesity showed relative stability in the 1960's and 1970's with striking increases in the 1980's and 1990's, followed by a trend toward stability during the first decade of the new century (Flegal 2010). In addition to contributing to staggering medical care and disability costs – $147 billion in 2008 (Finkelstein, 2009) – obesity substantially raises the risk of morbidity from a number of chronic diseases such as hypertension, type 2 diabetes, and coronary heart disease (CHD) (NIH guidelines 1998).

Observational studies indicate that overweight, obesity and excess abdominal fat are directly related to numerous cardiovascular risk factors, including increases in: blood pressure; total cholesterol; LDL cholesterol; triglycerides; and insulin levels (NIH guidelines 1998). Obesity is also associated with abnormal cardiac structure and function with the severity of the defects being associated with the severity and duration of the obesity (Klein 2004). Cardiovascular disease (CVD) death rates are also directly related to BMI in both men and women (Klein 2004). The risk of CVD mortality in obese persons with a BMI of greater than 35kg/m^2 is reported to be 2-to-3 times that of lean persons (BMI 18.5 to 24.9kg/m^2) (Calle 1999), with a 30% higher CHD mortality rate occurring for every 5-unit increase in BMI (Selmer 1995).

2.2.2 Physiological Mechanisms Linking Obesity and CVD/CHD

Obesity is associated with an increase in circulating inflammatory markers including CRP and various pro-inflammatory cytokines, such as interleukin-6 [IL-6] (Klein 2004). Adipose tissue is not a metabolically inert tissue, but synthesizes and secretes pro-inflammatory mediators, such as Tumor Necrosis Factor-α and IL-6 (Tziomalos, 2010,Fontana, 2007, Fain, 2004, Fried, 1998, Mohamed-Ali, 1997), with IL-6, in turn, stimulating the production of CRP by the liver (Heinrich 1990). The abnormal secretion of inflammatory mediators yields a chronic state of low-grade inflammation that underlies the metabolic and cardiovascular outcomes of obese populations (Ikeoka 2010).

The manner of regional distribution and metabolism of adipose tissue are crucial in determining the existence of an abnormal metabolic state is of particular interest to researchers (Mathieu 2010). The differentiation of preadipocytes into mature adipocytes is key to the development of adipocytes that promote inflammation (Mathieu 2010). If the differentiation of a preadipocyte is influenced by a chronic positive energy balance, it will promote, at some stage, the formation of a larger (hypertrophic), dysfunctional adipocyte (Mathieu 2010). These dysfunctional adipocytes will produce less of the anti-inflammatory adipokine adiponectin, (Mathieu 2010). Recent studies have begun to clarify the manner by which visceral adipose tissue, particularly the dysfunctional adipose tissue, may contribute to inflammation. Macrophages are present within adipose tissue and their density increases with obesity, particularly within the hypertrophic adipocytes that show reduced production of the anti-inflammatory adipokine adiponectin (Spalding, 2008; Gustafson, 2009). Hypertrophic adipocytes, such as those found in a state of positive energy balance, produce more free fatty acids (FFAs), which contribute to the synthesis and production of cytokines such as TNF-α

(Lygsø 2002), which activates the synthesis of IL-6, driving the production of CRP in the liver (Abeywardena, 2009).

The mechanisms that link chronic inflammation and obesity are becoming more understood with emergent research. Knowledge of these interactions may hold the key to improving the state of chronic inflammation and subsequent cardiovascular disease among the obese.

Atherosclerosis has been identified as an inflammatory disease. It has been documented that the lesions of atherosclerosis represent specific cellular and molecular responses that are best described as an inflammatory disease (Ross, 1986; Ross, 1973). The earliest lesions, a fatty streak common even in infants and young children, are in fact purely inflammatory lesions (Stary, 1994). Physiological observations in humans and animal models have led to the formation of the "response-to-injury" hypothesis of atherosclerosis (Ross, 1973) which emphasizes endothelial dysfunction and proposes that each characteristic lesion of atherosclerosis represents a different stage in the inflammatory process (Ross, 1999). If the inflammatory process is allowed to continue unabated, or if it is excessive, the result will be advanced and complicated lesions (Ross, 1999). Inflammatory related injury induces the endothelium to develop pro-coagulant properties, as opposed to anti-coagulant properties, and to form vasoactive molecules, cytokines, and growth factors (Ross, 1999). If the inflammatory process does not effectively neutralize or remove the offending agents, as occurs with obesity, chronic hypertension, or continued smoking, the process can continue indefinitely (Ross, 1999). The subsequent perpetual inflammatory state stimulates the migration and proliferation of smooth-muscle cells that become intermixed with the area of inflammation and form intermediate lesions (Ross, 1999). If this cycle continues inexorably, a thickening of the artery

wall begins to take place (Glagov, 1987). The artery wall compensates by gradually dilating so that, up to a point, the lumen is unaltered; a process called “remodeling” (Glagov, 1987). Beyond this maximal dilation stage, however, the lumen begins to narrow and blood flow becomes occluded (Glagov, 1987).

2.3 C-REACTIVE PROTEIN

C-reactive protein (CRP) is a calcium-dependent ligand-binding plasma protein in the pentraxin family(M. B. Pepys, and Gideon M. Hirschfield, 2003). The human CRP molecule is synthesized by hepatocytes (Clyne, 1999) and is composed of five identical nonglycosylated polypeptide subunits, containing 206 amino acid residues each (M. B. Pepys, and Gideon M. Hirschfield, 2003). Human CRP binds with highest affinity to phosphocholine residues, though it binds with a number of other autologous and extrinsic ligands; it aggregates or precipitates the cellular, particular, or molecular structure of these ligands (Thompson, 1999). Autologous ligands include native and modified plasma lipoproteins (M. B. Pepys, Rowe, I.F., and Balgtz, M.L., 1985), damaged cell membranes (Volanakis, 1979), numerous phospholipids and related compounds, small ribonucleoprotein particles (Du Clos, 1989), and apoptotic cells (Gershov, 2000). C-reactive protein was first detected by Tillet and Francis in 1930 with their identification of a substance in the sera of patients acutely infected by pneumococcal pneumonia that formed a precipitate when it combined with polysaccharide C of *Streptococcus pneumonia* (Tillet, 1930). This process was deemed evidence of the body’s chemical response to

inflammatory states when it was discovered that this reaction was not unique to pneumonia, but was found in numerous acute infections (Clyne, 1999). Since its discovery, CRP has been studied as a screening device for occult inflammation, as a marker of disease activity, and as a diagnostic tool (M. B. Pepys, 1981).

Chronic systemic inflammation is associated with the development of prevalent age- and inactivity-related diseases, such as diabetes and atherosclerosis (Stewart, et al 2007, Argiles, 2003Lechleitner, 2002; Smith2001) and the role of inflammation in theories that describe the atherosclerotic disease process has been well established (Tracy, 1998). Various inflammatory mediators are implicated in the induction of endothelial dysfunction, plaque formation and plaque instability; these constituting the primary mechanisms of vascular damage in atherosclerotic disease (Ikeoka, 2010). All stages of the pathological process of atherosclerosis (initiation, growth, and complication of the atherosclerotic plaque) could be considered to be an inflammatory response to injury (Pearson, 2003) with cigarette smoking, hypertension, atherogenic lipoproteins, and hyperglycemia being the major injurious factors to the endothelium (Pearson,2003***)***. Each of the steps in the atherosclerotic process is believed to involve cytokines, which are bioactive molecules that are characteristic of inflammation (Pearson, 2003). This pathophysiological knowledge offers potential measurement targets to identify and monitor the inflammatory process. These targets include pro-inflammatory risk factors such as oxidized low-density lipoproteins; proinflammatory cytokines such as Tumor Necrosis Factor-α; adhesion molecules such as intercellular adhesion molecule-1; inflammatory stimuli with hepatic effects, for example interleukin-6; and the products of hepatic stimulation, such as C-Reactive Protein (Pearson, 2003). Specifically, it is known that in response to infection or tissue inflammation, CRP is produced only by hepatocytes (M. B. Pepys, and Gideon M. Hirschfield, 2003) under the

transcriptional control of cytokines, particularly IL-6, IL-1, and tumor necrosis factor (Clyne, 1999; Jupe, 1996; Kolb-Bachofen, 1991). While systemic inflammation and the inflammatory cascade may have sources other than atherogenesis, increased recognition of the inflammatory component of the atherosclerotic process offers biological plausibility for the use of inflammatory markers as indicators of atherogenesis or atherosclerotic complications (Pearson, 2003).

In healthy individuals, levels of CRP are normally less than 10mg/dl (Clyne, 1999): the median concentration of CRP is 0.8 mg/l; the 90^{th} centile is 3.0mg/l; and the 99^{th} centile is 10mg/l (Shine, 1981). Following an acute phase stimulus, however, CRP values can increase from less than 50µg/dl to over 500mg/l, a 10,000-fold increase (Vigushin, 1993). The plasma half-life of CRP is constant under all conditions of health and disease, so the sole determinant of circulating CRP is the synthesis rate (Vigushin, 1993). This synthesis rate is a direct reflection of the pathological processes stimulating CRP production (M. B. Pepys, and Gideon M. Hirschfield, 2003). When the stimulus for increased production ceases, circulating CRP drops dramatically, at almost the rate of plasma CRP clearance (M. B. Pepys, and Gideon M. Hirschfield, 2003). CRP values tend to increase with age and this change is presumed to reflect an increase in subclinical pathologies (Hutchinson, 2000). A study including 4494 subject (2291 male; 2203 female) ranging in age from 25-74 years of age, Hutchinson, et al showed a significant ($p<0.001$) trend to higher CRP values with increasing age. This investigation showed a median CRP near 1.0mg/l in the youngest subject population (25-34years) and a median of approximately 2.0mg/l in the oldest participants (65-74years) (Hutchinson, 2000). The general population tends to have stable CRP concentrations characteristic for each individual and self-correlation coefficient of measurements that have been repeated years apart are approximately

0.5; this coefficient is very comparable to that of cholesterol (Pepys and Hirschfield, 2003). In most diseases the circulating value of CRP reflects ongoing inflammation and/or tissue damage (M. B. Pepys, and Gideon M. Hirschfield, 2003). It is important to note that acute-phase CRP values show no seasonal or diurnal variation and are unaffected by eating (M. B. Pepys, and Gideon M. Hirschfield, 2003). Liver failure is the only concurrent pathology that impairs CRP production, and very few drugs reduce CRP levels unless they act upon the underlying pathology that provides the acute-phase stimulus for CRP production (M. B. Pepys, and Gideon M. Hirschfield, 2003). Levels of circulating CRP correlate closely with other markers of inflammation that show similar, though less significant, predictive associations with coronary events (Danesh, 1998). The properties and behavior of CRP may sufficiently explain why it provides closer associations and better predictions of inflammation than other inflammatory markers, but it may also have particular and specific associations with cardiovascular disease (M. B. Pepys, and Gideon M. Hirschfield, 2003).

Research has documented that cardiovascular events often occur in people with no prior indication of clinical cardiovascular disease, clinical or subclinical, and no known risk factors for cardiovascular disease (Cesari,et al 2003, Braunwald, E. 1997). Add to this knowledge that the predictive validity of traditional cardiovascular disease decreases as age increases (Beckett, 2000; Casiglia, 1998*)*, and the necessity of finding better predictors of cardiovascular events are quite apparent. Inflammatory cytokine-induced CRP production observed in human coronary artery smooth muscle cells suggests that local production of CRP in the vessel may directly influence the development of atherosclerosis (Calobro 2003). In the Women's Health Study, where various inflammatory markers and their relationship to cardiovascular disease were

examined, individuals in the highest quartile of CRP levels showed a relative risk of future coronary event 3-times that of those in the lowest quartile (Ridker, 2000).

The American Heart Association and Centers for Disease Control released a Scientific Statement in 2003 indicating that inflammatory markers have the potential to augment risk assessment to identify persons who should be considered for lipid-lowering, anti-platelet, or other cardioprotective drug therapies (Pearson, 2003). Also included were those for whom there should be an increased emphasis on therapeutic lifestyle changes (Pearson 2003). Cesari, et al assessed the incidence of coronary heart disease (CHD), stroke, and CHF events according to serum levels of the inflammatory markers CRP, IL-6, and TNF-α in older, well-functioning subjects. Their findings showed that these inflammatory markers were as potent or more potent predictors of disease onset than traditional risk factors. All 3 inflammatory markers predicted the onset of cardiovascular events, and individuals with high levels of all 3 markers had the greatest risk of cardiovascular events, with IL-6 being the strongest and most consistent risk factor for cardiovascular events (Cesari, 2003). Because of the economic ease of testing for CRP, however, it maintains its importance the most suitable inflammatory marker for wide-spread testing (Cesari, 2003). Concurring evidence is given by Hawkins, who concluded that elevated levels of high-sensitivity CRP are associated with an increased cardiovascular disease Relative Risk of 4, providing considerable evidence that CRP can offer striking predictive significance for cardiovascular disease (Hawkins, 2004). The AHA/CDC document denotes that their literature review indicates most studies show a dose-response relationship between the level of CRP and risk of incident coronary disease (Pearson 2003).

While some have suggested with their research that inflammatory markers in general, and most specifically CRP, cause the development of elevated adiposity, obesity, and diabetes

(Bochud, 2009), a recent statistical investigation has concluded that it is indeed BMI which drives the levels of circulating CRP, with CRP being marker of elevated adiposity (Timpson, 2010).

New and emerging assays of other inflammatory markers may in the future provide precision, standardization, and other characteristics that are superior to those of current assays, but it appears that CRP is currently the best candidate for measures of inflammation (Pearson 2003). Individuals with CRP levels of <1 are categorized as being at a low relative risk of cardiovascular disease, those with levels 1.0 to 3.0 are categorized as being at an average relative risk of cardiovascular disease, and individuals with CRP levels >3 are categorized as being at a high relative risk of cardiovascular disease (Pearson, 2003).

2.4 WEIGHT LOSS AND INFLAMMATION

Lifestyle measures are the cornerstone of the management of obesity (Snow, 2005). Lifestyle modification has been proven effective at eliciting weight loss and is comprised, primarily, of three specific components: diet, exercise, and behavior therapy (Wadden, 2004). Behavior therapy includes techniques for modifying diet and exercise in order to achieve the desired weight loss. Behavior therapy teaches intervention participants how to better achieve their goals by such methods as physical activity and food consumption record keeping, and by addressing cues that lead to unwanted eating behavior (Wadden, 2004). Though some evidence suggests

that long-term adherence to exercise protocols is enhanced by increasing the amount of supervised exercise sessions (Cyarto, 2006), the majority of work indicates that participants who adhere to unsupervised exercise regimens have greater long-term success (Wadden, 2004).

Observational studies have shown that weight loss achieved with lifestyle changes was associated with a reduced incidence of coronary heart disease (CHD) (Eilat-Adar, 2005), and with lower all cause mortality (Gregg, 2003). Changes in inflammatory markers have been specifically correlated to changes in body weight (O'Brien, 2005) with several small studies noting a significant decrease in CRP after weight loss induced by dietary modification in otherwise healthy obese adults (Tziomalos 2010), and some showing the magnitude of decrease in CRP to be linearly correlated with the amount of weight lost (Tziomalos 2010).

A 12-month study by Dansinger et al, comparing four different weight loss diets[Atkins, Ornish, Weight Watchers, Zone], found decreases in CRP to be significantly associated with weight loss (Dansinger, 2005). The study randomized 160 adults with a mean age of 49 $\pm$ 11 and a mean BMI of 35 $\pm$ 3.9 to the four diet protocols (Dansinger, 2005). Greater adherence to any of the four protocols yielded greater weight loss and greater reductions in CRP (Dansinger, 2005). The participants had a mean kcal consumption of 1498kcal at month 1 and 1824kcal at month 12 (Dansinger, 2005). Following the intervention period, mean weight loss was 2.9 $\pm$5.75kg and yielded a mean decrease of 30% in CRP (Dansinger, 2005).

Studies have compared weight loss diets of different compositions and found that similar decreases in CRP are found among groups, indicating that weight loss *per se*, rather than diet composition, is the primary determinant of the decrease in inflammatory markers (Brinkworth, 2009, Tay, 2008). In the 2005 study by O'Brien, et al, 41 obese (BMI 33.6 $\pm$ 1.9) female participants were randomized to either a very low-fat diet or a very low-carbohydrate diet for a

period of 3-months (O'Brien, 2005). Low-fat dieters were asked to limit caloric consumption to 1200 Kcal per day; low-carbohydrate dieters were not given a caloric restriction, but were asked to consume no more than 20g/day of carbohydrates for the first two weeks and up to 60g/day for the duration of the study, so long as ketosis was maintained (O'Brien, 2005). Weight loss was substantially more in the low-carbohydrate group following the intervention [-7.6 $\pm$ 3.2 kg *v/s* -4.3 $\pm$3.5 kg, $p<0.01$], but change in CRP was not significantly different, with a mean decrease of 24% (O'Brien, 2005).

Esposito investigate the impact of fat removal via liposuction on inflammatory markers (Esposito, 2006). Mean aspiration volume was 3540 $\pm$ 890ml, equating to a mean fat removal of 2.7 + 0.7kg. This was associated with an average decrease in bodyweight of 3.5kg that was maintained in subjects through the 6-month to 1-year follow-up at which time participants showed a significant 26% ($p<0.05$) reduction in CRP.

Studies have also examined the impact of weight loss from bariatric surgery interventions on CRP levels. Agrawal (2009) showed that obese adults experienced a reduction in CRP after bariatric surgery, with a greater reduction in CRP being experienced with more surgical weight loss. Utilizing a 15-month median follow-up to bariatric surgery, the first tertile of participants had a mean BMI of 52.8 $\pm$ 11.5 at baseline, (n=20) lost 31.7 $\pm$ 10.6kg and lowered CRP by 0.51 $\pm$ 0.57mg/l. This body mass loss yielded a follow-up BMI of 41.3 $\pm$ 8.4. The second tertile of participants had a baseline BMI of 48.4 $\pm$ 6.2, (n=21) lost 39.4 $\pm$ 8.4kg and lowered CRP by 0.65 $\pm$ 0.76mg/l. The follow-up BMI for the second tertile was 33.7 $\pm$ 4.2. Finally, the third tertile of participants had a baseline BMI of 46.6 $\pm$ 6.7, decreased body weight by 51.9 $\pm$ 13.8kg and lowered CRP by 0.81 $\pm$ 0.67mg/l. The follow-up BMI for the third tertile was 27.7 $\pm$ 4.2.

The 2007 systematic review of the literature conducted by Selvin, et al determined that weight loss might be used as a primary nonpharmacologic method of reducing CRP levels (Selvin, 2007). Selvin (Selvin, 2007) concluded that weight loss was associated with a decline in CRP, and that across all studies (both lifestyle and surgical interventions) a 1-kg loss yielded a 0.013mg/dl decrease in CRP (weighted Pearson correlation, r=0.85). In 2002, Tchernoff and associates studied 61 obese (BMI 35.6), postmenopausal women (age: 56.4yrs) and found that among completers, adiposity was a significant predictor of plasma CRP, and that CRP was significantly reduced by weight loss (Tchernoff, 2002). The Tchernoff subjects lost an average of 14.5 $\pm$ 6.2kg, and reduced their CRP levels by an average of 32% (Tchernoff, 2002). The 2004 work of Xydakis, et al had shown similar results. The Xydakis study used 80 obese individuals (BMI 38.3) in a medically supervised 12-month program for rapid weight loss in which participants were assigned to a Very Low Calorie Diet (VLCD) of 600-800 kcal daily (Xydakis, 2004). Participants reduced their BMI by 2.6, their weight by 18lbs (8.2kg), and their CRP levels by 14% all of which were statistically significant (Xydakis, 2004). Moreover, decrease in CRP was significantly correlated with weight loss (Xydakis, 2004).

Tziomalos and fellows, in a more recent review with a larger scope, concurred with this report, indicating all but two of the twenty-six weight loss studies reviewed showed decreases in CRP (Tziomalos, 2010). Very recent research has only further confirmed these conclusions. The LIFE study included 212 obese men and women (BMI 30-39) and produced significant reductions in CRP after 6 months of lifestyle intervention focusing on weight loss with no prescribed exercise or physical activity goals (Yatsuya, 2010). In this study, changes in CRP were more strongly correlated to changes in BMI than to changes in other anthropometric measures, such as waist or hip circumference (Yatsuya, 2010).

2.4.1 Does Exercise Provide an Additive Effect to Weight Loss?

While physical activity has been shown to have a favorable association to weight-related outcomes (DHHS, 2008), physical activity alone has a very limited impact on inducing weight loss (Wadden, 2004). Various trials have shown that a physical activity dose of 13-26 MET-hours per week yields a modest 1%-3% reduction in weight (DHHS, 2008). In order to achieve 13 MET-hours per week, one would need to walk for 150 minutes at a 4 mile-per-hour pace, or jog at 6 miles-per-hour for 75 minutes (DHHS, 2008). Further, resistance training usually yields a weight loss of less than 1kg (2.2lbs) (DHHS, 2008). Slentz, et al showed a modest 3.5kg loss of body mass among those who participated in high amounts of vigorous activity, and about 1.1kg loss among those who participated in low amounts of moderate-intensity activity after an 8-month training intervention (Slentz, 2004). Numerous studies have found the effect of physical activity usually accounts for a 2-3kg loss during moderate-length diet and exercise interventions (ACSM, 2009), and very recent evidence from the RENEW study confirms this (Goodpaster, 2010). In the first 6-months of the RENEW study, Class II and Class III obese participants who received an exercise and diet intervention lost significantly more weight (10.9 kg) than those receiving a diet only intervention (8.2kg) (Goodpaster, 2010). There is an apparent dose effect of physical activity and weight loss, with higher doses capable of providing a 3% or greater weight loss from initial weight (ASCM, 2009); it is very difficult for most adults, and particularly overweight and obese adults, though, to induce a large enough calorie deficit via exercise to see dramatic weight loss. It is necessary, therefore, to induce weight loss through the management of caloric consumption.

2.4.2 Aerobic Exercise and C-Reactive Protein

The influence of exercise on circulating levels of CRP and IL-6 seems to be positive, but the existing literature is mixed in its results. Some cross-sectional studies have shown that regular exercise may significantly lower CRP and inflammatory cytokine concentrations (Goldhammer, 2005, Okita, 2004, and Toft, 2002), that there is a negative correlation between physical activity and CRP levels (Aronson, 2004; Pischon, 2003), and that interventions that include both diet and exercise tend to decrease inflammatory markers; however, interventions which include exercise alone or in combination with caloric maintenance diets are not as clear-cut (Tziomalos 2010). A 2006 meta-analysis of randomized controlled trials concluded that aerobic exercise does not reduce CRP levels in adults (Kelley and Kelley, 2006). The STRRIDE study concluded that while participants saw significant decreases in fat mass, no significant changes in CRP or IL-6 could be attributed to varying intensities and durations of aerobic exercise (Huffman 2007). More recently, however, Kadoglou showed that overweight and obese subjects saw significant decreases in CRP levels with a 6-month aerobic exercise intervention, despite a lack of significant change in body mass (Kadoglou, 2007). Kadoglou, et al used 60 overweight and obese (BMI>25; range 25.1-36.1) type 2 diabetic participants to assess inflammatory response to exercise training (Kadoglou, 2007). Participants randomized to the exercise group participated in 4 supervised aerobic training sessions per week, while the control group was encouraged not to change their daily activities (Kadoglou, 2007). Both groups were asked to maintain their eating patterns (Kadoglou, 2007). Despite a lack of significant weight loss, the exercise group

showed a significant 39.6% reduction in CRP (p=0.04). Kadoglou's work agreed with that of You, et al from 2004. The 6-month You study placed obese (BMI32.78 $\pm$ 4.73) women into groups participating in either weight loss, or weight loss and aerobic exercise (You, 2004). The study concluded that, while both the diet and diet plus exercise groups lost significant body weight (-4.7 $\pm$ 1.0kg and -7.3$^{\pm}$1.1kg, respectively) and fat-mass (-4.6$\pm$0.8kg and -5.4$\pm$1.1kg, respectively), only diet plus exercise was effective at reducing CRP in this population (You, 2004). A year-long study by Campbell et al showed a reduction in CRP among previously sedentary obese postmenopausal women with linear trends between CRP and 12-month changes in VO_2MAX, exercise adherence, body fat loss, weight loss, and intra-abdominal fat loss (Campbell, 2009). In a randomized controlled trial, Campbell and colleagues assigned 115 post-menopausal (age 60.7$\pm$6.9yrs), overweight and obese women (BMI = 30.3$\pm$3.9) to an aerobic exercise intervention wherein the participants exercised at 60-75% of observed maximal heart rate for >45 minutes per day 5 days per week (n=53), or a stretching control (n=62) with all participants being asked to maintain their usual diet (Campbell, 2009). At the end of the year-long intervention, the exercise group had decreased CRP by 10%, while the control group had seen a 12% increase in CRP (p=0.01)

Stewart and colleagues found that the use of a 6-month combined resistance and aerobic training program significantly reduced serum CRP concentrations in both young (age 25$\pm$4.7yrs) and old (age 71$\pm$4yrs) previously inactive participants (Stewart, 2007). Twenty-nine young physically inactive adults and 31 older inactive adult subjects completed an exercise intervention that included both aerobic (40min/session at 70-80% of Heart Rate Reserve) and resistance training (2 sets of 8 exercises at 70-80% of 1-repetition maximum) (Stewart, 2007). Both

younger and older exercise participants saw decreases of 42% ($p<0.01$) in CRP at the protocol conclusion (Stewart, 2007).

2.4.3 Resistance Training and C-Reactive Protein

Resistance training as a singular modality of exercise has been studied, though sparingly, for its impact upon markers of inflammation. No current studies have researched the impact of concurrent weight loss and resistance training on circulating CRP. While there is some indication that resistance training offers no benefit over weight loss for decreasing inflammatory markers among healthy overweight and obese subjects (Brochu, 2009, Levinger, 2009), the results, as with aerobic training interventions, are mixed. Donges, et al found that when aerobic and resistance training were compared over a 10-week intervention, only resistance training participants showed a reduction in CRP concentrations (Donges 2010). The Donges study assigned 35 participants to a periodized resistance training protocol, 41 participants to an aerobic protocol, and 26 participants to a control group, with all subjects being counseled to maintain their eating habits (Donges, 2010). All subjects were overweight and sedentary, with a mean BMI of 28.7 ± 4.5 (Donges, 2010). Following the 10-week protocol, the resistance trained participants decreased their CRP by 32.8% ($p<0.05$), while the aerobically trained participant saw a 16.1% reduction ($p=0.06$) (Donges, 2010). The results of a one-year study of resistance training among overweight, eumenorrheic women suggest that regular, moderate intensity resistance training *is* an effective non-phamacologic intervention to attenuate low-grade inflammation associated with overweight (Olson, 2007). The Olson study looked at 28 (12

control, 16 resistance trained) overweight (BMI 26.95±3.0) sedentary women (38.5±5.5yrs) studied before and after one year of resistance training (Olson, 2007). The resistance trained participants took part in at least two supervised sessions per week, using 3 sets of 8-10 repetitions with a progressive increase in the amount of weight lifted (Olson, 2007). Both resistance trained participants and controls were encouraged not to change their diets during the study (Olson, 2007). Resistance trained participants experiences a 9% decline in CRP ($p<0.01$), while there was no change in the control group (Olson, 2007).

2.5 CONCLUSIONS

It is clear that weight loss has a positive effect on C-reactive protein levels and though some research shows a positive impact of exercise, further study is necessary. To date, studies have used sedentary overweight and obese participants. Study of exercise and weight loss to impact CRP among Class II and Class III obese participants has not been attempted. It is necessary to address this population, as they make up a significant segment of the population that is considered overweight and obese.

3.0 METHODOLOGY

3.1 SUBJECTS

Subjects were 24 healthy, sedentary, obese (BMI: 35.0 to <45 kg/m^2) females with an average age of 45.4 $\pm$ 6.9 years. Subjects were excluded from the study if classified in the High Risk stratification based on ACSM guidelines, or if they meet any of the following additional exclusionary criteria:

1. Currently pregnant, pregnant in the last 6 months, or plan on becoming pregnant in the next 6 months

   RATIONALE: Women who are currently pregnant have different nutritional needs and potentially have limitations to physical activity than non-pregnant women. Moreover, vigorous exercise is not recommended for women who are pregnant, which would limit the ability of these women to participate in the graded exercise test in this study. Pregnancy is also a contraindication for performing a DXA analysis of body composition. Women who have been pregnant within the past 6 months or who are breast feeding may not be weight stable, which would potentially confound the influence of the proposed interventions on changes in body weight and body composition in this study.
2. Currently participating in regular exercise for over 60 minutes/week.

RATIONALE: An important component of the design of this study is to examine the effects of either aerobic or resistance exercise to an energy restricted diet. Therefore, to minimize the potential of a confounding effect, it is important to include only sedentary individuals in this study, which will also allow for maximal effects of the prescribed exercise on outcome measures.

3. Taking any medications that affect body weight or metabolism (e.g. synthroid).

   RATIONALE: This study is to examine the effect of a behavioral lifestyle intervention on weight loss, with the intervention including changes in physical activity and/or dietary behaviors. Therefore, medications that affect body weight could confound the results of this study, and are excluded.

4. Have any physical limitations that would prevent exercise.

   RATIONALE: Exercise is a component of the intervention for two of the intervention conditions, and there are measures of fitness included in this study. Therefore, it is imperative that study participants are able to exercise at the prescribed doses and complete the assessments of fitness in this study.

5. History of myocardial infarction or a cardiac procedure (e.g., bypass, etc.), or currently being treated for coronary heart disease, diabetes mellitus, hypertension, or cancer.

   RATIONALE: Individuals with these conditions may require additional medical clearance and supervision that is outside the scope of the proposed study, or may require alterations in the intervention that would limit their ability to comply to the standardized intervention that is proposed.

6. Have a resting systolic blood pressure $\geq$ 150 mmHg or diastolic blood pressure of $\geq$ 100 mmHg or currently taking any medications that affect blood pressure or heart rate (e.g. beta

blockers). RATIONALE: Hypertension at these levels may contraindicate participation the exercise prescribed as part of the interventions in this study. Moreover, cardiorespiratory fitness in this study is being assessed using a sub-maximal exercise test to 85% of age-predicted maximal heart rate that is not supervised by a physician. Therefore, blood pressure at these levels may pose a safety concern in this study. Moreover, if taking medication that affect the heart rate response to exercise, the termination heart rate during the graded exercise test will not be able to be determined.

7. Currently enrolled in a commercial weight loss program, participating in a current weight loss study, or participated in a weight loss study completed in the previous 6 months.

   RATIONALE: Enrollment in an outside weight loss study, or recent participation in a weight loss study may confound the weight loss interventions proposed for this study.

8. Have lost >5% of current body weight in the past 6 months.

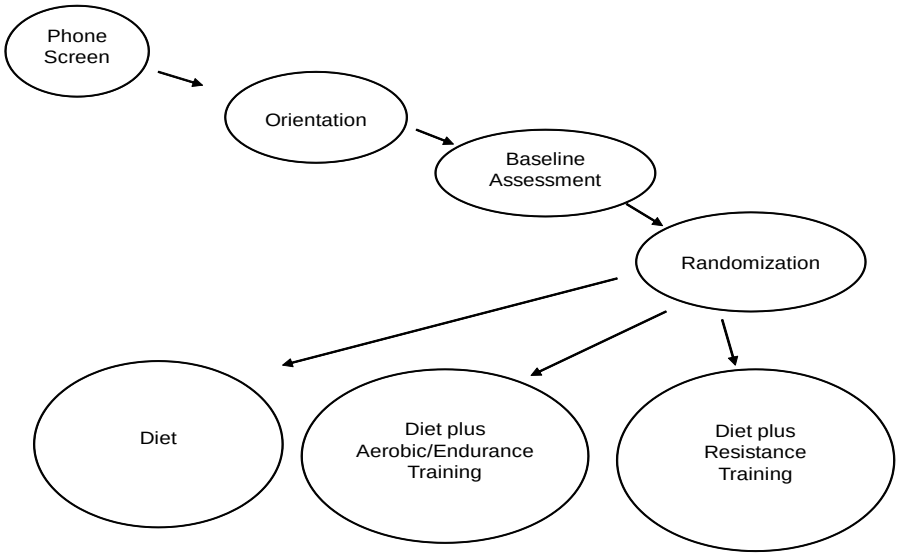
   RATIONALE: This may confound the effects of the intervention on weight loss, body composition, and the other outcomes measures in this study. Moreover, based on our experience, the intervention may shift to a focus on prevention of weight regain rather than continued weight loss in these individuals.

9. Currently being treated for any psychological issue or taking any prescription psychotropic medication. RATIONALE: Receiving an intervention for psychological issues may confound the effect or may affect compliance to the intervention proposed for this study. Moreover, many psychotropic medications can affect body weight, and therefore should be excluded from this study.

3.2 RECRUITMENT AND SCREENING

Subjects were recruited through advertisements in various traditional and electronic media outlets in the greater Pittsburgh area that could have included television and radio advertisements, direct mail, posting of fliers, newspaper advertisements, etc. The University of Pittsburgh Institutional Review Board approved of all recruitment materials. Interested participants were instructed to call the University of Pittsburgh's Physical Activity and Weight Management Research Center where trained staff and graduate students conducted a brief telephone screening to determine initial eligibility. The telephone screening included a description of the study, and after obtaining the participant's verbal consent, staff asked questions regarding the potential participant's medical history along with other questions relevant to the exclusion/inclusion criteria.

Individuals who appeared to be eligible based on this telephone screening were invited to attend an orientation session where complete details of the study were provided by the Principal Investigators and Director of the University of Pittsburgh Physical Activity and Weight Management Research Center. At this orientation, subjects were encouraged to ask questions on the study's procedures so that each participant entered the intervention with full knowledge of the protocol and expectations. Interested subjects provided written informed consent to participate. Participants also completed a Physical Activity Readiness Questionnaire (PAR-Q) (Thomas, 1992) and provided a medical history to confirm initial eligibility. In addition, participants provided written approval from their personal physician confirming no contraindications to study participation prior to undergoing additional baseline assessments and participation in the intervention. This study utilized a computer generated randomization


Phone Screen
Orientation
Baseline Assessment
Randomization
Diet
Diet plus Aerobic/Endurance Training
Diet plus Resistance Training

3.3 EXPERIMENTAL DESIGN

This study was a randomized control trial to examine the response of high sensitivity C-Reactive Protein (hs-CRP) in obese adults to energy restriction or energy restriction plus exercise. This study included a 12-week intervention conducted at the University of Pittsburgh Physical Activity and Weight Management Research Center, with assessments performed at pre-intervention (baseline) and post-intervention (12 weeks). Following baseline assessments, eligible participants were randomized to one of three intervention protocols: 1) Standard Behavioral Weight Loss Program focused on energy intake restriction with no prescribed exercise (DIET); 2) Standard Behavioral Weight Loss focused on energy intake restriction plus Aerobic/Endurance Training (DIET+AT); 3) Standard Behavioral Weight Loss focused on energy intake restriction plus Resistance Training (DIET+RT). All study procedures were approved by the Institutional Review Board of the University of Pittsburgh prior to implementation, and subjects provided informed consent prior to engaging in these study procedures. The study timeline and study progression are illustrated in Figures 3.2 and 3.1, respectively.

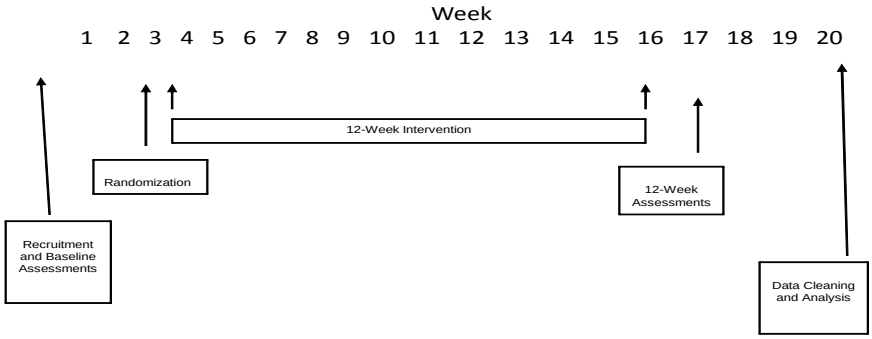


Figure 3.2. Study Timeline

3.3.1.1 INTERVENTION

3.3.1.2 DIET

The behavioral weight loss program was conducted at the University of Pittsburgh Physical Activity and Weight Management Research Center. The intervention included a 12-week program in which participants attend group sessions as illustrated in Figure 3.3. Group sessions were approximately 30-45 minutes in length and conducted by trained staff and graduate students with academic training in nutrition, exercise physiology, or behavior modification. The intervention focused on modification of weight loss behavior, with the intervention based on

multi-theoretical approaches such as Social-Cognitive Theory (Bandura, 1990), Problem Solving Theory (D'Zurilla, 1971), and Relapse Prevention (Marlatt, 1984). Participants were weighed at each intervention session to provide them feedback on progress towards a minimum weight loss goal of 5 percent of initial body weight. In the event a participant missed a regularly scheduled session, interventionists contacted the participant to try to schedule a make-up session prior to the next regular group or individual session so that the participant maintained the orderly flow of lessons and did not miss important information.

	Intervention Week											
Session Type	1	2	3	4	5	6	7	8	9	10	11	12
Group Session	X	X	X	X	X	X	X	X	X	X	X	X
Individual Sessions as Necessary												

Figure 3.3. Type of intervention session by intervention week

Dietary Intervention Component (DIET): Participants were given a calorie and fat gram goal according to their initial bodyweight (Table 3.1). Subject goals changed through the course of the intervention based upon reduction to a lower weight classification. The fat gram goals were set at 20% to 30% of total caloric intake. Sample meal plans, recipes, and a copy of the 2009 edition of The Calorie King Calorie, Fat, and Carbohydrate Counter (Borushek, 2009) were provided for participants to assist with making healthy eating decisions that also helped to achieve the calorie and dietary fat intake goals.

Each week participants were provided a food diary to record daily food type and both calorie and fat content of foods consumed. Diaries were to be turned in weekly and were reviewed by trained staff. Feedback was provided on the diary contents by the staff prior to the diary being returned to participants at the next intervention session. In the case of a subject not

returning a weekly diary or the diary being incomplete, an interventionist consulted with the participant at the next group or individual session or contacted the participant by telephone if she missed the scheduled intervention session, to consider strategies to improve compliance to self-monitoring

Table 3.1. Calorie and Fat Goals for Baseline Weights

Starting Weight	Calorie Goal	Fat gram Goal
≤175	1200	27
175-219	1500	33
220-249	1800	40
250+	2100	47

3.3.1.3 DIET+AT

Participants in the DIET+AT group received all of the components as described above for the DIET group in the manner described: Additionally, this group participated in guided progressive aerobic training 3 days per week as shown in Figure 3.4. Participants were asked to attend 3 sessions per week at the Physical Activity and Weight Management Research Center of the University of Pittsburgh where they participated in supervised exercise sessions progressing from 60 to 180 minutes per week of moderate-intensity (3.0-6.0 METS) aerobic exercise (e.g., treadmill walking, recumbent cycling, adaptive motion trainer, elliptical trainer). 180 minutes per week of exercise meets the American College of Sports Medicine and the American Heart Association recommendations of 450-750 $METS \cdot min \cdot wk^{-1}$ (Haskell, 2007). These exercise sessions were overseen by staff instructed not to provide any additional information relative to weight loss or dietary change to the participants during the sessions.

	Intervention Week											
	1	2	3	4	5	6	7	8	9	10	11	12
Supervised Sessions Per Week												
Length of Supervised Session (minutes)	20	30	40	50	60	60	60	60	60	60	60	60
Minutes per Week Supervised Exercise	60	90	120	150	180	180	180	180	180	180	180	180
Percent of Age-predicted Maximal Heart Rate	60-65	60-65	60-65	65-70	65-70	65-70	65-70	65-70	65-70	65-70	65-70	65-70

Figure 3.4. Progressive Aerobic Training Protocol

3.3.1.4 DIET +RT

Participants in the DIET+RT group received all of the components as described above for the DIET group in the manner described. DIET+RT also participated in a guided resistance training protocol 3 days per week. The protocol started the participants at a low volume of exercise at an intensity of 50-55% of 1-repetition maximum, and oscillated in both volume and intensity during the intervention (Table 3.2), with a general trend toward increases in intensity as outlined in the ACSM Position Stand: Progression Models in Resistance Training for Healthy Adults (2009). During the first week of sessions the participants were introduced to the resistance training

equipment at the Physical Activity and Weight Management Research Center and instructed in its' appropriate use. The resistance exercises used included, but was not limited to: Leg Press; Seated Row; Chest Press; Pulldown; Shoulder Press; Bicep Curl; Tricep Extension; Leg Extension; and Seated Leg Curl. Some resistance exercises utilizing resistance bands/tubes, and dumbbells were also be used. The schedule of introduction of exercises can be viewed in Table 3.3. The training sessions were overseen by interventionists trained in the use of the equipment and qualified to respond to untoward events.

Table 3.2. Progressive Resistance Training Protocol for DIET+RT.

Week	Sets times Repetitions	Workout Day and Daily Intensity (% of Repetition Maximum)
		Day: 1 2 3
1	2x8	65 – 65 – 60
2	2x8	65 – 70 – 65
3	3x10	70 – 70 – 65
4	3x10	70 – 75 – 70
5	4x6	70 – 75 – 65
6	4x6	75 – 80 – 70
7	4x6	75 – 85 – 70
8	4x6	70 – 70 – 70
9	6x4	75 – 80 – 70
10	6x4	70 – 80 – 70
11	6x4	80 – 90 – 70
12	6x4	75 – 75 – 75

Table 3.3. Timeline for introduction of various resistance exercises for DIET+RT.

Exercise	Intervention Week											
	1	2	3	4	5	6	7	8	9	10	11	12
Leg Press	X	X	X	X	X	X	X	X	X	X	X	X
Leg Extension				X	X	X	X	X	X	X	X	X
Leg Curl												

				X	X	X	X	X	X	X	X	X
Back Exercise												
Seated Row	X	X	X				X	X			X	X
Pulldown				X	X	X			X	X		
Chest Press	X	X	X	X	X	X	X	X	X	X	X	X
Shoulder Exercise												
Shoulder Press							X	X	X			X
D-bell Upright Row				X	X	X					X	
Tubing Lateral Raise	X	X	X							X		
Bicep Curls												
Machine				X	X	X						
Dumbbell										X	X	X
Tubing							X	X	X			
Tricep Extension												
Machine				X	X	X				X		X
Dumbbell							X	X	X		X	

3.4 ASSESSMENT VISITS

Assessments were performed at 0 and 12 weeks at the University of Pittsburgh Physical Activity and Weight Management Research Center. The assessment procedures took approximately 120 to 150 minutes to complete. In addition, participants were given questionnaires to complete at

home, and these questionnaires were returned to the investigators on the day of their in-person assessment.

3.4.1 Assessment Components

Height

Height was measured to the nearest 0.01 cm at the baseline assessment using a wall-mounted stadiometer (Perspective Enterprises, Portage, MI). Participants removed their shoes for this measurement.

Weight

Weight: Body weight was measured to the nearest 0.1 kg on a Tanita WB-110A electronic scale (Tanita Corporation, Arlington Heights, IL) with participants wearing only a lightweight hospital gown.

Body Mass Index

Body mass index was computed from measures of weight and height as kg/m^2.

Anthropometric Measures

Waist girth, hip circumference, and computed waist-to-hip ratio was used to represent abdominal adiposity. Waist and hip girth were measured to the nearest 0.1 cm using a Gulick tape measure. Waist circumference is measured on the horizontal plane at the level of the iliac crest. Hip circumference is measured at the largest part of the hips. Waist-to-hip ratio is calculated by dividing the waist measurement by the hip measurement. Two measures were taken at each site, with these measures considered valid if they differ by $\leq$2.0 centimeters. If these criteria were not achieved, additional measures were taken until this criteria was achieved.

Body Composition

Body fat percent was assessed with a GE Lunar iDXA dual-energy x-ray absorptiometer (DXA) (GE Healthcare, Madison, WI). Calibration using a photon is performed daily according to manufacturer specifications. Women were required to undergo a urine pregnancy test just prior to this procedure to confirm non-pregnancy, with women who were pregnant excluded from further participation in this study. Subjects wore a lightweight hospital gown and removed any jewelry or metal items prior to this procedure. The scanning speed and mode was determined according to manufacturer recommendations based on body size. Participants were instructed to lay motionless on the DXA scanning table during the total body scan, which took approximately 10 to 15 minutes to complete. Analysis of the scan was performed by trained personnel and provided information on fat mass, lean mass, percent body fat, bone mineral content, and bone mineral density. Body fat percentage is reported as tissue percent fat and total body percent fat.

Tissue percent fat measures body fat as a percent of total body mass without bone mineral content mass. Total body percent fat measures body fat as a percent of total body mass.

Resting Heart Rate and Blood Pressure

Heart rate and blood pressure were measured by utilizing a Dinamap automated blood pressure and heart rate monitor (GE Healthcare, Madison, WI) by personnel trained in the use of the equipment. Two blood pressure measures were performed with a 1-minute waiting period between the measurements. If the systolic pressures between the two measures differed by > 10mmHg, or the diastolic by >6mmHg, additional readings were taken until the criteria were met. Individuals with systolic blood pressure >150 mmHg or diastolic blood pressure >100 mmHg were excluded from the study.

Cardiorespiratory Fitness

Cardiorespiratory fitness was assessed using a submaximal graded exercise test on a treadmill, with this test being similar to the test used for the multi-center Look AHEAD Study (Curtis, 2010). All exercise testing was conducted by an American College of Sports Medicine (ACSM) Certified Exercise Specialist.

During the exercise test, the speed of the treadmill was held constant at 80.4 m/min (3.0 mph), with the grade of the treadmill starting a 0% grade and increasing by 1.0% at 1-minute intervals. Heart rate was assessed during the final 10 seconds of each minute using a 12-lead electrocardiogram (ECG) with standard 10 electrode placement. Blood pressure was assessed

during the final 45 seconds of each even minute (e.g., 2 minutes, 4 minutes, etc.) using a manual sphygmomanometer and stethoscope. Rating of perceived exertion (RPE) was assessed at the end exercise stage (each minute) using the 15-category Borg Scale (6=no exertion, 20=maximal exertion). Heart rate, blood pressure, and RPE were also be assessed at the point of test termination. Breath-by-breath analysis of expired gas concentrations and volume were assessed using a CareFusion Vmax Encore metabolic cart (CareFusion, Yorba Linda, CA). This metabolic cart was calibrated using known gas concentrations and volumes according to manufacture specifications.

The graded exercise test was terminated when the participant achieved 85% of age-predicted maximal heart rate, which is computed as [(220 minus age) multiplied by 0.85]. In addition, ACSM criteria for test termination was used to identify situations that required termination of the test prior to achieving the target of 85% of age-predicted maximal heart rate. For participants achieving 85% of age-predicted maximal heart rate, the test was following by a 7 minute recovery period that included a 3-minute cool-down phase (2.0 mph, 0% grade) and a 4 minute seated phase. Heart rate was measured during the each minute and blood pressure measured at minutes 1, 3, 5, and 7. Each exercise test was reviewed by a board certified cardiologist. Cardiorespiratory fitness was represented as: 1) oxygen consumption (ml/kg/min) at test termination, and 2) time until test termination.

Muscular Strength Assessment

Muscular strength assessment was performed at 0 and 12 weeks on the Leg Press, Chest Press, and Seated Row, utilizing a modified ACSM 1-repetition maximum protocol (ACSM, 2010).

Leg Press: The subject was seated on a Precor™ leg press apparatus and placed feet on the press platform. Assessment staff assisted the subject in placing the seat of the apparatus at a setting that allows the knee to maintain a 90-degree angle of flexion. The subject performed a warm up with a single set of 5 repetitions with a single weight plate of 25 pounds (Fonzi, 2008). A second warm-up set of 5 repetitions was performed with an increase in resistance based upon the subject's Rate of Perceived Exertion (RPE) using the 10-point OMNI scale (Robertson, 2003). If the subject had an RPE of <5 the resistance was increased by two plates (30 pounds); an RPE of 5-6 resulted in an increase of a single plate (15 pounds); an RPE of >7 resulted in no second warm-up set. At this point, single-repetition efforts were performed with the resistance progressively increased by 5-20 lbs until the subject could no longer complete the selected repetitions. The goal was to determine 1-Repetition Maximum within 4-6 trials, with rest periods of 30-60 seconds between trials. All repetitions of the assessment were performed at the same speed and range of motion remained consistent throughout the trial. The final weight successfully lifted was recorded as the absolute 1-Repetition Maximum.

Chest Press: The subject was seated on the Precor™ vertical chest press machine adjusted for height such that the handles of the machine were at mid-chest level and the feet could be placed flat on the floor. The hands were placed on the machine handles at either a prone grip or a neutral grip, depending upon participant preference. The subject performed a warm up with a single set of 5 repetitions with a single weight plate of 25 pounds (Fonzi, 2008). A second warm-up set of 5 repetitions was performed with an increase in resistance based upon the subject's Rate of Perceived Exertion (RPE) using the 10-point OMNI scale (Robertson, 2003). If the subject had an RPE of <5 the resistance was increased by 10 pounds; an RPE of 5-6 resulted in an increase of 5 pounds; an RPE of >7 resulted in no second warm-up set. At this point,

single-repetition efforts were performed with the resistance progressively increased by 5-20 lbs until the subject could no longer complete the selected repetitions. The goal was to determine 1-Repetition Maximum within 4-6 trials, with rest periods of 30-60 seconds between trials. All repetitions of the assessment were performed at the same speed and range of motion remained consistent throughout the trial. The final weight successfully lifted was recorded as the absolute 1-Repetition Maximum).

Seated Row: The subject sat on the Precor™ seated row machine at a seat height that allowed the chest pad to strike the participant just below shoulder level. The participant utilized a neutral grip during the testing session. The subject performed a warm up with a single set of 5 repetitions with a single weight plate of 25 pounds (Fonzi, 2008). A second warm-up set of 5 repetitions was performed with an increase in resistance based upon the subject's Rate of Perceived Exertion (RPE) using the 10-point OMNI scale (Robertson, 2003). If the subject had an RPE of <5 the resistance was increased by 10 pounds; an RPE of 5-6 resulted in an increase of 5 pounds; an RPE of >7 resulted in no second warm-up set. At this point, single-repetition efforts were performed with the resistance progressively increased by 5-20 lbs until the subject could no longer complete the selected repetitions. The goal was to determine 1-Repetition Maximum within 4-6 trials, with rest periods of 30-60 seconds between trials. All repetitions of the assessment were performed at the same speed and range of motion remained consistent throughout the trial. The final weight successfully lifted was recorded as the absolute 1-Repetition Maximum.

The 1-Repetition Maximum weights achieved during initial assessments were used to develop the strength training protocol for each participant, ensuring that each person was trained to their individual ability.

Physical Activity

Physical activity was subjectively assessed using the Paffenbarger Physical Activity Questionnaire (Paffenbarger, 1986) and objectively assessed using a Body Media SenseWear Pro Armband. The Paffenbarger questionnaire is used to determine energy expenditure during participation in leisure-time physical activity. Participants are queried on daily average number of flights of stairs walked up, average daily walking performed for the purpose of exercise, and any sport, recreational, or fitness activities the subject engaged in over the previous week typical week. Activity is converted to energy expenditure based on metabolic equivalents (METs) provided in the compendium of physical activity (Paffenbarger, 1986). The Paffenbarger questionnaire has been shown to provide an acceptable level of validity and reliability (Pereira, 1997).

Physical activity was also assessed objectively using the SenseWear Pro Armband (BodyMedia, Inc., Pittsburgh, PA). The SenseWear Pro Armband (BodyMedia) includes an accelerometer, a heat flux sensor, a galvanic skin response sensor, a skin temperature sensor and a near-body ambient temperature sensor (Berntsen, 2010). These data are used in conjunction with body weight, height, handedness, and smoking status data to calculate energy expenditure (St-Onge, 2007). The monitor was worn on the right arm over the triceps brachii muscle at the midpoint between the acromion and olecranon processes, and was only removed for bathing purposes or other water activities (St-Onge, 2007). The data from the monitor was downloaded and calculated with software developed by the manufacturer (Innerview Professional Research Software V.6.1, BodyMedia) (Berntsen, 2010). The SenseWear Pro_2 Armband has been

validated against doubly-labeled water to provide an accurate depiction of total daily energy expenditure (St-Onge, 2007).

Venous Blood Sample

The primary outcome of the intervention was change in hs-C-Reactive Protein level. Venous blood samples were taken at the baseline and follow-up assessments. Blood samples were taken following a 12-hour fast and abstention from moderate-to-vigorous physical activity. Blood was drawn into evacuated tubes containing EDTA, sodium heparing, or SST clot activator [Becton-Dickinson, Franklin Lakes, NJ] (Stewart, 2007). Baseline and 12-week samples were stored in a -70° C freezer so that each participant's baseline and 12-week samples could be measured using the same assay kit (Church, 2010). hs-CRP was measured by a solid-phase, chemiluminescent immunometric assay (Immulite 2000 High-Sensitivity CRP; Diagnostics Products Corporation, Los Angles, CA) (Church, 2010).

Dietary Intake

Dietary intake was measured using the most recent version of the Block Food Frequency Questionnaire (FFQ), which is Version 2005. This questionnaire assesses the usual frequency consumption of specific foods and typical portion sizes over a certain time period. The FFQ obtains information regarding daily energy intake and nutrient intake estimates and has been previously validated (Block, 1990; Subar, 2001).

Weight Loss Behaviors

Behaviors recommended for weight loss were measured using the Eating Behavior Inventory (EBI) (O'Neil, 1979). This questionnaire assesses behaviors that may be related to weight loss such as self-monitoring of food intake and weight, refusing offers of food, shopping from a list, and eating in response to emotions. The EBI consists of 26 items that are rated with a 5-point scale ranging from never or hardly ever to always or almost always. The EBI has been established as a valid tool for measuring changes in weight related behaviors (O'Neil, 1979; O'Neil 2005).

3.5 PRIMARY OUTCOME MEASURES

3.5.1 hs-C-reactive Protein

Venous blood samples were taken at the baseline and follow-up assessments. Blood samples were taken following a 12-hour fast and abstention from moderate-to-vigorous physical activity. Blood was drawn into evacuated tubes containing EDTA, sodium heparing, or SST clot activator [Becton-Dickinson, Franklin Lakes, NJ](Stewart, 2007). Baseline and 12-week samples were stored in a -70° C freezer so that each participant's baseline and 12-week samples could be measured using the same assay kit (Church, 2010). CRP was measured by a solid-phase, chemiluminescent immunometric assay (Immulite 2000 High-Sensitivity CRP; Diagnostics Products Corporation, Los Angles, CA) (Church, 2010). All blood samples were processed at

the Heinz Nutrition Laboratory in the Graduate School of Public Health at the University of Pittsburgh.

3.5.2 Weight Loss

Body weight was measured both at baseline and at follow-up assessments to the nearest 0.1 kg on a Tanita WB-110A electronic scale (Tanita Corporation, Arlington Heights, IL) with participants wearing only a lightweight hospital gown. End-point weight was subtracted from baseline weight to calculate total weight loss from baseline to follow-up assessment.

3.5.3 Body Composition

Body composition was assessed with a GE Lunar iDXA dual-energy x-ray absorptiometer (DXA) (GE Healthcare, Madison, WI). Analysis of the scan was performed by trained personnel and provided information on fat mass, lean mass, percent body fat, bone mineral content, and bone mineral density.

3.6 STATISTICAL ANALYSIS

Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) software, version 17.0. Statistical significance will be set at $P<0.05$. Analyses will be conducted using individuals completing the assessment protocol at 0 and 12 weeks, and with intention-to-treat analysis carrying the baseline data forward. The following analyses will be performed:

1. Descriptive analysis to examine the mean baseline characteristics (age, weight, BMI), body composition, daily physical activity, and dietary intake.
2. Descriptive analysis to examine process measures, including: session attendance; dietary logging; self-reported caloric intake; and self-weighing frequency. One-way analysis of variance (ANOVA) will be used to examine any differences in the process measures among groups
3. 3 x2 mixed ANOVA will be performed on CRP levels as a function of group and time to determine differences between the variables. The main effect of time will be examined between 0 and 12 weeks; the main effect of group will examine any differences among randomized groups. The group x time interaction will be examined to determine patterns of difference among groups for weight loss between 0 and 12 weeks. Post hoc pairwise comparisons will be used, if needed, to determine differences in weight loss between the randomized groups.
4. 3 x 2 mixed ANOVA will be performed on weight loss as a function of group and time to determine differences between the variables. The main effect of time will be examined between 0 and 12 weeks; the main effect of group will examine any differences among randomized groups. The group x time interaction will be examined to determine patterns of difference among groups for weight loss between 0 and 12 weeks. Post hoc pairwise

comparisons will be used, if needed, to determine differences in weight loss between the randomized groups.

5. 3 x 2 mixed ANOVA will be performed on dietary intake by group to determine if there are differences between the variables. The main effect of time will determine any differences between 0 and 12 weeks. The main effect of group will determine any differences between randomized group. Group x time interaction will be examined to determine patterns of difference between the groups for dietary intake between 0 and 12 weeks. Post hoc pairwise comparisons will be used, if needed, to determine differences in weight loss between the randomized groups.
6. 3 x 2 mixed ANOVA will be performed on body composition by group to determine if there are differences between the variables. The main effect of time will determine any differences between 0 and 12 weeks. The main effect of group will determine any differences between randomized group. Group x time interaction will be examined to determine patterns of difference between the groups for body composition between 0 and 12 weeks. Post hoc pairwise comparisons will be used, if needed, to determine differences in weight loss between the randomized groups

3.7 POWER ANALYSIS

The primary aims of this study were to examine the effect of the interventions (DIET, DIET+AT, DIET+RT) on inflammatory measures that have been shown to be associated with increased risk of CVD. The results from this study were intended to fulfill the dissertation requirement of the investigator and to provide important pilot data that can be used to support an appropriately

powered larger study. The pilot data of importance that were to be obtained from this study included the following: 1) effect sizes of differences between the intervention conditions for the proposed inflammatory measures, 2) measures of compliance to the proposed doses of exercise in the DIET+AT and DIET+RT groups, 3) measures of compliance and feasibility for attendance at supervised exercise sessions, 4) data on recruitment of subjects to participate in a study of this nature. Based on these goals, it is proposed that 30 subjects be recruited and complete the intervention, with 10 subjects completing the study in each condition. Therefore, we propose a conservative attrition rate of 20%, which would require the recruitment of 36 subjects with 12 randomly assigned to each intervention condition. With alpha defined as 0.05 and statistical power defined as 80%, this would permit the detection of larger effect sizes on the outcome measures for between group comparisons. However, this would also provide the pilot and feasibility data as described above to support a larger clinical study in this area.

4.0 RESULTS

The purpose of this study was to evaluate the additive effect of aerobic or resistance exercise training to caloric restriction for weight loss on high sensitivity C-Reactive Protein changes compared to dietary restriction alone in class II and class III obese individuals: Caloric restriction weight loss program with no exercise (DIET); Caloric restriction with aerobic endurance training (DIET+AT); and Caloric restriction with resistance training (DIET+RT). The study also compared changes between these interventions for measures of waist circumference, hip circumference, waist-to-hip ratio, and body composition.

4.1 SUBJECTS

36 obese women between the ages of 30 and 55 signed written informed consent documents to participate in this study. Eight (8) recruited subjects were unable to commit to their initial assessment and/or were unable to obtain physician permission to participate within the appropriate time-frame and were therefore excluded from study participation. Three (3) subjects were found to be outside of the required BMI criteria and were thereby deemed to be ineligible to participate. One subject underwent the baseline assessment and subsequently decided that she would prefer not to participate. A total of 24 (BMI: 39.0$\pm$2.7; Age: 45.4$\pm$6.9) subjects

completed baseline assessments, were deemed eligible, and were randomized for participation in this study.

Descriptive statistics are shown in Table 4.1 for both the total sample and for each intervention group. A series of one-way analysis of variance revealed there was no significant difference between intervention groups at baseline for: age; weight; BMI; waist circumference; hip circumference; waist-to-hip ratio; body composition; dietary intake; eating behavior inventory; and self-reported physical activity. Further, no baseline differences between groups were detected in oxygen consumption (VO_2) at 85% of age-predicted maximal heart rate (AMHR); treadmill time to 85% of AMHR; muscular strength on leg press, chest press, and seated row; or high-sensitivity C-reactive protein (hs-CRP).

Table 4.1. Differences in Baseline Characteristics by Treatment Group

Characteristics	Total (N=24) (Mean±S.D.)	DIET (N=8) (Mean±S.D.)	DIET+AT (N=8) (Mean±S.D.)	DIET+RT (N=8) (Mean±S.D.)	p-value for group comparisons
Age (years)	45.4 ± 6.9	46.6 ± 8.8	43.9 ± 5.9	45.5 ± 6.1	0.741
Weight (kg)	104.2 ± 10.6	104.2 ± 11.3	107.2 ± 13.3	101.3 ± 6.8	0.757
Body Mass Index (kg/m^2)	39.0 ± 2.7	39.7 ± 2.2	39.0 ± 3.2	38.4 ± 2.7	0.622
Waist Circumference (cm)	111.9 ± 7.2	115.3 ± 8.5	109.9 ± 7.1	110.6 ± 5.2	0.284
Hip Circumference (cm)	128.6 ± 8.9	126.3 ± 3.9	132.6 ± 12.4	127.0 ± 7.9	0.316
Waist-to-hip ratio	0.87 ± 0.07	0.91 ± 0.06	0.83 ± 0.07	0.87 ± 0.05	0.061
†Body Composition %Fat Total (%) %Fat Tissue (%)	 48.9 ± 3.6 50.2 ± 3.5	 48.2 ± 3.8 49.5 ± 3.8	 49.8 ± 4.4 51.1 ± 4.4	 48.6 ± 3.7 49.9 ± 3.8	 0.715 0.708
Race %African American %Caucasian %Other	 20.8 (N=5) 75.0 (N=18) 4.2 (N=1)	 0.0 (N=0) 100.0 (N=8) 0.0 (N=0)	 25.0 (N=2) 75.0 (N=6) 0.0 (N=0)	 37.5 (N=3) 50.0 (N=4) 12.5 (N=1)	0.508
Dietary Intake (kcal/day) %Fat %Protein %Carbohydrate	2056.0±644.2 38.1±5.5 15.2±3.3 64.7±8.6	2024.6±264.4 37.2±2.2 13.5±0.9 49.3±3.5	2088.3±276.4 35.8±1.7 15.4±0.8 49.3±2.7	2055.3±154.4 41.3±1.5 16.6±1.6 41.5±2.2	0.982 0.107 0.175 0.108
Eating Behavior Inventory	69.2 ± 9.7	67.6 ± 13.1	69.6 ± 8.9	70.6 ± 6.6	0.844
Self Reported Physical Activity (kcal/week)	473.7 ± 445.6	322.1 ± 300.8	570.9 ± 516.7	528.1 ± 504.4	0.511
‡VO_2 at 85% AMHR (ml/kg/min)	20.4 ± 3.6	21.8 ± 4.3	20.1 ± 3.7	19.2 ± 2.6	0.372
§Time to Reach 85% AMHR (min)	5.8 ± 2.7	7.0 ± 2.7	4.9 ± 3.4	5.3 ± 1.7	0.286
Muscular Strength by 1-RM (kg) Leg Press Chest Press Seated Row	 66.1 ± 37.9 39.7 ± 13.5 38.4 ± 14.8	 55.4 ± 35.8 33.8 ± 15.1 33.5 ± 11.4	 71.6 ± 40.4 44.0 ± 13.7 39.8 ± 18.6	 71.3 ± 40.3 41.2 ± 10.6 41.8 ± 14.3	 0.640 0.304 0.531
*hs-CRP (mg/dl)	0.79 ± 0.62	0.66 ± 0.31	1.07 ± 0.88	0.65 ± 0.51	0.326

†%Fat Total includes Bone Mineral Content (BMC), %Fat Tissue is exclusive of BMC
*high-sensitivity C-reactive Protein
‡Oxygen consumption at 85% of Age-predicted Maximal Heart Rate (AMHR)
§Time of Graded Exercise Test to achieve 85% of Age-predicted Maximal Heart Rate (AMHR)

4.2 RETENTION RATES

Twenty-four subjects were randomized to one of three treatment groups (DIET, DIET+AT, DIET+RT). A total of 18 participants completed baseline and 12-week follow-up assessments and will be referred to as “completers”. Participants who did not complete the follow-up assessment will be considered “non-completers”. Three of the non-completers were official withdrawals: one participant was injured in a car accident at week 7 and could no longer participate, a second was injured during a recreational activity at week 9 and was under the care of a physician at the time of follow-up assessments, and a third was forced to drop the program due to an unanticipated move. Three participants stopped communication with the study and were lost to follow-up.

Figure 4.1 shows subject enrollment, retention, and reason for withdrawal. Retention rate overall and by group was 75%.

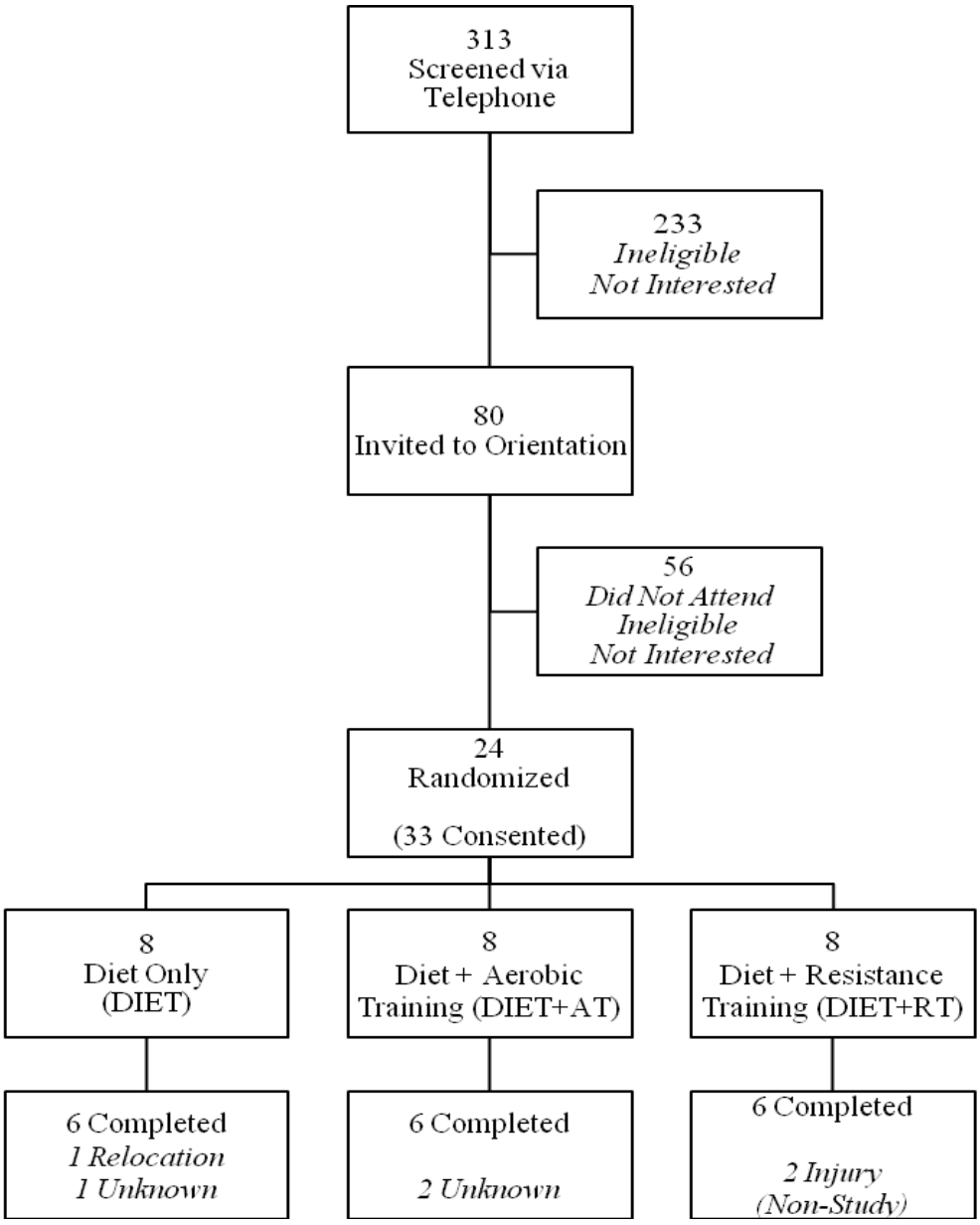


Figure 4.1. Recruitment and Screening

4.3 BASELINE CHARACTERISTICS

Analysis of Variance (ANOVA) at baseline yielded no significant differences between completers and non-completers for any of the baseline measures, including: age, weight, BMI, waist and hip circumference, waist-to-hip ratio, percent body fat with and without bone mineral content, dietary intake, eating behavior, self-reported physical activity, VO_2 at 85% AMHR, Treadmill time to 85% of AMHR, muscular strength measures, and hs-CRP.

When ANOVA was performed on completers and non-completers by treatment group, significant differences were found between the completers and non-completers in the DIET and DIET+AT groups for VO_2 at 85% of AMHR ($p<0.05$) and between completers and non-completers in all three treatment groups for Treadmill time to 85% of AMHR ($p<0.05$). The results of the analysis of variance on completers and non-completers by treatment group are shown in Table 4.3.

Baseline characteristics for completers and non-completers are presented in Table 4.2.

Table 4.2. Differences in Baseline Characteristics by Completers and Non-Completers

Characteristics	Total (N=24) (Mean±S.D.)	Completers (N=18) (Mean±S.D.)	Non-Completers (N=6) (Mean±S.D.)	p-value for group comparisons
Age (years)	45.4 ± 6.9	44.9 ± 6.6	46.7 ± 7.9	0.598
Weight (kg)	104.2 ± 10.6	105.0 ± 10.9	101.9 ± 10.1	0.550
Body Mass Index (kg/m^2)	39.0 ± 2.7	39.3 ± 2.6	38.1 ± 2.9	0.352
Waist Circumference (cm)	111.9 ± 7.2	113.6 ± 7.2	107.2 ± 4.6	0.056
HipCircumference (cm)	128.6 ± 8.9	129.3 ± 9.5	126.7 ± 7.1	0.551
Waist-to-hip ratio	0.87 ± 0.07	0.88 ± 0.07	0.85 ± 0.04	0.280
†Body Composition %Fat with BMC (%) %Fat w/o BMC (%)	 48.9 ± 3.6 50.2 ± 3.5	 48.9 ± 4.0 50.2 ± 4.0	 48.7 ± 3.5 50.0 ± 3.5	 0.937 0.924
Race %African American %Caucasian %Other	 20.8 (N=5) 75.0 (N=18) 4.2 (N=1)	 27.8 (N=5) 72.2 (N=13) 0.0 (N=0)	 0.0 (N=0) 83.3 (N=5) 16.7 (N=1)	 0.339
Dietary Intake (kcal/day) %Fat %Protein %Carbohydrate	2056.0±644.2 38.1±5.5 15.2±3.3 64.7±8.6	2201.9±157.3 38.6±1.4 15.8±0.8 45.9±2.1	1618.5±120.9 36.4±1.9 13.3±1.1 49.3±3.1	0.052 0.407 0.103 0.418
Eating Behavior Inventory	69.2 ± 9.7	68.6 ± 9.6	71.2 ± 9.7	0.578
Self Reported Physical Activity (kcal/week)	473.7± 445.6	466.4 ± 428.9	495.5 ± 535.8	0.894
‡VO_2 at 85% AMHR (ml/kg/min)	20.4 ± 3.6	20.2 ± 3.8	20.9 ± 3.4	0.701
§Time to Reach 85% AMHR (min)	5.8 ±2.7	5.8 ±2.9	6.3 ± 2.3	0.597
Muscular Strength by 1-RM (kg) Leg Press Chest Press Seated Row	 66.1 ± 37.9 39.7 ± 13.5 38.4 ± 14.8	 66.7 ± 39.3 39.8 ± 13.6 39.0 ±16.2	 36.9 ± 15.0 14.4 ± 5.9 36.4 ± 12.9	 0.902 0.954 0.713
*hs-CRP (mg/dl)	0.79 ± 0.62	0.79 ± 0.62	0.79 ± 0.62	1.0

†%Fat Total includes Bone Mineral Content (BMC), %Fat Tissue is exclusive of BMC
*high-sensitivity C-reactive Protein
‡Oxygen consumption at 85% of Age-predicted Maximal Heart Rate (AMHR)
§Time of Graded Exercise Test to achieve 85% of Age-predicted Maximal Heart Rate (AMHR)

Table 4.3. Baseline Characteristics by Treatment Group and Completion

Characteristics	DIET		DIET+AT		DIET+RT	
	Completers (N=6) (Mean±S.D.)	Non-Completers (N=2) (Mean±S.D)	Completers (N=6) (Mean±S.D.)	Non-Completers (N=2) (Mean±S.D)	Completers (N=6) (Mean±S.D.)	Non-Completers (N=2) (Mean±S.D)
Age (years)	48.7±7.8	40.3±11.4	41.9±5.2	49.8±4.9	44.1±5.8	50.0±6.4
Weight (kg)	105.8±12.7	99.2±5.1	108.9±12.0	101.9±21.1	100.2±7.6	104.6±2.2
Body Mass Index (kg/m^2)	40.3±2.1	38.0±2.0	39.0±3.1	39.3±5.0	38.8±2.7	37.2±2.9
Waist Circumference (cm)	118.3±7.1	106.2±5.8	110.2±7.9	109.3±6.3	112.2±4.7	106.1±4.6
Hip Circumference (cm)	127.6±3.6	122.4±2.0	133.6±13.2	129.4±13.4	126.6±9.2	128.3±3.3
Waist-to-hip ratio	0.93±0.05	0.87±0.06	0.83±0.09	0.85±0.04	0.89±0.05	0.83±0.01
†Body Composition %Fat with BMC (%) %Fat w/o BMC (%)	 49.2±3.1 50.5±3.1	 45.3±4.3 46.5±4.3	 49.8±5.2 51.1±5.2	 49.6±1.3 50.8±1.4	 47.7±3.9 49.0±4.0	 51.4±1.0 52.7±1.0
Race %African American %Caucasian %Other	 0.0 (N=0) 100.0 (N=6) 0.0 (N=0)	 0.0 (N=0) 100.0 (N=2) 0.0 (N=0)	 16.7 (N=1) 83.3 (N=5) 0.0 (N=0)	 50.0 (N=1) 50.0 (N=1) 0.0 (N=0)	 50.0 (N=3) 50.0 (N=3) 0.0 (N=0)	 0.0 (N=0) 50.0 (N=1) 50.0 (N=1)
Dietary Intake (kcal/day) %Fat %Protein %Carbohydrate	2216.2±316.3 36.3±2.4 14.3±1.0 50.5±3.8	1449.9±130.2 39.7±5.6 11.3±0.1 45.8±10.2	2183.5±367.4 36.2±2.2 15.3±0.8 48.7±3.5	1802.7±75.9 34.6±2.2 15.7±2.9 51.3±4.2	2206.0±131.9 43.4±0.9 17.9±1.8 38.5±1.3	1603.1±366.1 35.0±1.3[a] 12.8±0.7 50.7±1.8[a]
Eating Behavior Inventory	67.8±14.7	67.0±11.3	67.5±7.0	76.0±14.1	70.5±6.3	70.5±7.8
Self Reported Physical Activity (kcal/week)	350.2±349.5	238.0±59.4	471.0±358.4	870.5±993.5	578.2±586.1	378.0±59.4
‡VO_2 at 85% AMHR (ml/kg/min)	20.9±4.6	24.5±1.3[a]	21.1±3.8	17.2±0.4[a]	18.6±2.7	21.0±1.1
§Time to Reach 85% AMHR (mins)	6.39±2.87	8.8±0.7[a]	5.3±3.9	3.7±0.5[a]	5.0±1.9	6.3±0.0[a]
Muscular Strength by 1-RM (kg) Leg Press Chest Press Seated Row	 50.0±26.4 31.0±11.6 32.2±10.5	 71.6±69.1 42.0±27.3 37.5±17.7	 78.8±43.5 46.2±15.5 43.2±20.5	 50.0±25.7 37.5±1.6 29.5±6.4	 71.2±46.0 42.0±10.1 41.7±15.0	 71.6±27.3 38.6±16.1 42.0±17.7
*hs-CRP (mg/dl)	0.77 ± 0.28	0.34 ± 0.08	1.03 ± 1.03	1.18 ± 0.36	0.58 ± 0.41	0.85 ± 0.94

a = mean difference significant at $p < 0.05$ for completers and non-completers within treatment group
†%Fat Total includes Bone Mineral Content (BMC), %Fat Tissue is exclusive of BMC
*high-sensitivity C-reactive Protein
‡Oxygen consumption at 85% of Age-predicted Maximal Heart Rate (AMHR)
§Time of Graded Exercise Test to achieve 85% of Age-predicted Maximal Heart Rate (AMHR)

4.4 INTERVENTION OUTCOME MEASURES

4.4.1 Weight

A two-factor repeated measures ANOVA (two-factor R-ANOVA) was performed to examine changes in weight from baseline to 12-weeks. The results from both completers analysis and intent-to-treat analysis revealed significant decreases in body weight among all groups ($p<0.001$), with no significant differences between groups ($p=0.40$), and no significant group-by-time effect ($p=0.04$). Utilizing a completers analysis, the DIET group lost 3.1 $\pm$ 2.4kg; the DIET+AT group lost 5.9 $\pm$ 3.1kg; and the DIET+RT group lost 4.9 $\pm$ 4.1kg. Intent-to-treat analysis revealed a loss of 2.4 $\pm$ 2.5kg in the DIET group; a loss of 4.4 $\pm$ 3.8kg in the DIET+AT group; and a loss of 3.7 $\pm$ 4.2kg in the DIET+RT group. Weight loss data is shown in Tables 4.4 and 4.5.

Table 4.4. Intervention Outcome Differences By Treatment Groups at 12 Weeks Completers

Outcome Variable	DIET (N=6) (Mean±S.D.)	DIET+AT (N=6) (Mean±S.D)	DIET+RT (N=6) (Mean±S.D.)	Group Effect	Time Effect	Group x Time Effect
Weight (kg)						
0 Weeks	105.8±12.7	108.9±12.0	100.2±7.6	.400	<0.001	0.376
12 Weeks	102.7±12.5	103.1±12.2	95.4±6.1			
Body Mass Index (kg/m^2)						
0 Weeks	40.3±2.1	39.0±3.1	38.8±2.7	.387	<0.001	0.468
12 Weeks	39.1±2.2	36.9±3.6	36.9±1.8			
Eating Behavior Inventory						
0 Weeks	67.8±14.7	67.5±7.0	70.5±6.3	0.133	<0.001	0.291
12 Weeks	88.7±7.7	82.8±7.5	96.5±7.0			
Self Reported Physical Activity (kcal/week)				.506	.057	0.730
0 Weeks	350.2±349.5	471.0±358.4	578.2±586.1			
12 Weeks	522.3±849.7	925.8±780.3	1084.0±1009.6			
‡VO_2 at 85% AMHR (ml/kg/min)						
0 Weeks	20.9±4.6	21.1±3.8	18.6±2.7	.454	.128	0.883
12 Weeks	21.3±4.6	22.2±3.9	19.6±2.1			
§Time to Reach 85% AMHR (mins)						
0 Weeks	6.4±2.9	5.3±4.0	5.0±1.9	.690	<0.001	0.094
12 Weeks	7.3±2.8	7.9±3.5	6.1±1.1			
Muscular Strength by 1-RM (kg)						
Leg Press						
0 Weeks	50.0±26.4	78.8±43.5	71.2±46.0			
12 Weeks	52.6±20.8	75.8±44.0	94.3±52.9	0.364	0.098	0.060
Chest Press						
0 Weeks	31.0±11.6	46.2±15.5	42.0±10.1			
12 Weeks	38.6±13.5	49.2±15.5	50.8±10.2	0.189	<0.001	0.179
Seated Row						
0 Weeks	32.2±10.5	43.2±20.5	41.7±15.0			
12 Weeks	40.1±11.8	46.6±17.0	51.9±13.0	0.431	<0.001	0.204

†%Fat Total includes Bone Mineral Content (BMC), %Fat Tissue is exclusive of BMC
‡Oxygen consumption at 85% of Age-predicted Maximal Heart Rate (AMHR)
§Time of Graded Exercise Test to achieve 85% of Age-predicted Maximal Heart Rate (AMHR)

Table 4.5. Intervention Outcome Differences By Treatment Group at 12 Weeks - Intent to Treat

Outcome Variable	DIET (N=8) (Mean±S.D.)	DIET+AT (N=8) (Mean±S.D)	DIET+RT (N=8) (Mean±S.D.)	Group Effect	Time Effect	Group x Time Effect
Weight (kg)						
0 Weeks	104.2±11.3	107.2±13.3	101.3±6.8	0.582	<0.001	0.521
12 Weeks	101.8±10.9	102.8±13.0	97.7±6.7			
Body Mass Index (kg/m^2)						
0 Weeks	39.7±2.2	39.0±3.2	38.4±2.7	0.478	<0.001	0.593
12 Weeks	38.8±2.1	37.5±3.7	36.9±1.9			
Eating Behavior Inventory						
0 Weeks	67.6±13.1	69.6±8.9	70.5±6.1	0.437	<0.001	0.515
12 Weeks	83.3±12.7	81.1±8.9	90.0±13.7			
Self Reported Physical Activity (kcal/week)						
0 Weeks	322.1±300.8	570.9±516.7	528.1±504.4	0.379	0.055	0.735
12 Weeks	451.3±730.4	912.0±759.3	907.5±914.0			
‡VO_2 at 85% AMHR (ml/kg/min)						
0 Weeks	21.8±4.3	20.1±3.7	19.2±2.6	0.397	0.122	0.882
12 Weeks	22.1±4.2	20.9±4.1	19.9±1.9			
§Time to Reach 85% AMHR (mins)						
0 Weeks	7.0±2.7	4.9±3.4	5.3±1.7	0.395	0.001	0.161
12 Weeks	7.7±2.5	6.8±3.6	6.1±1.0			
Muscular Strength by 1-RM (kg)						
Leg Press						
0 Weeks	55.4±35.8	71.6±40.4	71.3±40.3	0.485	0.109	0.067
12 Weeks	57.4±32.7	69.3±40.2	88.6±47.1			
Chest Press						
0 Weeks	33.8±15.1	44.0±13.7	41.2±10.6	0.384	<0.001	0.282
12 Weeks	39.5±15.5	46.3±14.2	47.7±12.0			
Seated Row						
0 Weeks	33.5±11.4	39.8±18.6	41.8±14.3	0.458	0.001	0.296
12 Weeks	39.5±12.1	42.3±16.6	49.5±13.6			

†%Fat Total includes Bone Mineral Content (BMC), %Fat Tissue is exclusive of BMC
‡Oxygen consumption at 85% of Age-predicted Maximal Heart Rate (AMHR)
§Time of Graded Exercise Test to achieve 85% of Age-predicted Maximal Heart Rate (AMHR)

4.4.2 Body Mass Index

A two-factor R-ANOVA was performed on participants who completed the study for Body Mass Index (BMI) to examine changes from baseline to 12-weeks between groups. Analysis showed that there was a significant decrease ($p<0.001$) in BMI over time, but no group effect ($p=0.39$) and no group-by-time interaction ($p=0.47$). Completers in the DIET group had a decrease in BMI of 1.2 ± 0.9; completers in the DIET+AT group decreased BMI by $2.1 \pm 1.1 kg/m^2$; and the DIET+RT completers group decreased BMI by $1.9 \pm 1.7 kg/m^2$.

Two-factor R-ANOVA was also executed for Intent-to-treat analysis of BMI. The results indicate that there was a significant decrease in BMI over time ($p<0.001$). There were no group ($p=0.48$) or group-by-time ($p=0.59$) effects for BMI in the intent-to-treat analysis. The DIET group decreased BMI by $0.9 \pm 0.9 kg/m^2$; the DIET+AT group decreased BMI by $1.6 \pm 1.3 kg/m^2$; and the DIET+RT group decreased BMI by $1.7 \pm 1.3 kg/m^2$. BMI data can be found in Table 4.4 for Completers and 4.5 for Intent-to-treat.

4.4.3 Eating Behavior Inventory

A two-factor R-ANOVA was performed on eating behavior based on scores from the Eating Behavior Inventory (EBI). Completers and intent-to-treat analyses showed that there was a significant increase in EBI score across time ($p<0.001$), though no group effect was seen for completers ($p=0.13$) or intent-to-treat ($p=0.44$) and there was no group-by-time interaction for completers ($p=0.29$) or for intent-to-treat ($p=0.52$). Completers in the DIET group had an increase in EBI score of 20.83 ± 12.3 and intent to treat in the DIET group showed an increased score of 15.6 ± 14.2. Results are shown in Tables 4.4 for Completers and 4.5 for Intent-to-treat.

4.4.4 Physical Activity

Based on two-factor R-ANOVA there was a trend for a time effect for self-reported Physical Activity for both completers (p=0.057), and intent-to-treat (p=0.055). There were no significant group effects for completers (p=0.51) or for intent to treat (p=0.38). There were also no group-by-time effects for completers (p=0.73) or for intent-to-treat (p=0.74). Self-reported Physical Activity information is presented in Table 4.4 for Completers and Table 4.5 for Intent-to-treat.

4.4.5 Cardiorespiratory Fitness

A two-factor R-ANOVA was performed to examine changes in oxygen consumption (VO_2 ml/kg/min) at 85% of AMHR from baseline to 12-weeks. The results from both completers analysis and intent-to-treat showed no significant change in VO2 across time, no significant group effect, and no group-by-time effect. Data for VO_2 at 85% of AMHR is shown in Tables 4.4 and 4.5 for Completers and Intent-to-treat, respectively.

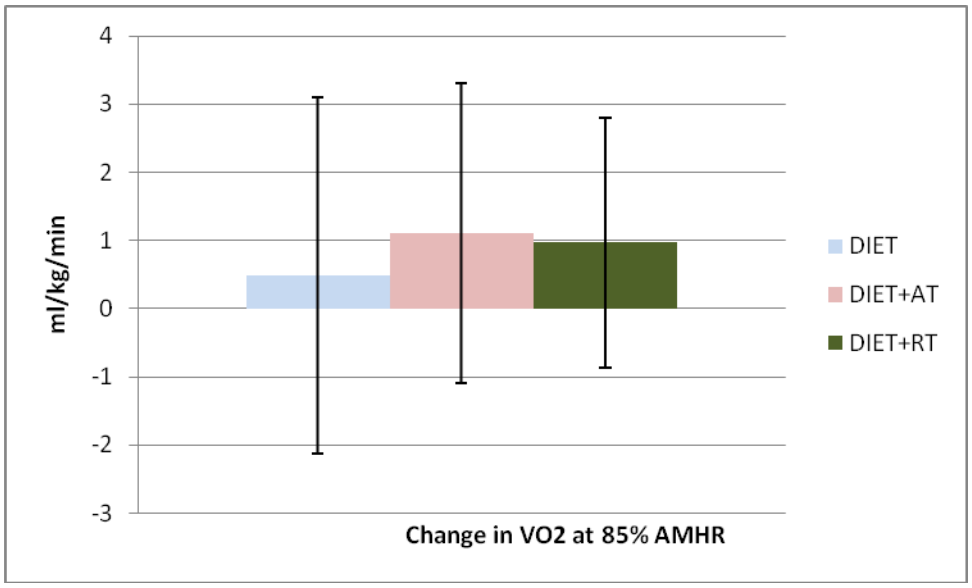
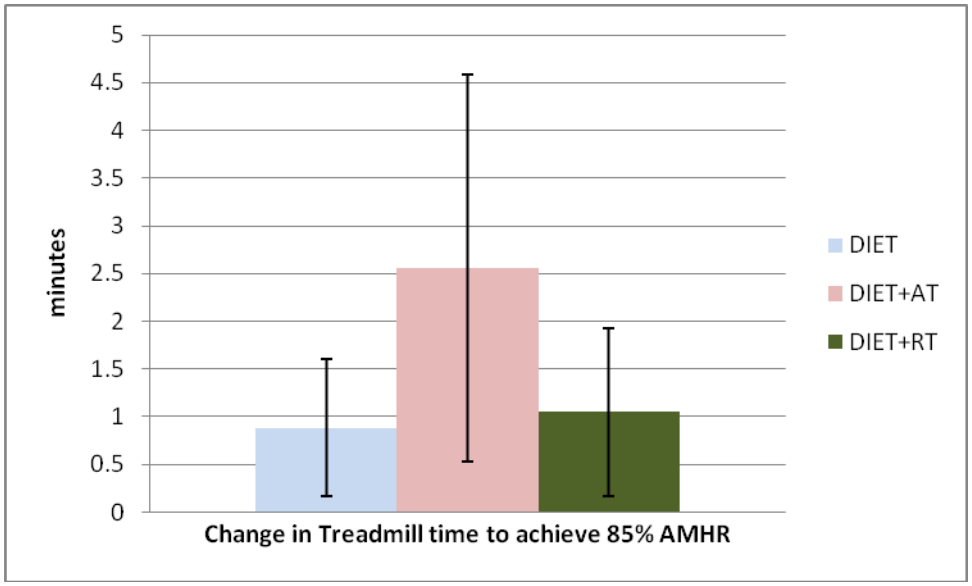


Figure 4.2. Completers VO2 change Baseline to 12-weeks

Two-factor R-ANOVA was performed on treadmill time to 85% of AMHR. Completers and intent-to-treat analyses showed that there was a significant increase in treadmill time to 85% of AMHR across time ($p<0.001$), though no group effect was seen for completers ($p=0.69$) or intent-to-treat ($p=0.40$) and there was no group-by-time interaction for completers ($p=0.09$) or for intent-to-treat ($p=0.16$). Completers in the DIET group had an increase in treadmill time of 0.9 $\pm$ 0.7 minutes and intent to treat in the DIET group showed an increase of 0.7 $\pm$ 0.7 minutes. Completers in the DIET+AT group had an increase of 2.6 $\pm$ 2.1 minutes with intent-to-treat showing an increase of 1.9 $\pm$ 2.1 minutes. The DIET+RT group had an increase of 1.1 $\pm$ 0.9 minutes among completers, and an increase of 0.8 $\pm$ 0.9 minutes with intent-to-treat. Results are shown in Tables 4.4 for Completers and 4.5 for Intent-to-treat. It should be noted that among the completers in the DIET+AT group, 141 of the possible 168 hours of aerobic training were

completed. 118 of the hours exercised took place on the treadmill, 19 hours took place on the elliptical or Adaptive Motion Trainers, and 4 hours of exercise took place on the recumbent bicycles. Treadmills were used as the mode of exercise for 84% of the time completed.



*significant at p<0.001

Figure 4.3. Completers Change in Treadmill Time Baseline to 12-weeks

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4.4.6 Muscular Strength Assessments

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Muscular strength changes were analyzed for changes from baseline to 12 weeks with a two-factor R-ANOVA. No significant changes were found in the leg press for time (p=0.10), group (p= 0.36), or for group-by-time (p=0.06) among completers. With intent-to-treat analysis, no significant changes were found for the leg press across time (p=0.11), between groups (p=0.49),

and no group-by-time interactions were found (p=0.07). Results are shown in Tables 4.4 for Completers and 4.5 for Intent-to-treat.

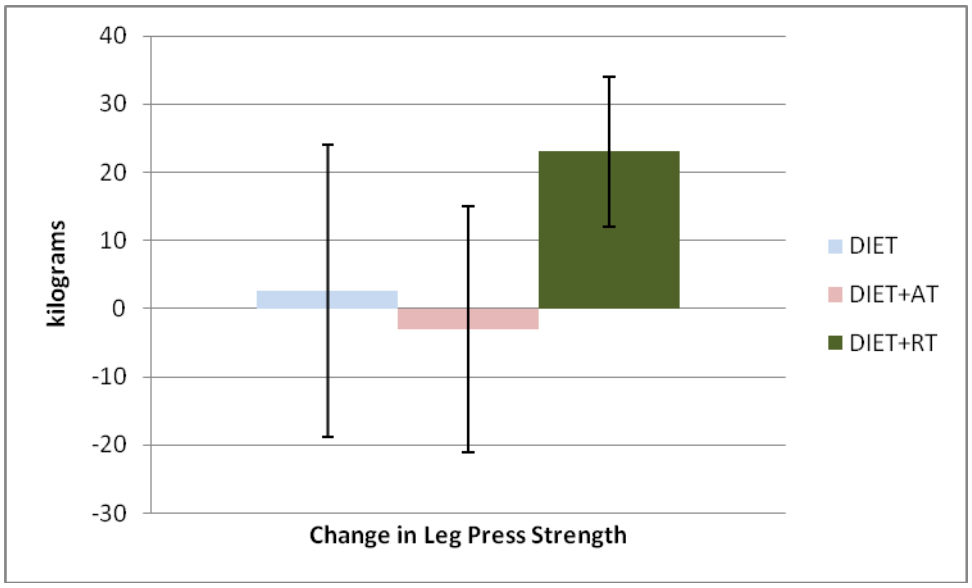
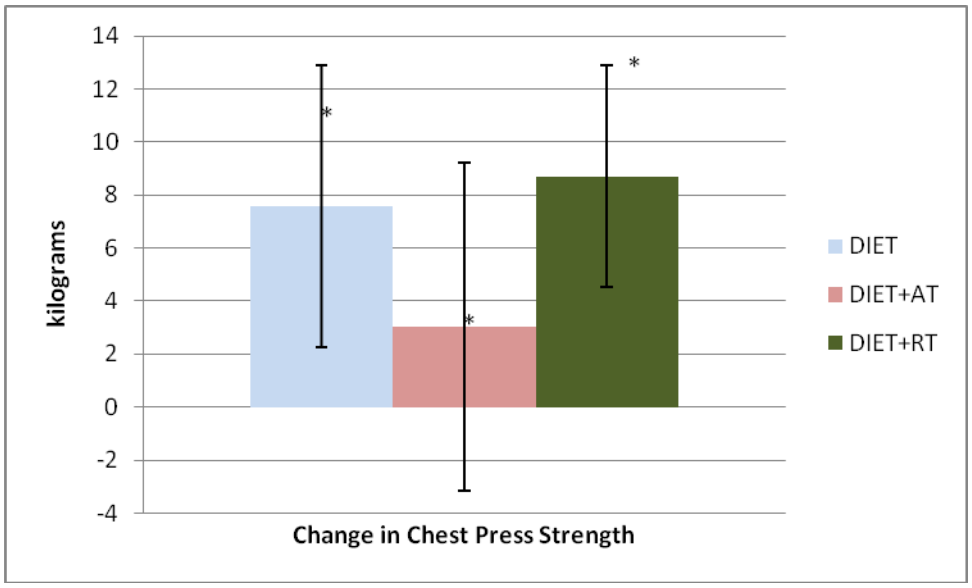


Figure 4.4. Completers Change in Leg Press Strength Baseline to 12-weeks

Two-factor R-ANOVA for the chest press showed significant increases across time among completers (p<0.001) without a significant group effect (0.19) or a group-by-time effect (p=0.18) effect. With intent to treat analysis, the chest press showed a significant increase across time (p<0.001) without a significant group (p=0.38) or group-by-time effect (p=0.28). Within the completers, the DIET group increased chest press by 7.58 $\pm$ 5.31kg, the DIET+AT group increased by 3.03 $\pm$ 6.21kg, and the DIET+RT group increased by 8.71 $\pm$ 4.17kg. Intent-to-treat analysis showed an increase in chest press in the DIET group of 5.68 $\pm$ 5.70kg, in the DIET+AT group of 2.27 $\pm$ 5.43 kg, and in the DIET+RT group of 6.54 $\pm$ 5.35kg. Results are shown in Tables 4.4 for Completers and 4.5 for Intent-to-treat.

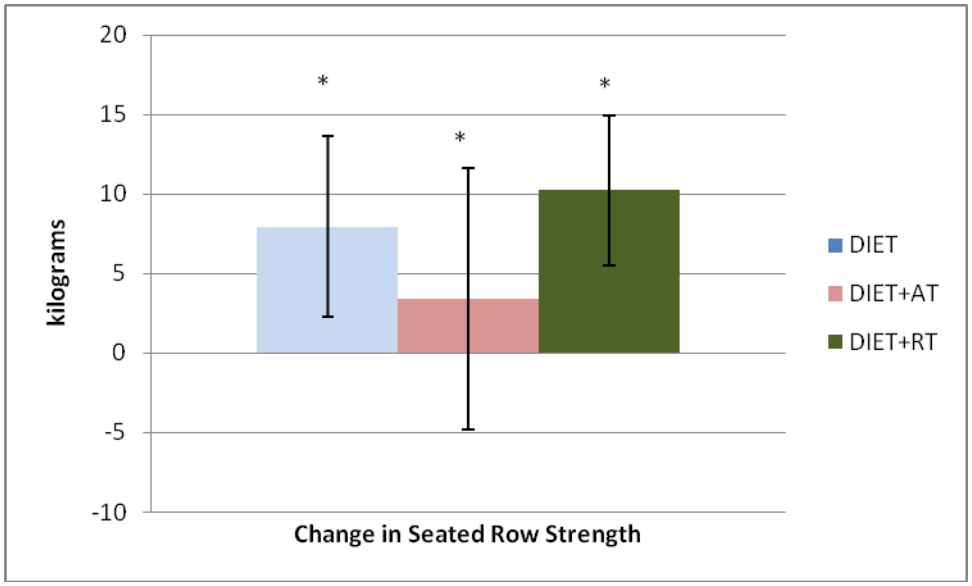


*significant at p<0.001

Figure 4.5. Completers Change in Chest Press Strength Baseline to 12-weeks

A two-factor R-ANOVA was also performed among completers for the seated row exercise and showed a significant time effect ($p<0.001$), but no group effect ($p=0.43$) and no group-by-time interaction ($p=0.20$). The DIET group had an increase of 5.97 $\pm$ 6.07kg, the DIET+AT group experienced an increase of 2.56 $\pm$ 7.13kg, and the DIET+RT group had and increase of 7.67 $\pm$ 16.18kg. Intent-to-treat two-factor R-ANOVA showed a significant increase ($p<0.001$) in strength on the seated row exercise also. There were no group effects ($p=0.46$) and no group-by-time effects ($p=0.30$). The intent-to-treat DIET group had an increase of

5.97 ± 6.07kg, the DIET+AT group had an increase of 2.56 ± 7.13kg, and the DIET+RT group had an increase of 7.67 ± 16.18kg. Results are shown in Tables 4.4 for Completers and 4.5 for Intent-to-treat.



*significant at p<0.001

Figure 4.6. Completers Change in Seated Row Strength Baseline to 12-weeks

4.4.7 Dietary Intake

A two-factor R-ANOVA showed a significant reduction in dietary intake for completers and intent-to-treat from 0 to 12 weeks in all treatment groups (p=0.001). Weight loss eating behaviors were measured using the Eating Behavior Inventory, and showed significant improvement in all treatment groups from baseline to 12 weeks (p<0.001). The completers

DIET group increased EBI score by 20.8 ± 12.3; DIET+AT increased EBI score by 15.3 ± 11.2; and DIET+RT improved EBI score by 26.0 ± 10.2. No differences were observed between groups (p=0.133).

Intent-to-treat analysis also showed significant reductions in dietary intake from baseline to 12 weeks in all groups (p=0.001). Further, weight loss eating behaviors improved over 12 weeks (p<0.001), although no group differences (p=0.478) were found. The intent-to-treat DIET group improved EBI score by 15.6 ± 14.2; intent-to-treat DIET+AT improved EBI score by 11.5 ± 11.8; and intent-to-treat DIET+RT improved EBI score by 19.5 ± 14.8.

Completers analysis revealed a significant reduction in calorie intake (DIET:-725.6 ± 799.0; DIET+AT: -402.2 ± 712.2; DIET+RT: -467.4 ± 406) and percent of calories from fat (DIET: -7.2 ± 6.9; DIET+AT: -3.2 ± 7.7; DIET+RT: -9.6 ± 8.0) and a significant increase in percent of calories from carbohydrates (p<0.024) across all groups (DIET: 5.0 ± 13.9; DIET+AT: 1.4 ± 7.6; DIET+RT: 12.0 ± 8.4). There was no significant change in protein intake over 12 weeks (p=0.571). Dietary intake data can be found in Table 4.6 for Completers and for Intent-to-treat.

Table 4.6. Dietary Intake Completers and Intent to Treat

Completers	DIET (N=6) (Mean±S.D.)	DIET+AT (N=6) (Mean±S.D)	DIET+RT (N=6) (Mean±S.D.)	Group Effect	Time Effect	Group x Time Effect
Dietary Intake (kcal/day)						
0 Weeks	2216.2±774.7	2183.5±899.9	2206.0±323.1	0.985	0.001	0.905
12 Weeks	1490.6±593.2	1630.1±634.8	1565.9±336.7			
%Fat						
0 Weeks	36.3±2.4	36.2±2.2	43.4±0.9	0.306	0.002	0.339
12 Weeks	31.7±2.8	33.0±2.7	33.8±3.3			
%Protein						
0 Weeks	14.3±1.0	15.3±0.8	17.9±1.8	0.131	0.617	0.670
12 Weeks	15.9±2.2	16.5±1.7	17.1±1.1			
%Carbohydrate						
0 Weeks	50.5±3.8	48.7±3.5	38.5±1.3	0.320	0.019	0.172
12 Weeks	55.5±4.9	50.1±4.2	50.4±3.3			
Intent to Treat	DIET (N=8) (Mean±S.D.)	DIET+AT (N=8) (Mean±S.D)	DIET+RT (N=8) (Mean±S.D.)	Group Effect	Time Effect	Group x Time Effect
Dietary Intake (kcal/day)						
0 Weeks	2024.6±747.9	2088.3±781.8	2055.3±436.8	0.969	<0.001	0.994
12 Weeks	1480.0±481.3	1673.2±533.2	1575.2±299.7			
%Fat						
0 Weeks	37.2±2.2	35.8±1.7	41.3±1.5	.344	0.003	0.417
12 Weeks	31.7±2.8	33.4±2.0	34.1±2.5			
%Protein						
0 Weeks	13.5±0.9	15.4±0.8	16.6±1.6	.339	0.563	0.646
12 Weeks	14.8±1.8	16.3±1.4	16.0±1.1			
%Carbohydrate						
0 Weeks	49.3±3.5	49.3±2.7	41.5±2.2	.358	0.026	0.253
12 Weeks	53.1±4.4	50.4±3.2	50.5±2.5			

4.4.8 Body Composition

Percent Body Fat: ($\%FAT_{TISSUE}$): $\%FAT_{TISSUE}$ measures body fat as a percent of total body mass without the mass of bone mineral content; that is, percent fat of total soft tissue. Completers

analysis to examine tissue percent fat change was performed with two-factor R-ANOVA. There was a significant decrease in tissue percent fat over time ($p<0.05$), with no significant differences between groups ($p=0.63$) and no group-by-time interaction ($p=0.44$). Decrease in $\%FAT_{TISSUE}$ for the DIET group was 0.6 ± 0.1; decrease in the DIET+AT group was 1.5 ± 1.6; and decrease for the DIET+RT group was 1.6 ± 1.5.

Intent-to-treat analysis of change in $\%FAT_{TISSUE}$ was also performed with two-factor R-ANOVA. This analysis revealed a significant change in $\%FAT_{TISSUE}$ over time ($p<0.05$) and no significant differences between groups ($p=0.79$) and no group-by-time effect ($p=0.48$). Decrease in $\%FAT_{TISSUE}$ with intent-to-treat analysis showed a decrease in the DIET group of 0.5 ± 1.0; a decrease in the DIET+AT group of 1.2 ± 1.5; and a decrease in the DIET+RT group of 1.2 ± 1.5. Body composition data is shown in Table 4.6 for Completers and Table 4.7 for Intent-to-treat.

Fat Mass: Results of a two-factor R-ANOVA revealed a significant decrease in fat mass from baseline to week 12 for completers ($p<0.001$). There were no differences between groups ($p=0.33$) and no group-by-time effect ($p=0.56$) for fat mass loss. The completers in the DIET group lost a fat mass of 2.2 ± 2.3kg; the DIET+RT group lost a fat mass of 3.6 ± 2.4kg; and the DIET+RT group lost fat mass of 3.8 ± 3.4kg.

Lean Mass: Among completers, a two-factor R-ANOVA showed a trend toward significant lean mass loss ($p=0.053$), and no differences between groups and no group-by-time effect. Completers in the DIET group lost $1.0 \pm .9$kg of lean mass; completers on the DIET+AT group lost 0.61 ± 2.5 kg of lean mass; and completers in the DIET+RT group lost 0.87 ± 1.2kg of lean mass. Intent-to-treat analysis with a two-factor R-ANOVA indicated a significant loss of fat mass over time ($p<0.001$) with no group ($p=0.52$) or group-by-time ($p=0.65$) effect. The DIET

group lost 1.6 ± 2.2kg; the DIET+AT group lost 2.7 ± 2.6kg; and the DIET+RT group lost 2.8 ± 3.4 kg.

Intent-to-treat analysis of lean mass loss with two-factor R-ANOVA revealed that there was a significant loss of lean mass (p=0.05), with no difference in group (p=0.75) or group-by-time (p=0.92). Intent-to-treat analysis for lean mass revealed that the DIET group lost 0.8 ± 0.9kg of lean mass; the DIET+AT group lost 0.5 ± 2.1kg of lean mass; and the DIET+RT group lost 0.7 ± 1.1kg of lean mass. Information of fat and lean tissue loss can be found in Tables 4.6 for Completers and 4.7 for Intent-to-treat.

Table 4.7. Body Composition and Anthropometric Differences By Treatment Group

Outcome Variable	DIET (N=6) (Mean±S.D.)	DIET+AT (N=6) (Mean±S.D)	DIET+RT (N=6) (Mean±S.D.)	Group Effect	Time Effect	Group x Time Effect
Waist Circumference (cm)						
0 Weeks	118.3±7.1	110.2±7.9	112.2±4.7	0.047	<0.001	0.440
12 Weeks	113.9±3.4	105.4±7.6	104.4±6.1			
Hip Circumference (cm)						
0 Weeks	127.6±3.6	133.6±13.2	126.6±9.2	0.352	<0.001	0.583
12 Weeks	123.6±3.7	128.2±12.9	120.3±6.6			
Waist-to-hip ratio						
0 Weeks	0.93±0.05	0.83±0.09	0.89±0.05	0.040[a]	0.197	0.628
12 Weeks	0.92±0.03	0.83±0.07	0.87±0.06			
Bone Mineral Content (kg)						
0 Weeks	2.7±0.5	2.8±0.4	2.7±0.4	0.789	0.064	0.601
12 Weeks	2.7±0.5	2.8±0.4	2.7±0.4			
Body Fat with BMC (%)						
0 Weeks	49.2±3.1	49.8±5.2	47.7±3.9	0.617	0.002	0.441
12 Weeks	48.6±3.4	48.3±6.0	46.1±3.0			
Body Fat w/o BMC (%)						
0 Weeks	50.5±3.1	51.2±5.2	49.0±4.0	0.625	0.003	0.437
12 Weeks	49.9±3.3	49.6±6.0	47.4±3.1			
Fat Mass (kg)						
0 Weeks	51.3±6.3	54.2±10.2	51.0±7.8	0.330	<0.001	0.560
12 Weeks	49.2±6.1	50.6±11.8	43.7±4.5			
Lean Mass (kg)						
0 Weeks	50.6±7.4	51.2±5.7	49.2±4.6	0.822	0.053	0.921
12 Weeks	49.6±7.3	50.6±6.9	48.3±3.9			
Bone Mineral Density (g/cm^3)						
0 Weeks	1.32±0.14	1.32±0.14	1.33±0.13	0.978	0.753	0.533
12 Weeks	1.32±0.13	1.32±0.14	1.32±0.12			

Table 4.8. Body Composition and Anthropometric Differences By Treatment Group

Outcome Variable	DIET (N=8) (Mean±S.D.)	DIET+AT (N=8) (Mean±S.D)	DIET+RT (N=8) (Mean±S.D.)	Group Effect	Time Effect	Group x Time Effect
Waist Circumference (cm) 0 Weeks 12 Weeks	115.3±8.5 111.9±5.1	110.0±7.1 106.3±7.1	110.6±5.2 104.8±5.5	0.119	<0.001	0.542
Hip Circumference (cm) 0 Weeks 12 Weeks	126.3±3.9 123.3±3.3	132.6±12.4 128.5±12.1	127.0±7.9 122.3±6.8	0.289	<0.001	0.697
Waist-to-hip ratio 0 Weeks 12 Weeks	0.91±0.06 0.91±0.04	0.83±0.07 0.83±0.06	0.87±0.05 0.86±0.05	0.041	0.155	0.775
Bone Mineral Content (g) 0 Weeks 12 Weeks	2.7±0.4 2.7±0.4	2.7±0.5 2.7±0.5	2.7±0.3 2.7±0.3	0.953	0.062	0.608
Body Fat with BMC (%) 0 Weeks 12 Weeks	48.2±3.6 47.7±3.6	49.8±4.4 48.6±5.1	48.6±3.7 47.4±3.6	0.786	0.003	0.501
Body Fat w/o BMC (%) 0 Weeks 12 Weeks	49.5±3.5 49.0±3.6	51.2±4.4 50.0±5.1	50.0±3.8 48.8±3.6	0.786	0.003	0.483
Fat Mass (kg) 0 Weeks 12 Weeks	49.7±6.6 48.1±6.1	53.2±9.9 50.5±10.9	48.9±5.9 46.1±5.9	0.518	<0.001	0.648
Lean Mass (kg) 0 Weeks 12 Weeks	50.7±6.3 50.0±6.2	50.5±6.0 50.1±6.8	48.8±4.0 48.2±3.3	0.748	0.050	0.923
Bone Mineral Density (g/cm^3) 0 Weeks 12 Weeks	1.32±0.12 1.32±0.11	1.31±0.15 1.31±0.15	1.32±0.11 1.32±0.11	0.966	0.840	0.424

4.4.9 Anthropometric Measures

A two-factor R-ANOVA was performed on waist circumference for both completers and intent-to-treat. The completers analysis showed there was a significant time effect ($p<0.001$) and a significant group effect ($p<0.05$), but no group-by-time interaction ($p=0.44$). Completers in the DIET group achieved a non-significant ($p=0.13$) reduction of 4.4 $\pm$ 6.1cm, the DIET+AT group saw a significantly larger decrease of 4.9 $\pm$ 4.2cm, and the DIET+RT group had a significantly larger ($p<0.05$) decrease in waist circumference of 7.8 $\pm$ 4.0cm. Intent-to-treat analysis with a two-factor R-ANOVA showed a significant time effect ($p<0.001$), but no group ($p=0.12$) or group-by-time effect ($p=0.54$). The intent-to-treat DIET group decreased waist circumference by 3.3 $\pm$ 5.5cm, the intent-to-treat DIET+AT group had a decrease of 3.6 $\pm$ 4.2cm, and the intent-to-treat DIET+RT group decreased waist circumference by 5.9 $\pm$ 4.9cm.

Two-factor R-ANOVA was performed on hip circumference changes over time. Completers analysis showed that there was a significant time effect ($p<0.001$), but no group ($p=0.35$) and no group-by-time differences ($p=0.58$). Similarly, intent-to-treat analysis showed a time effect ($p<0.001$), but no group ($p=0.30$) or group-by-time ($p=0.70$) effect. Completers in the DIET group decreased hip circumference by 4.0 $\pm$ 2.7cm, completers in the DIET+AT group decreased hip circumference by 5.4 $\pm$ 4.8cm, and DIET+RT completers decreased hip circumference by 6.3 $\pm$ 3.5cm. The intent-to-treat DIET group decreased hip circumference by 3.0 $\pm$ 2.9cm, the intent-to-treat DIET+AT group decreased hip circumference by 4.1 $\pm$ 4.8cm, and the intent-to-treat DIET+RT group decreased hip circumference by 4.7 $\pm$ 4.1cm.

Two-factor R-ANOVA was performed on change to waist-to-hip ratio. Intent-to-treat and completers analysis showed that there was no time effect (Intent-to-treat: $p=0.20$, Completers : $p=0.15$) and no group-by-time effect (Intent-to-treat: $p=0.63$, Completers: $p=0.70$),

but a significant effect between groups (Intent-to-treat: p=0.04, Completers: p=0.04) with DIET+RT improving waist-to-hip ratio more than DIET and DIET+AT in both intent-to-treat and completers groups. Anthropometric outcomes for completers can be found in Table 4.6 and for intent-to-treat in table 4.7.

4.4.10 hs-CRP

The baseline hs-CRP levels of the participants in the current study were not above clinically significant levels. In healthy individuals, levels of CRP are normally less than 10mg/dl (Clyne, 1999): the median concentration of CRP is 0.8 mg/l; the 90^{th} centile is 3.0mg/l; and the 99^{th} centile is 10mg/l (Shine, 1981). The mean hs-CRP in the cohort at baseline was 0.79 $\pm$ 0.62mg/dl for total participants, as well as for completers and non-completers. When viewed by treatment group, the DIET group had a mean of 0.66 $\pm$ 0.31mg/dl; the DIET+AT had a mean of 1.07 $\pm$ 0.88mg/dl; and the DIET+RT group had a mean of 0.65 $\pm$ 0.51mg/dl.

Spearman correlations were performed for baseline hs-CRP and body composition and anthropometric measures at baseline: baseline weight; baseline BMI; baseline waist circumference; baseline hip circumference; baseline waist-to-hip ratio; baseline fat mass; baseline lean mass; and baseline tissue percent fat. No significant correlations were found between hs-CRP and any of these parameters. Further, baseline hs-CRP levels were divided among participants into those having a hs-CRP at 1mg/dl or below and those having a hs-CRP of greater than 1mg/dl. The mean of the 17 participants below 1 was 0.5 $\pm$ 0.1mg/dl; for the 7 participants above 1, the mean hs-CRP was 1.6 $\pm$ 0.2mg/dl. The baseline measures of body composition and anthropometric measures between the two groups (hs-CRP<1, hs-CRP>1) were

then examined with T-tests. Again, no significant differences between the two groups were found at baseline. The finding that of no association between hs-CRP and baseline BMI is in contrast to prior literature. Danesh (1998), reported a strong positive association between baseline CRP concentration and BMI. Results can be found in Table 4.9.

Table 4.9. Baseline Correlations Between hs-CRP and Body Composition Measures

Baseline Variable	Spearman Correlation Coefficient with hs-CRP	p-value
Weight	0.03	0.90
BMI	0.14	0.51
Waist Circumference	-0.02	0.94
Hip Circumference	-0.05	0.82
Waist-to-Hip Ratio	-0.05	0.83
Fat Mass	0.12	0.59
Lean Mass	-0.32	0.13
Percent Body Fat (Tissue)	0.37	0.08

High-sensitivity C-reactive Protein (hs-CRP) was examined for changes from baseline to 12 weeks for completers and as intent-to-treat. The hs-CRP data was found to lack normality, therefore the data were log-transformed and this resulted in normality. The log-transformed data was normal and was analyzed with a two-factor R-ANOVA. The results of the two-factor R-ANOVA for both completers and intent-to-treat yielded no significant group effect, no significant time effect, and no group-by-time interaction. Data are shown in Table 4.10.

Table 4.10. hs-CRP(mg/dl) Outcome Differences Between Treatment Groups at 12-Weeks

Analysis	Groups	Assessment Period 0 weeks	12 weeks	Group Effect	Time Effect	Group x Time Effect
Completers	DIET (N=6)	0.77±0.28	0.58±0.18	0.572	0.511	0.379
	DIET+AT (N=6)	1.03±1.03	1.29±0.96			
	DIET+RT (N=6)	0.58±0.41	0.38±0.21			
Intent-to-treat	DIET (N=8)	0.66+0.31	0.52+0.19	0.441	0.506	0.369
	DIET+AT (N=8)	1.07+0.88	1.26+0.83			
	DIET+RT (N=8)	0.65+0.51	0.50+0.45			

Spearman correlations were performed between the change in hs-CRP among completers and all other outcome measures. Changes in hs-CRP were significantly associated with change in leisure time physical activity, indicating that an increase in physical activity is associated with (Spearman r = .489, p=0.40) an increase in hs-CRP. An increase in treadmill time to achieve 85% AMHR was positively associated (Spearman r = 0.568, p=0.01) to increases in hs-CRP. Positive changes in chest press strength were significantly associated (Spearman r = -0.55, p=0.02) to decreases in hs-CRP. Finally, decreases in lean body tissue were significantly associated (Spearman r = 0.50, p=0.04) to decreases in hs-CRP. The correlation between change in hs-CRP and waist-to-hip ratio showed a trend toward significance (p=0.09). Surprisingly, hs-CRP change was not significantly correlated with change in body weight (p=0.67), change in BMI (p=0.46), change inVO_2 at 85% of AMHR (p=0.39), changes in waist circumference (p=0.18), or change in fat mass (p=0.60). Correlation data can be found in Table 4.11.

Table 4.11. Correlations Between Change in hs-CRP and Change in Outcome Variables

Outcome Variable	Spearman Correlation Coefficient with hs-CRP	p-value
Bodyweight (kg)	0.19	0.46
BMI (kg/m^2)	0.19	0.46
Lean Tissue (kg)	0.50	0.04
Fat Mass (kg)	0.13	0.60
Waist Circumference (cm)	0.33	0.18
Waist-to-Hip Ratio (cm)	0.41	0.09
Paffenbarger Exercise Habits Questionnaire	0.49	0.04
VO2 at 85% AMHR (ml/kg/min	0.22	0.39
Treadmill Time to 85% AMHR (min)	0.57	0.01
Chest Press Strength (kg)	-0.55	0.02
Leg Press (kg)	0.13	0.61
Seated Row (kg)	0.00	0.99

4.4.11 Process Measures

Descriptive analysis was used to examine process measures including: group attendance; exercise attendance; daily dietary logging; self-reported calorie consumption; and self weighing frequency. All data sets except exercise attendance were found to lack normality, therefore Kruskal-Wallis analysis of data was performed. The Kruskal-Wallis test replaces data with their rank, and performs analysis of variance based on the group median, rather than the group mean. The Kruskal-Wallis test found no significant differences between groups for the process measures of group attendance ($p= 0.14$), daily dietary logging ($p=1.0$), self reported calorie consumption ($p=1.0$), or self-weighing frequency ($p=0.73$). One-way ANOVA was used to

examine exercise attendance between the two exercise groups and did not show a significant difference between groups (p=0.81). Data for process measures is found in Table 4.12.

Table 4.12. Process Measure Differences Between Groups at Week 12

Outcome Variable	DIET (N=6) (Mean±S.D.)	DIET+AT (N=6) (Mean±S.D)	DIET+RT (N=6) (Mean±S.D.)	p-value for group comparisons
Intervention Behavioral Session Attendance (%)	88.9+4.3	91.7+12.9	87.5+11.5	0.144
Exercise Session Attendance (%)		85.7+13.9	83.5+15.8	0.806
£Food Diary Days (%)	87.3+10.2	81.0+15.3	67.9+35.4	1.00
Self Reported Calories Per day	1436.3+313.3	1306.8+260.9	1289.1+125.4	1.00
Self-Weighing Days Per Week	0.47+0.98	0.73+1.8	2.2+3.0	0.725

£ Number of days per week food consumption was logged in self-monitoring diaries

5.0 DISCUSSION

The purpose of the current investigation was to evaluate the additive effect of aerobic or resistance exercise training to caloric restriction for weight loss on high-sensitivity C-Reactive Protein (hs-CRP) changes compared to dietary restriction alone in class II and class III obese individuals: Caloric restriction weight loss program with no exercise (Diet); Caloric restriction with aerobic endurance training (Diet+AT); and Caloric restriction with resistance training (Diet+RT). The study also compared changes between these interventions for measures of weight, BMI, dietary intake, self-reported physical activity, cardiorespiratory fitness, muscular fitness,waist circumference, hip circumference, waist-to-hip ratio, and body composition.

Research has shown that weight loss has a positive effect on C-reactive protein levels and though some research shows a positive impact of exercise, data has been equivocal. Prior studies had used sedentary overweight and obese participants. This study sought to expand upon the current literature by utilizing Class II and Class III obese participants to evaluate change to CRP with diet and diet plus exercise.

5.1 RETENTION

A total of 18 out of 24 randomized subjects completed both baseline and 12-week assessments. The retention rate was 75% for total participants in the study, as well as 75% in each of the treatment groups, as each randomized group of eight saw an attrition of two subjects. This is slightly lower that the 80% completion rate expected of 6-month behavioral intervention programs (Wadden, 2005). Church, et al (2010) recently showed an 80% retention rate with a 4-month exercise study. In contrast, Bourke (2011) experienced a 44% attrition rate in a recent 6-month lifestyle intervention that included exercise.

5.2 WEIGHT LOSS AND C-REACTIVE PROTEIN

Prior research has shown that adipose tissue is not a metabolically inert tissue, but synthesizes and secretes pro-inflammatory mediators, such as Tumor Necrosis Factor-α and IL-6 (Tziomalos, 2010,Fontana, 2007, Fain, 2004, Fried, 1998, Mohamed-Ali, 1997), with IL-6, in turn, stimulating the production of CRP by the liver (Heinrich 1990). The abnormal secretion of inflammatory mediators yields a chronic state of low-grade inflammation that underlies the metabolic and cardiovascular outcomes of obese populations (Ikeoka 2010). Changes in inflammatory markers have been specifically correlated to changes in body weight (O'Brien, 2005) with several small studies noting a significant decrease in CRP after weight loss induced by dietary modification in otherwise healthy obese adults (Tziomalos 2010), and some showing

the magnitude of decrease in CRP to be linearly correlated with the amount of weight lost (Tziomalos 2010).

A meta-analysis of the literature conducted by Selvin (Selvin, 2007) concluded that weight loss was associated with a decline in CRP, and that across all studies (both lifestyle and surgical interventions) a 1-kg loss yielded a 0.013mg/dl decrease in CRP (weighted Pearson correlation, r=0.85). The result of the meta-analysis was that weight loss may be an effective strategy for lowering CRP without pharmacologic intervention. In a more recent review with a larger scope, Tziomalos concurred with this report, indicating all but two of the twenty-six weight loss studies reviewed showed decreases in CRP (Tziomalos, 2010). Further, Esposito has investigated the impact of fat removal via liposuction on inflammatory markers (Esposito, 2006). The procedure produced an average decrease in bodyweight of 3.5kg that was maintained in subjects through the 6-month to 1-year follow-up at which time participants showed a significant 26% ($p<0.05$) reduction in CRP. The results of this study may differ from the current investigation, in that the participants of the Esposito study had a baseline CRP of 3.1mg/dl. Further, Agrawal found a decrease of 0.015mg/dl in CRP per kilogram of weight loss following bariatric surgery in obese participants. The participants in the Agrawal study showed a baseline CRP of 1.1 + 0.98mg/dl.

The baseline hs-CRP levels of the participants in the current study were not above clinically critical levels, with the maximal level seen in any participant in this study at baseline being 2.75mg/dl. Individual hs-CRP levels at baseline and follow-up can be viewed in Appendix D. In healthy individuals, levels of CRP are normally less than 10mg/l (Clyne, 1999): the median concentration of CRP is 0.8 mg/l; the 90^{th} centile is 3.0mg/l; and the 99^{th} centile is 10mg/l (Shine, 1981). The mean hs-CRP in the cohort at baseline was 0.79 $\pm$ 0.62mg/dl for total

participants, as well as for completers and non-completers. When viewed by treatment group, the DIET group had a mean of 0.66 ± 0.31mg/dl; the DIET+AT had a mean of 1.07 ± 0.88mg/dl; and the DIET+RT group had a mean of 0.65 ± 0.51mg/dl. The baseline CRP values of the participants in the current study did not move significantly with a weight loss that was statistically significant. Further, the participants in the current study did not see the extreme weight losses seen in the Agrawal study (> 30kg), and this lack of excessive weight loss may also have muted the decrease in hs-CRP. The individual data for participants in the current study can be found in Appendix D. Figures with individual change data can be found in Appendix C.

In contrast to findings in the literature, the current study showed a significant weight loss in Class II and Class III obese individuals without a significant reduction in circulating CRP levels. When examined by group, the DIET and DIET+RT groups experienced non-significant decreases in CRP levels, though the DIET+AT group experienced a non-significant increase in CRP. Dansinger (2005) found a decrease in CRP over a 1-year intervention in which 160 participants lost a mean of 2.96 ± 5.75kg. The weight loss results of this investigation was 4.6 ± 3.3kg among completers, providing a greater average loss of body mass than that of the Dansinger study, yet no accompanying decreases of CRP were found. Further, the Dansinger study was a 1-year longitudinal study, and the current study was 12-weeks. The relatively short duration of the current inquiry is likely to have also contributed to the discrepancy between studies. O'Brien (2005), however, did note significant decreases in CRP in a 3-month diet study. The weight losses in the O'Brien Study were greater than those found in the current investigation, however (6.0 ± 3.4kg vs 4.6 ± 3.3kg). The difference in weight loss, while small, may have had an impact on the CRP differences seem between the two studies. An even greater weight loss in the present investigation may have further impacted circulating CRP such that a

significant difference would have been observed. Moreover, the mean baseline BMI of the participants in the Dansinger (2005) study was 35.0 $\pm$ 3.9kg/m^2 and the mean BMI of the O'Brien study was 33.6 + 1.9kg/m^2 compared to the 39.0 $\pm$ 2.7kg/m2 mean baseline BMI of the participants in the current study. The discrepancy between the BMI of the individuals used in this study versus the size of participants used in other studies may further exacerbate the lack of a significant decrease in circulating CRP.

The participants in the O'Brien study (2005) had baseline levels of CRP that, like the participants in the current study, were not at clinically critical levels with a median [interquartile range] baseline CRP = 0.35mg/dl (25^{th} percentile = 0.17mg/dl, 75^{th} percentile = 0.75mg/dl), yet saw significant decreases in CRP with significant weight loss. The Dansinger participants also had a baseline CRP mean of 0.41 + 0.34mg/dl. The current study recruited subjects based upon criteria of Class II and Class III obesity. As presented in section 4.3 (Table 4.1) and stated previously in this section, the baseline hs-CRP levels of the participants in this investigation had a mean of 0.79 $\pm$ 0.62mg/dl. It is unlikely, therefore, that the levels of baseline CRP found in the participants of this study impacted the lack of significant reductions at follow-up.

5.3 AEROBIC EXERCISE TRAINING AND C-REACTIVE PROTIEN

Studies have shown that exercise can cause acute increase in C-reactive protein (Mendham, 2011), but that higher fitness levels may be associated with lower levels of CRP (Lavie, 2011). LaMonte, (2002) assessed CRP and fitness via maximal treadmill exercise testing and found that among the 135 participants studied, CRP decreased among tertiles of fitness indicating that the more fit a participant was, the lower their CRP. While higher levels of physical activity have

been associated with lower levels of CRP (Geffken, 2001), the existing literature on exercise and CRP remains controversial. A 2006 meta-analysis of randomized controlled trials concluded that aerobic exercise does not reduce CRP levels in adults (Kelley and Kelley, 2006). The STRRIDE study concluded that while participants saw significant decreases in fat mass, no significant changes in CRP could be attributed to varying intensities and durations of aerobic exercise (Huffman 2007). More recently, however, Kadoglou showed that overweight and obese subjects saw significant decreases in CRP levels with a 6-month aerobic exercise intervention, despite a lack of significant change in body mass (Kadoglou, 2007). Notwithstanding a lack of significant weight loss, the exercise group showed a significant 39.6% reduction in CRP (p=0.04). Some studies indicate that interventions that include both diet and exercise tend to decrease inflammatory markers. The 2004 study by You (2004) concluded that, while both the diet and diet plus exercise groups lost significant body weight and fat-mass, only diet plus exercise was effective at reducing CRP in this population (You, 2004). The current investigation's findings contrast those you You (2004) and Kadoglou (2007). No conclusive evidence was found that adding aerobic exercise to a dietary intervention decreased circulating CRP level.

Participants in this investigation who took part in the DIET+AT group did lose significant amounts of weight, but no significant decreases in CRP were shown. Additionally, the participants in the current study who took part in aerobic exercise training showed a non-significant *increase* in CRP. The positive associations between activity and increased CRP are a surprise: prior literature has indicated that higher levels of physical activity and fitness are associated with lower levels of CRP (Gefken, 2001, Lavie, 2011). It is suggested that the one of the factors influencing this finding, and driving the positive correlation found between increased treadmill time and increased CRP, is volume of aerobic exercise. The participants in this study

were Class II and Class II obese individuals who were not participating in regular exercise at baseline. The participants who were randomized to the DIET+AT group increased their weekly exercise volume from 60 to 180 minutes per week during the first five weeks of the intervention, and 180 minutes per week was maintained for the duration of the investigation. It is possible that the DIET+AT group experienced an increase in CRP associated with increased exercise volume that never diminished during the time-frame of the investigation. The studies of You (2004) and Kadoglou (2007) were both of 6-month duration. It is possible that given an additional 3-months to physiologically adapt to the demands of regular exercise, the increase in CRP seen in the DIET+AT group would be eliminated.

5.4 RESISTANCE EXERCISE TRAINING AND C-REACTIVE PROTEIN

A paucity of studies exists regarding the impact of resistance training on markers of inflammation, and no prior studies have researched the impact of concurrent weight loss and resistance training on circulating CRP. While there has been some indication in the literature that resistance training offered no benefit over weight loss for decreasing inflammatory markers among healthy overweight and obese subjects (Brochu, 2009, Levinger, 2009), the results have been mixed. In the 10-week Donges, intervention, when aerobic and resistance training were compared, only resistance training participants showed a reduction in CRP concentrations (Donges 2010), with the resistance trained participants decreasing their CRP by 32.8% ($p<0.05$), while the aerobically trained participant saw a 16.1% reduction (Donges, 2010). A one-year

study by Olson (2007) concluded that resistance training lead to decreases in CRP without concurrent weight loss.

The current research study has findings in agreement with those of Brochu (2009) and Levinger (2009). The resistance trained group showed non-significant decreases in circulating CRP after the 12-week intervention. The Levinger study was a 10-week protocol, and showed no decrease in CRP after resistance training. The Brochu study was a 6-month intervention, and even after this significant duration showed no decrease in circulating CRP levels among participants. Had the current investigation continued for the span of a year, it is possible that significant decreases in CRP would have been seen among the DIET+RT participants. It is possible that the participant BMI in the current study had an impact on CRP outcomes as well. The subject populations for the Donges (2010) and Olson (2007) studies had lower BMI (Donges = 28.7 $\pm$ 4.5kg/m2, Olson = 27.0 $\pm$ 3.0) than the current investigation. The use of larger participants may have had some influence on the change in CRP.

While it is unclear from the results of this study if exercise has the potential to significantly lower hs-CRP levels in Class II and Class III obese adults, it is very clear that the addition of exercise to the lifestyle of the sedentary population used for this study did not cause a significant increase in hs-CRP. It would appear that the minimal increase in hs-CRP seen in the DIET+AT group coupled with the increased loss of weight seen in this group can be viewed as a positive outcome and can offer insight into the prescription of exercise to Class II and Class III obese individuals. Further, the DIET+RT group showed a non-significant decrease in hs-CRP. If orthopedically capable, Class II and Class III obese individuals can exercise at an intensity that can assist with weight loss and improve markers of fitness such as treadmill time on a graded exercise test.

5.5 WEIGHT LOSS

Physical activity has been shown to have a favorable association to weight-related outcomes (DHHS, 2008), though physical activity alone has a very limited impact on inducing weight loss (Wadden, 2004). Numerous studies have found the effect of physical activity usually accounts for a 2-3kg loss during moderate-length diet and exercise interventions (ACSM, 2009). Recent evidence from the RENEW study substantiates prior research (Goodpaster, 2010). In the first 6-months of the RENEW study, Class II and Class III obese participants who received an exercise and diet intervention lost significantly more weight (10.9 kg) than those receiving a diet only intervention (8.2kg) (Goodpaster, 2010).

The results of the current investigation show some conflict with prior research. This investigation showed a significant decrease in weight in all groups, but no between group effects. Though the weight loss seen between groups was not significantly different, when absolute amounts of weight lost are examined, the exercise groups in the current investigation lost 2.2kg more weight than the DIET only group. This is in very close agreement with the results of the Goodpaster findings and the 2009 ACSM statement.

The additional weight loss experienced by the participants assigned to the exercise treatments in this study (DIET+AT, DIET+RT) compared to the DIET group may reflect additional difference in the intervention across treatment arms. The participants in the exercise arms engaged in supervised exercise sessions, resulting in an additional 36 hours of contact with the intervention staff (48 for exercise groups vs. 12 for DIET only). Perri (2002) has suggested

the additional contact time may be important for increasing weight loss. While the instructors for the exercise sessions were told not to participate in discussions about weight loss or diet during the exercise sessions, it is possible that this could have occurred. Therefore, future studies should confirm the differences in weight loss observed in this study with the addition of exercise in a design that equates for contact time between participants and intervention staff.

CRP values tend to increase with age and this change is presumed to reflect an increase in subclinical pathologies (Hutchinson, 2000). A study including 4494 subject (2291 male; 2203 female) ranging in age from 25-74 years of age, Hutchinson, et al showed a significant ($p<0.001$) trend to higher CRP values with increasing age. The current study utilized participants from 30-55 years of age with a mean of 45.4 $\pm$ 6.9. To accommodate for the possible increases in CRP associated with age, future studies should limit the age range to a more homogeneous subset, for instance participants from 45-55 years of age.

Finally, study of greater length might have served to increase the differences seen between the diet plus exercise groups, and may have yielded a difference in weight loss between exercise groups.

5.6 CARDIORESPIRATORY FITNESS

Aerobic exercise has been shown to increase cardiorespiratory fitness in sedentary, overweight and obese populations in as little as 72 $\pm$ 12.3 minutes per week (Church, 2007), though the American College of Sports Medicine recommends a minimum of 150 minutes of moderate-intensity aerobic physical activity per week to promote and maintain health (Haskell, 2007). The

participants in the current study took part in 3 guided training sessions per week at the Physical Activity and Weight Management Research Center of the University of Pittsburgh progressing from 60 to 180 minutes per week of moderate-intensity (3.0-6.0 METS) aerobic exercise (e.g., treadmill walking, recumbent cycling, adaptive motion trainer, elliptical trainer). Participants began with 60 minutes of exercise per week and increased by 30 minutes per week for five weeks, achieving 180 minutes of exercise per week at week 5 of the intervention (Figure 3.4). 180 minutes of exercise per week was maintained for the duration of the 12-week intervention. 180 minutes per week of exercise meets the American College of Sports Medicine and the American Heart Association recommendations of 450-750 $METS \cdot min \cdot wk^{-1}$ (Haskell, 2007). The participants in the current study did not have significant improvement in maximal oxygen uptake at 85% of AMHR.

This is in contrast to the findings of Church (2007) who showed significant improvements in Peak VO_2 in participants taking part in 72 $\pm$ 12.3, 135.8 $\pm$ 19.5, and 191.7 $\pm$ 33.7 minutes of exercise per week for 6-months. The discrepancy in findings may have to do with the length of the current study, as this investigation was only 3-months in duration. The subjects in the current study showed non-significant increases in VO_2, and an additional 3 months might have resulted in greater improvements. Further, this study terminated the graded exercise test at the fixed sub-maximal load of 85% of AMHR; the difference in measurement technique may also have an impact on the discrepancy of results between the Church (2007) study and the current investigation.

The participants in the current investigation demonstrated significant increases in treadmill time to achieve 85% of AMHR. This increase can be quantified as an improvement of 20.7% in time to achieve 85% of AMHR for all participants, and an increase of 32.4% for

participants in the DIET+AT group. This is in agreement with the findings of Jakicic (2003), who showed an increase of 22.3% in time to achieve 85% of maximal heart rate in participants who performed aerobic exercise at a moderate intensity for a moderate duration during a 6-month intervention.

5.7 MUSCULAR FITNESS

The participants in the current study randomized to the DIET+RT group participated in a guided resistance training protocol 3 days per week. The protocol started the participants at a low volume of exercise at an intensity of 50-55% of 1-repetition maximum, and oscillated in both volume and intensity during the intervention (Table 3.3), with a general trend toward increases in intensity as outlined in the ACSM Position Stand: Progression Models in Resistance Training for Healthy Adults (2009). During the first week of sessions the participants were introduced to the resistance training equipment at the Physical Activity and Weight Management Research Center and instructed in its' appropriate use. Research has shown a significant increase in both leg press and chest press strength in participants of a 10-week protocol (Donges, 2010). In contrast to these findings, this current investigation found a significant increase only in the chest press exercise. These findings agree with those of Olson (2007), who showed a significant increase in upper body strength measured with the chest press but not a significant increase in lower body strength with the leg press after 1 year of resistance training in 28 overweight female participants.

As indicated previously, the protocol undertaken for this investigation utilized a one week familiarization period to allow participants to become accustomed to the exercises being performed, and began the protocol with a low level of intensity to avoid delayed-onset muscle soreness and the inability of participants to take part in activity due to muscle soreness. This approach may have delayed the anticipated improvements in strength and eliminated the opportunity to see significant strength changes in all measures. Though significance was not found in leg press and seated row strength changes, improvements were experienced by the DIET+RT group. The leg press increased by 50.8 $\pm$ 24.2kg, and the seated row increased by 22.5 $\pm$ 10.4kg. The absolute changes are very similar to those of the Donges (2010) study, who found a leg press increase of 44.7 $\pm$ 18.4kg.

5.8 PROCESS MEASURES

In the current intervention, behavioral session attendance was high, with a mean of 89.3 $\pm$ 9.8 percent session attendance. The high percentage of session attendance was to be expected, as the participants have the expectation of accountability to the study interventionists at these sessions. Jakicic (2003) showed a 79.2% attendance rate for group sessions over six months, and Anton (2011) experienced an 83% group session attendance in a 24-week study. Exercise session attendance was 84.6 $\pm$ 14.2 percent. This is less than the 91% training session completion achieved by the Donges study (2010) and the 92% seen in the Levinger (2009) study. Days of dietary monitoring in the provided food diaries (78.7 + 23.2 percent) and days of self-weighing behavior (1.1 days per week) were less than might have been expected. The process measures

findings reveal that participants were compliant to the prescribed intervention, therefore compliance was unlikely to have had a detrimental impact on the study outcome.

5.9 LIMITATIONS AND FUTURE RESEARCH

This study examined the additive effect of aerobic or resistance exercise training to caloric restriction for weight loss on C-Reactive Protein changes compared to dietary restriction alone in class II and class III obese individuals. There are several limitations to the study that may have impacted the main outcomes. Therefore, the results should be interpreted cautiously, and future studies should strive to address the limitations associated with this investigation.

1. The sample size was small. This small sample size may not have been sufficient to detect changes in CRP without exceptionally high effect sizes. The effect sizes found in this study should be used to determine the sample size needed for an appropriately powered study on this topic.
2. The duration of the study was short. A majority of studies examining the effect of weight loss with and without exercise intervention are greater than 6-months in duration. The 12-week duration of this study may not have been sufficient to assess chronic changes in circulating CRP in this population of Class II and Class III obese adults. Future studies should address this time issue by conducting interventions of at least 6-months.

3. In this intervention, the DIET group visited the research facility a total of 12 occasions and the exercise groups had the opportunity for face-to-face interaction with the study investigators 36 occasions. There is a clear lack of equivalent contact. Future investigations should address alternative methods of exercise provision so that investigators can equate contact among groups.
4. This study used only female participants and no controls were in place for hormonal variability in the blood draw protocol. hs-CRP has been shown to have no daily or seasonal variation, however control of blood draws to a predetermined time during the menstrual cycle would allow for greater precision of data.
5. The current study utilized only female participants. This study cannot be generalized to males and sex differences may exist within participants of similar BMI levels. Future studies should utilize both male and female participants.
6. Participants in the current study had a mean age of 45.4 $\pm$ 6.9 years. Prior studies have used older participants (Church, 2010), post-menopausal participants (Campbell, 2009; Stewart, 2010) and younger participants (Ziccardi, 2002). Age appears to have an impact on circulating CRP levels (Hutchinson, 2000), therefore the results of this study can be generalized only to a population of similar age.
7. The current study used a sample population that was 75% Caucasian (18 of 24 participants). Because of a lack participation in this study by

a larger percentage of minorities, findings cannot be generalized to all races. Future studies should emphasize the use of minority populations.

8. The current study utilized an oxygen consumption assessment that collected heart rate and oxygen consumption at 85% of AMHR. It is possible that lack of a maximal oxygen consumption and heart rate led to a program of exercise that lacked the appropriate intensity to yield significant changes. Future research in this area should utilize a maximal graded exercise test to assess oxygen consumption and heart rate and use this information for exercise prescription.
9. The volume of training experienced by the exercise groups differed. The DIET+AT group trained for 180 minutes per week. The DIET+RT group achieved their exercise prescription in approximately 90 minutes per week. Future exercise studies should address the need to more formally equate total exercise volume between exercise groups.
10. Waist circumference was used in the current study as a measure of abdominal and visceral adiposity. This measure lacks the accuracy of MRI or CT scanning for visceral adiposity. Future studies should utilize the MRI or CT scan for visceral adiposity.
11. The blood analysis in this study was for hs-CRP. The limitation of assays for a single marker of inflammation should be addressed in

future research. Future studies should measure other markers of systemic inflammation, such as TNF-α and IL-6.

12. At the time of blood draws, the current investigation did not control for the short-term use of anti-inflammatory medications. Control of the use of aspirin, ibuprofen and other anti-inflammatory medications in future studies at both baseline and follow-up will help to ensure homeostatic levels of CRP.

5.10 CONCLUSIONS

In conclusion, this investigation was successful in producing weight loss, BMI decreases, decreases in body fat percentage, and positive changes in fitness markers. The primary hypothesis that all groups would experience significant decreases in CRP attributable to weight loss and that the DIET+RT group would experience a further increase attributable to the training program was not supported, however several important findings can be taken from this study. Primarily, the use of a behavioral weight loss intervention with an exercise component can be successfully implemented in a population of Class II and Class III obese individuals with relatively high levels of exercise adherence and behavioral session attendance. Second, this type of behavioral intervention is successful in producing significant weight loss in as little as 12 weeks among this population. Finally, a reduction of weight in this population can lead to a decrease in waist circumference, thereby reducing risk of mortality. This investigation can offer a foundation for the design, magnitude of exercise stimulus, and appropriate power for further

investigations into the influence of weight loss and exercise on CRP levels among Class II and Class III individuals.

APPENDIX A

INFORMED CONSENT DOCUMENT

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School of Education
Physical Activity and Weight Management Research Center

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412-488-4184
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CONSENT TO ACT AS A SUBJECT IN A RESEARCH STUDY

TITLE: The Effect of Weight Loss and Exercise on Cardiovascular Disease Risk Factors in Class II and III Obese Women

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SOURCE OF SUPPORT: Department of Health and Physical Activity University of Pittsburgh & Physical Activity and Weight Management Research Center

DESCRIPTION:

The number of overweight and obese adults in the United States has been increasing at a rapid rate. Of particular concern is the rate at which obesity of the highest levels are increasing. Excess body weight is associated with a number of adverse health consequences including cardiovascular disease. Developing programs to treat obesity which can significantly improve health and improve interventions to help you lose weight are needed to increase success within weight loss programs. In addition, these interventions may improve exercise participation, which is an important component of weight loss programs. This study will examine the effect of diet alone or the combination of diet and exercise on weight loss and cardiovascular disease risk. These interventions include the following:

1. A Diet intervention that involves reducing your calorie intake by changing your eating behaviors and attending regular group-based weight loss meetings.
2. A Diet intervention that involves reducing your calorie intake by changing your eating behaviors and attending regular group-based weight loss meetings. In addition, you will increase your physical activity by accumulating three days per week of supervised aerobic training that will be conducted at the Physical Activity and Weight Management Research Center.
3. A Diet intervention that involves reducing your calorie intake by changing your eating behaviors and attending regular group-based weight loss meetings. In addition, you will increase your physical activity by accumulating three days per week of supervised resistance training that will be conducted at the Physical Activity and Weight Management Research Center.

This study will also investigate the effect of these interventions on fitness, body composition, cardiovascular disease risk factors and other behavioral factors related to weight control. In addition, a portion of your blood sample will be stored indefinitely for future genetic research.

You are being invited to take part in this research study because you are within the body weight range for this study, and do not have any medical conditions that would prohibit you from reducing your calorie intake to 1200 or to 2100 calories per day or participating in moderate to vigorous intensity activity. Moderate intensity activity is defined as activity similar to brisk walking where you can also have a conversation. Vigorous intensity activity is defined as walking at a faster pace such that you cannot have a conversation because you are breathing deeper and faster. People invited into this study have to be women between 30-55 years of age. This study is being performed on a total of 36 individuals.

If you decide to take part in this research study, you will undergo the following procedures that are not part of your standard medical care:

Screening Procedures:

Procedures to determine if you are eligible to take part in a research study are called “screening procedures”. For this research study the screening procedures will be completed at the Physical Activity and Weight Management Research Center at the University of Pittsburgh and will take place on a day prior to any experimental procedure. These screening procedures will include:

You will complete a physical activity readiness questionnaire (PAR-Q), and this will take approximately 5 minutes to complete. You will also complete a detailed medical history, and this will take approximately 20 minutes to complete. These questionnaires will allow the investigators to determine if you have any significant medical condition that would indicate that exercise is unsafe for you. You will also be required to provide medical clearance from your personal physician before starting this study. Participants cannot be pregnant, and you will be required to accurately report whether you are pregnant to the investigators prior to beginning this study and during the study if your status should change. You will also have a urine pregnancy test prior to starting this study and then 12 weeks after you start this study.

Experimental Procedures:

If you qualify to take part in this research study, you will undergo assessment procedures at the University of Pittsburgh Physical Activity and Weight Management Research Center on two separate occasions that will take approximately two hours to complete. Further, you will undergo assessment procedures at UPMC-Presbyterian Hospital on two separate occasions that will take approximately one and one-half hours to complete. These experimental assessment procedures include:

A. Body Weight and Height (10 minutes): Your body weight will be measured using a calibrated electronic scale. Your height will be measured with a ruler that is attached to a flat wall. These will be measured 0 and 12 weeks.

B. Body Composition (30 minutes): Your body composition is the amount of fat weight and lean weight (muscle and bone) that you have on your body. Your body composition will be measured using a dual-energy x-ray absorptiometer (DXA) which assesses the amount of bone, fat tissue, and lean tissue in your body. This scan is done similar to an x-ray but it gives more information. You will lie flat on your back on the DXA scanning table in a private room for approximately 20 minutes. There are no injections, IVs or blood samples, and the test is painless. You may feel slightly uncomfortable lying on the hard surface of the scanning table. In addition, because small levels of radiation are involved with this procedure, you will undergo a urine sample pregnancy test prior to the scan. Pregnant women will be excluded from the study. Your body composition will also be measured using a technique known as Bioelectrical Impedance Analysis (BIA). This procedure requires that a small electrode be placed on your hand, wrist, ankle, and foot. A low-level signal that is not harmful to you and that you will not feel is transmitted between the electrodes. Measurements of your hip and waist areas will also be taken using a measuring tape. These will be measured at 0 and 12 weeks.

C. Resting Blood Pressure and Heart Rate (10 minutes): Your blood pressure will be measured using a blood pressure cuff that is connected to a machine that will record the values from the blood pressure cuff. This device will also record your resting heart rate. You will be in a seated position for this measurement. These will be measured at 0 and 12 weeks.

D. Blood Sample (10 minutes): A fasting blood sample will be taken at the 0 and 12 week assessment visits. Blood will be collected using a needle stick and obtained by a trained phlebotomist or medical technician. Each blood sample will be approximately 3 tablespoons of blood (45ml), with a total of approximately 6 tablespoons of blood being collected at this site for the study. Your blood will be analyzed for C-Reactive Protein, which provides an indication of inflammation in your blood vessels. Inflammation in the blood vessels of the heart causes those vessels to become narrow and decreases blood flow to the heart, which may increase the risk of cardiovascular disease. Your blood will be analyzed at a later date for other chronic disease risk factors related to, but not limited to, diabetes, cancer and others. This will include, but not limited to, lipids, glucose, insulin, and others. In addition, a portion of your blood sample will be stored indefinitely and will be used for future genetic research. For example, this genetic research may involve, but is not limited to, the study of weight, physical activity, dietary intake, and various chronic diseases such as heart disease, diabetes, and cancer. However, you will not be informed of the results of this genetic research because this information cannot be interpreted or applied in a clinically relevant or meaningful manner. The blood sample will be under the control of the principal investigators of this research project. To protect your confidentiality, all personal identifiers (i.e. name, social security number, birth date) will be removed (de-identified) and replaced with a specific code number. The information linking these code numbers to the corresponding subjects' identities will be kept in a separate, secure location. Your de-identified samples may also be shared with other investigators outside of the University of Pittsburgh/UPMC and may be utilized in future studies. You can decline to have your blood samples stored for future analysis, and this will not affect your ability to participate in all other components of this study. To decline to have your blood samples stored for future analysis you should respond "No" to the question on Page 13 of this consent document. If you permit your blood samples to be stored for future analysis you should respond "Yes" to the question on Page 13 of this consent document.

E. Cardiorespiratory Fitness (30 minutes): Measurement of your cardiorespiratory fitness will provide information about how fit your heart and lungs are to perform exercise. Your fitness will be estimated by having you walk on a treadmill. The speed of the treadmill will be kept at 3.0 mph (a brisk pace), however the grade of the treadmill will increase 3.0% every three minutes so that it feels like you are walking up a hill. As you are walking, your heart rate, blood pressure, and perception of physical exertion will be measured. Your heart rate will be measured using an electrocardiogram, which is also known as an ECG. The ECG will require that electrodes be placed on the chest and abdomen areas of your body. You will continue to walk on this treadmill until you reach a heart rate that is 85 percent of your maximal capacity, which is less than your maximal capacity, and then the test will be stopped. During this test, you will breathe in and out through a sterilized (cleaned to prevent the spread of germs and disease) mouthpiece and will wear a set of nose clips so that no air flows through your nose. The air that you breathe will be measured by a machine known as a metabolic cart. This will provide information about the amount of oxygen that you need when you are exercising. A staff member who is certified as an Exercise Specialist by the American College of Sports Medicine and at least one additional staff member will conduct this test. No other study participants will be in the testing room during this assessment. If during this test it is determined that you have a medical condition that makes it unsafe for you to

exercise, you will no longer be permitted to participate in this study, and you will be referred to your primary care physician for medical follow-up. This will be measured at 0 and 12 weeks.

F. Muscular Strength: Muscular strength refers to the maximal amount of weight that can be lifted for a single attempt. Your muscular strength will be assessed for upper and lower body by having you perform one resistance training exercise each for the chest, mid-back, and thighs. You will be asked to perform a maximal lift for the chest press exercise machine (for the chest muscles), the seated row exercise machine (for the mid-back), and the leg press exercise machine (for the thighs).

G. Physical Activity: You will also wear a small device that will measure how active you are. This involves wearing a monitor that is worn on your upper right arm that will track the amount of energy you burn and the amount of physical activity that you perform. You will wear this monitor during all of your waking hours and will remove the monitor when you sleep or participate in water activities such as showering, bathing, or swimming. You will wear this device for 7 consecutive days at 0 and 12 weeks. You will return the activity monitor at your next scheduled visit that will occur at least 7 days after wearing the activity monitor. If you are unable to return the activity monitor in person within this period of time, you will be provided a postage paid envelop by the investigators to return the monitor at no expense to you. In addition, you will be asked to complete a brief structured interview to provide additional information about your physical activity patterns on the day that you receive this small device. This interview will involve a staff member asking you a series of questions related to your physical activity patterns over the past week. This intervention will take 10 minutes to complete. Wearing the activity monitoring device and completing the structured interview about your activity patterns will occur at 0 and 12 weeks.

H. Dietary Intake: You will be asked to complete a questionnaire about your eating patterns over the prior 6 months. You will complete this questionnaire at the 0 and 12 week assessment periods. It will take you about 30 minutes to complete this questionnaire.

I. Cardiac Structure and Function: You will receive a cardiovascular magnetic resonance imaging (CMRI) scan at the 0 and 12 week assessment visits. The CMRI will be conducted at the Cardiovascular Magnetic Resonance Center within UPMC Presbyterian Hospital. This scan will take approximately 60 to 90 minutes and it may not occur on the same day as the assessments that are conducted at the Physical Activity and Weight Management Research Center. The CMRI will allow us to examine the structure and function of the tissues that make up your heart and blood vessels. Each scan requires that approximately 3 tablespoons (45ml) of your blood be collected (a total of 6 tablespoons of blood over two visits) to establish normal ranges for the CMRI measurements. The CMRI will require that a contrast dye be injected into your vein.

Weight Control and Exercise Procedures

After completing these assessments, if you are still eligible to participate, you will be randomly assigned to one of three interventions to assist you with your weight loss and/or exercise

behaviors. Random assignment is similar to flipping a coin to determine the group that you will be in. These groups are described below:

A. Diet Only Intervention:

1. Meetings and Contacts: You will attend weekly group meetings for 12 weeks. Each group meeting will last approximately 45 minutes. The intervention meetings will be held on the same night every week and the group will have approximately 10-12 members that will be dieting to lose weight. These meetings will involve discussions with the staff and other members of the group about your diet to help you to lose weight.

2. Diet: You will be placed on a diet that encourages you to decrease the amount of total calories and fat that you eat. If you are less than 175 pounds, you will be placed on a 1200 calorie per day diet. If you are 175-219 pounds, you will be placed on a 1500 calorie per day diet. If you are 220-249 pounds, you will be placed on an 1800 calorie per day diet. If you are greater than 250 pounds, you will be placed on a 2100 calorie diet. You will also be taught how to decrease the amount of fat that you eat, and will be encouraged to decrease fat intake to 20-30% of your total calories. You will record the food that you eat in a diary, and this information will be provided to the investigators weekly.

3. Exercise: You will not be given an exercise program, but will be encouraged to maintain your current level of physical activity throughout the 12 weeks of this study.

B. Diet Plus Aerobic Training:

1. Meetings and Contacts: You will attend weekly meetings as described above for the Diet Only Group.

2. Diet: You will be placed on the same diet as described above for the Diet Only Group.

3. Exercise: You will be given an exercise program that will be performed at the Physical Activity and Weight Management Research Center. You will participate in 3 individual aerobic exercise sessions per week under the supervision of the investigators with the duration of each day increasing from 20 to 60 minutes over the 12 week program, yielding an increase of 60 minutes per week to 180 minutes per week. The facility will be available from 6:30-8:30am and 11:00am-1:30pm Monday - Friday, 4:00-7:00pm Monday –Thursday, and Saturday mornings from 8:30-10:30am for your exercise participation. You will exercise at an intensity of 60-70% of your maximal capacity, which is the equivalent of taking a brisk walk for most individuals. You may use any of the aerobic training equipment at the facility (i.e. treadmills, ellipticals, recumbent bicycles, adaptive motion trainers). You will record your exercise in a diary, and this information will be provided to the investigators weekly.

C. Diet Plus Resistance Training:

1. Meetings and Contacts: You will attend weekly meetings as described above for the Diet Only Group.

2. Diet: You will be placed on the same diet as described above for the Diet Only Group.

3. Exercise: You will be given an exercise program that will be performed at the Physical Activity and Weight Management Research Center. You will participate in 3 exercise sessions per week that involve resistance exercise, which is also known as weight training. These sessions will be supervised by the staff at the Physical Activity and Weight Management Research Center. The facility will be available from 6:30-8:30am and 11:00am-1:30pm Monday -Friday, 4:00-7:00pm Monday –Thursday, and Saturday mornings from 8:30-10:30am for your exercise participation. During these sessions you will perform 4-8 consecutive lifts for each of the exercises, and after a brief rest, you will repeat this between 2-6 times. The weight that you will lift will be set at 60% to 90% of you maximal strength that is determined for each of the exercises that you will perform. You will record your exercise in a diary, and you will provide this to the investigators each week.

RISKS and BENEFITS:

The possible risks of this research study may be due to the exercise that you will be performing and the assessments that will be performed.

Risks

A. Risks of Exercise Testing and Exercise Participation: There are moderate risks associated with participating in an exercise test and a regular exercise program. During exercise, you may experience a serious cardiac (affecting your heart) event, an arrhythmia (your heart beats at a pace that is not normal), or chest pain. An example of a cardiac event would be a heart attack or another medical condition that causes damage to your heart or cardiovascular system. The possibility of experiencing a serious cardiac event has been estimated to be approximately 6 per 10,000 in exercising adults. Therefore, the risk is of this happening to you is rare. In addition, during exercise, you may experience an increase in heart rate, an increase in blood pressure, shortness of breath, general fatigue, and in some cases muscle soreness or injury to your muscle, bone, or joints. The risk of this happening to you is likely because these occur in more that 25% of people (more than 25 out of 100 people). In the event that you experience a serious medical condition during your exercise session, the session will be stopped and appropriate emergency medical care will be provided. This may include providing CPR until Paramedics or other appropriate medical personnel arrive. If you elect to participate in exercise in addition to the required supervised sessions for this study conducted at the Physical Activity and Weight Management Research Center, the staff will not be able to assist you.

B. Risk of having the air that you breathe in and out measured by a metabolic cart: When measuring the air that you breathe in and out during exercise, you may experience a dry mouth. The risk of this happening to you is likely.

C. Risk of Electrocardiogram (ECG): You may experience skin irritation or skin redness from electrodes being placed on your skin. The risk of this happening to you is likely.

D. Risk Associated with DXA Radiation Exposure: The DXA scan involves exposure to radiation which is 0.05 mrem (a unit of radiation exposure) per total body scan. (These radiation exposures will be present at both the baseline and 12-week assessments). For comparison, radiation workers are permitted, by federal regulation, a maximum annual whole body radiation exposure of 5,000 mrems. In addition, this exposure to radiation is less than the radiation exposure from a standard chest x-ray or the radiation exposure received during a transcontinental airplane flight. There is no known minimum level of radiation exposure that is recognized as being totally free of the risk of causing genetic defects (abnormal cells) or cancer. However, the risk associated with the amount of radiation exposure that you will receive from this study is considered to be low and comparable to everyday risks. If you are a woman who is able to have children, you will have a urine pregnancy test performed less than 24 hours prior to the DXA scan. If we find that you are pregnant, you cannot be in the study. If you become aware that you are pregnant during any time you are in this research study, you must not have a DXA scan or complete any of the tests we do in this research study.

E. Risk Associated with Wearing the Physical Activity Monitor: Some people may experience mild skin irritation where the activity monitor is worn. One cause of skin irritation has already been identified in people who wear the armband for extensive periods of time (i.e., more than 24 hours). Specifically, the build-up of sweat that can be trapped between the skin and the armband, or not properly cleaning the armband, can cause pink pustules or pimples to appear. This condition is named miliaria, or prickly heat. This condition is common in people who wear the armband. To help prevent this condition you should clean your arm using rubbing alcohol before putting on the activity monitor. Also, you should use soap and water to clean the elastic strap that attaches the monitor to your arm before each use. You should also wipe off the monitor using rubbing alcohol and allow this to dry before putting it on your arm.

F. Risk of Drawing Blood: The risks of drawing blood from a vein include discomfort at the site of puncture and possible bruising and swelling around the puncture site. The risks of this happening to you are likely. Although rare, it is possible that you may develop an infection or experience faintness from the procedure. The risk of an infection or fainting occurring is rare. You may also experience the rare occurrence of breach of confidentiality with regard to stored blood samples.

G. Risk of Cardiac Magnetic Resonance Imaging: There are risks associated with the MRI scans. These risks are rare. Risks include claustrophobia (fear of enclosed spaces), discomfort, peripheral nerve stimulation (numbness, tingling, or pain in an arm or leg), loss of hearing since scanners can be loud, and skin burns. Every precaution is taken to prevent injury, and the likelihood of such events is extremely rare. For example, we use a short and extra wide scanner that minimizes feelings of enclosure, provide ear protection, remove metal jewelry prior to scans to minimize the chances of a burn, and ensure that participants do not have conditions or devices that would render scanning unsafe. The UPMC CMR Center scanner and personnel are dedicated to the care of patients with cardiovascular disease. All of the MRI scanning settings follow safety requirements of the US Food and Drug Administration (FDA). MRI contrast dye agents can cause a rare condition called “nephrogenic

systemic fibrosis" (NSF), a potentially fatal complication whereby increased tissue deposition of collagen occurs, often resulting in thickening and tightening of the skin. NSF has occurred mainly in those with severe kidney disease. We minimize this risk by excluding those with severe kidney disease or dialysis, and by choosing a MRI contrast agent that poses a significantly less of a risk for developing NSF. If the MRI contrast agent leaks out of the vein during injection, painful swelling may occur. We minimize this risk by ensuring the in-dwelling catheter (tubing inserted into your blood vessels) is well positioned and functioning properly before giving any MRI contrast. Conventional cardiovascular MRI scanning requires you to hold your breath which may cause you to become tired. If you find breath holding difficult, we will perform a scan without the need for breath holding.

H. Risk Associated with Completion of Questionnaires: You may experience non-physical risks such as boredom, frustration, stress, and time constraints when completing the questionnaire. The risk of this happening to you is likely.

I. Risks of Reducing Your Calorie and Fat Intake: Consuming a moderately low fat and low calorie diet appears to be safe and effective for weight loss. However, if you reduce your calorie or fat intake below recommended levels, you may experience dry skin and thinning of your hair. This is common. You may also experience rare problems with your gall bladder, including the development of gall stones that may include symptoms such as intense abdominal pain that increases from a few minutes to hours, back pain, nausea or vomiting. Other symptoms may include rare bloating, gas, and indigestion.

J. Risks Associated with Participating in the Group Intervention: Attending group sessions has been shown to be effective for weight loss. However, attendance at these sessions may involve you sharing information about yourself and your weight loss efforts to other group members. You can elect not to share this private information about yourself to other group members. Members of the group will be instructed to keep all information shared in the group sessions confidential. However, because the investigators cannot guarantee that all group members will keep this information confidential, there is risk that group members may share information about the group session with individuals not participating in this study. The risk of this happening to you is likely.

K. Risk of Pregnancy During the Intervention: Participants cannot be pregnant, and you will be required to accurately report whether you are pregnant to the investigators prior to beginning this study and during the study if your status should change. It is possible that weight loss may increase fertility. You will also have a urine pregnancy test prior to starting this study and 12 weeks after you start this study.

Benefits:

There are also possible benefits of this research study that may be due to the exercises that you will be performing and the diet that will reduce the amount and types of foods that you will be eating. However, there is no guarantee that any or all of these changes will occur as a result of you participating in this study.

A. Benefits of Exercise: The benefits of participating in an exercise program have been shown to include improvements in physical fitness, weight loss, improvements in blood pressure, and improvements in blood cholesterol levels. However, there is no guarantee that any or all of these changes will occur as a result of your participation in this study.

B. Benefits of Reducing your Calorie and Fat Intake: Consuming a low fat and low calorie diet appears to be safe and effective for weight loss. Additional benefits of eating this type of diet can be improvements in blood pressure and improvements in blood cholesterol levels. However, there is no guarantee that any or all of these changes will occur as a result of your participation in this study.

If we should find out about a medical condition you were unaware of, with your written permission, this information will be shared with the doctor of your choice.

NEW INFORMATION:

You will be promptly notified if any new information develops during the conduct of this research study, which may cause you to change your mind about continuing to participate.

COSTS and PAYMENTS:

Neither you, nor your insurance provider, will be charged for the costs of any of the procedures performed for the purpose of this research study. These costs will be paid by the sponsor of this research study.

You may be compensated up to $300 for your participation in this study, with funds distributed to you based on your completion of 3 components of this study as described below. These payments will be made to you at the completion of the study and will be provided to you in the form of a debit card issued by the University of Pittsburgh solely for the purpose of compensating you for your participation in this study.

1. You will receive $100 for completion of the assessment procedures conducted at 12 weeks at the Physical Activity and Weight Management Research Center. As described above these assessments include measures of height, weight, body composition, resting blood pressure, resting heart rate, collection of blood samples, cardiorespiratory fitness, strength, physical activity, and dietary intake.
2. You will receive $100 for completion of CMRI assessments conducted at 12 weeks at the Cardiovascular Magnetic Resonance Center within UPMC Presbyterian Hospital.
3. If you complete the 12 week assessments you will also be compensated for the intervention sessions that you attended, and you will receive $100 for attendance at 100% of the intervention sessions as described above. For the Diet Only intervention this will require attendance at 12 weekly meetings. For both the Diet Plus Aerobic Training and Diet Plus Resistance Training groups, this will require attendance at 12 weekly group meetings plus 3 supervised exercise sessions per week. If you are not able to achieve 100% compliance, your compensation will be based on a percentage of the sessions you do attend according to the following:
    a. Attendance at 90-99% of sessions will be compensated at $90.
    b. Attendance at 80-89% of sessions will be compensated at $80.
    c. Attendance at 70-79% of sessions will be compensated at $70.
    d. Attendance at 60-69% of sessions will be compensated at $60.

e. Attendance at 50-59% of sessions will be compensated at $50.
f. Attendance at 40-49% of sessions will be compensated at $40.
g. Attendance at 30-39% of sessions will be compensated at $30.
h. Attendance at 20-29% of sessions will be compensated at $20.
i. Attendance at 10-19% of sessions will be compensated at $10.

Your biological sample or genetic material may lead, in the future, to new inventions or products. If the research investigators are able to develop new products from the use of your biological sample or genetic material, there are currently no plans to share with you any money or other rewards that may result from the development of the new product.

COMPENSATION FOR INJURY:

University of Pittsburgh researchers and their associates who provide services at the University of Pittsburgh Medical Center (UPMC) recognize the importance of your voluntary participation in their research studies. These individuals and their staffs will make reasonable efforts to minimize, control, and treat any injuries that may arise as a result of this research. If you believe that you are injured as a result of the research procedures being performed, please contact immediately the Principal Investigator listed on the first page of this form.

Emergency medical treatment for injuries solely and directly related to your participation in this research study will be provided to you by the hospitals of UPMC. It is possible that UPMC may bill your insurance provider for the costs of this emergency treatment, but none of these costs will be charged directly to you. If your research-related injury requires medical care beyond this emergency treatment, you will be responsible for the costs of this follow-up care unless otherwise specifically stated below. There is no plan for monetary compensation. You do not, however, waive any legal rights by signing this form.

CONFIDENTIALITY:

Any information about you obtained from this research will be kept as confidential (private) as possible. All records related to your involvement in this research study will be stored in a locked file cabinet. Your identity on these records will be indicated by a case number rather than by your name, and the information linking these case numbers with your identity will be kept separate from the research records. In addition, all research databases will have password controlled access, and this will be controlled by the researchers. Only the researchers listed on the first page of this form and their staff will have access to your research records. However, other scientists may request data obtained by this study. We will allow data to be released to qualified researchers only after ensuring that your name and other identifying information is not given to these researchers. You will not be identified by name in any publication of research results unless you sign a separate form giving your permission (release).

This research study will not involve the recording of current and/or future identifiable medical information from your hospital and/or other (e.g. physician office) records. The information that will be recorded will be limited to information concerning medical clearance from your physician to participate in this research study. The information obtained from this study will not be communicated to your physician except in the case of medical necessity, such as an abnormal EKG during assessment procedures.

In addition to the investigators listed on the first page of this authorization (consent) form and their research staff, the following individuals will or may have access to identifiable information related to your participation in this research study:

Authorized representatives of the University of Pittsburgh Research Conduct and Compliance Office may review your identifiable research information for the purpose of monitoring the appropriate conduct of this research study.

In unusual cases, your research records may be required to release identifiable information related to your participation in this research study in response to an order from a court of law. If the researchers learn that you or someone with whom you are involved is in serious danger or potential harm, they will need to inform, as required by Pennsylvania law, the appropriate agencies.

A cardiologist in the University of Pittsburgh School of Medicine and UPMC will review the treadmill exercise tests that are completed as part of your participation in this study, and he/she will have access to your identifiable medical information.

The investigators may continue to use and disclose, for the purposes described above, identifiable information related to your participation in this research study for 6 years following the completion of this study, as per University policy, or when such is approved by the sponsor of this study, whichever should occur last.

RIGHT TO PARTICIPATE or WITHDRAW FROM PARTICIPATION:

Your participation in this research study, to include the use and disclosure of your identifiable information for the purposes described above, is completely voluntary. (Note, however, that if you do not provide your consent for the use and disclosure of your identifiable information for the purposes described above, you will not be allowed, in general, to participate in the research study.) Whether or not you provide your consent for participation in this research study will have no effect on your current or future relationship with the University of Pittsburgh. Whether or not you provide your consent for participation in this research study will have no effect on your current or future medical care at a UPMC hospital or affiliated health care provider or your current or future relationship with a health care insurance provider.

You may withdraw, at any time, your consent for participation in this research study, to include the use and disclosure of your identifiable information for the purposes described above. (Note, however, that if you withdraw your consent for the use and disclosure of your identifiable information for the purposes described above, you will also be withdrawn, in general, from further participation in this research study.) Any identifiable research information recorded for, or resulting from, your participation in this research study prior to the date that you formally withdrew your consent may continue to be used and disclosed by the investigators for the purposes described above. Blood samples collected prior to withdrawal from the study will be rendered anonymous and held in storage for possible future study use. “Rendered anonymous” means that the information linking your identity to the samples will be destroyed so that your samples can never be traced back to you. To decline to have blood samples stored for future analysis you should respond “No” to the question on the consent document found below. If you permit blood samples to be stored for future analysis, please respond “Yes” to the question on the consent document.

To formally withdraw your consent for participation in this research study you should provide the notice of this decision to the principal investigator listed on the first page of this form in one of the following ways: 1) provide a written and dated notice of this decision, or 2) send an email of this decision, or 3) contact the investigator by telephone to inform him of this decision. This written letter, email, or record of this telephone notice to withdraw your consent from the study will be retained by the investigator.

Your decision to withdraw your consent for participation in this research study will have no effect on your current or future relationship with the University of Pittsburgh. Your decision to withdraw your consent for participation in this research study will have no effect on your current or future medical care at a UPMC hospital or affiliated health care provider or your current or future relationship with a health care insurance provider.

It is possible that you may be removed from the research study by the researchers if, for example, your health status changes and it does not appear that it is safe for you to continue to reduce your food intake, participate in exercise, or lose weight. You will also be removed if you should become pregnant during this study.

****

VOLUNTARY CONSENT

I give my permission to have a portion of my de-identified blood sample stored indefinitely and used for future genetic research.

YES _____ NO _____

The above information has been explained to me and all of my current questions have been answered. I understand that I am encouraged to ask questions, voice concerns or complaints about any aspect of this research study during the course of this study, and that such future questions, concerns or complaints will be answered by a qualified individual or by the investigator(s) listed on the first page of this consent document at the telephone number(s) given. I understand that I may always request that my questions, concerns or complaints be addressed a listed investigator. I understand that I may contact the Human Subject Protection Advocate of the IRB Office, University of Pittsburgh (1-866-212-2668) to discuss problems, concerns, and questions; obtain information; offer input; or discuss situations in the event that the research team is unavailable.

By signing this form, I agree to participate in this research study. A copy of this consent form will be given to me.

_____ _____

Participant's Signature Date

CERTIFICATION OF INFORMED CONSENT

I certify that I have explained the nature and purpose of this research study to the above-named individual, and I have discussed the potential benefits and possible risks of study participation. Any question the individual has about this study has been answered, and we will always be available to address future questions, concerns or complaints as they arise. I further certify that no research component of this protocol was begun until after this consent form was signed.

Printed Name of Person Obtaining Consent	Role in Research Study
Signature of Person Obtaining Consent	Date

APPENDIX B

PHYSICIAN CONSENT DOCUMENT

University of Pittsburgh

School of Education
Physical Activity and Weight Management Research Center

Suite 600 Birmingham Towers
2100 Wharton Street
Pittsburgh, PA 15203
412-488-4184
Fax: 412-488-4174

PHYSICIAN PERMISSION TO PARTICIPATE IN A DIET AND EXERCISE PROGRAM AT THE UNIVERSITY OF PITTSBURGH

O: _____
Physician's Name

_____
Address

_____
City S Z
tate ip

( )
_____
Telephone Number

RETURN TO: (envelope provided)

John M. Jakicic, Ph.D.
University of Pittsburgh
Department of Health and Physical Activity
Physical Activity and Weight Management Research Center
2100 Wharton Street, Suite 600
Pittsburgh, PA 15203
Telephone: (412) 488-4184

FAX: (412) 488-4174

Your patient _____ has asked to participate in a diet and exercise program at the University of Pittsburgh. This is a 12 week research study designed to help patients to change their eating and exercise habits and to examine the impact that this will have on weight loss and cardiovascular disease risk. This will involve the following:

1. A diet program that will reduce energy intake to 1200-2100 calories per day, with dietary fat reduced to 20-30% of total energy intake.
2. A walking program that will be supervised or a supervised resistance training program. The exercise will gradually be progressed from 20 minutes per day to as much as 60 minutes per day, 3 days per week. Exercise intensity will be set at 60-70% of the patient's maximal heart rate or 60-90% of participants' maximal strength.
3. A graded exercise test which involves walking on a motorized treadmill, with the workload gradually increasing 3% every 3 minutes. The test will be terminated when the patient achieves 85% of age-predicted maximal heart rate, or prior to this level if the individual experiences signs or symptoms that would indicate that exercise is contraindicated. Both blood pressure, heart rate, and EKG will be monitored continuously. The ACSM Guidelines for Exercise Testing will be followed.
4. Behavioral modification techniques for changing eating and exercise behaviors.
5. A list of additional factors that are exclusionary criteria for this study that you should consider are listed on the attached sheet.

****
******

Please indicate below if this program seems appropriate for your patient or if you see any contraindications for her participation (*please check the appropriate box below)*.

☐ I know of no contraindications to this patient participating in any of the above components of the program.

☐ I feel that this program would not be appropriate for this patient for the following reason(s):

_____

_____ _____

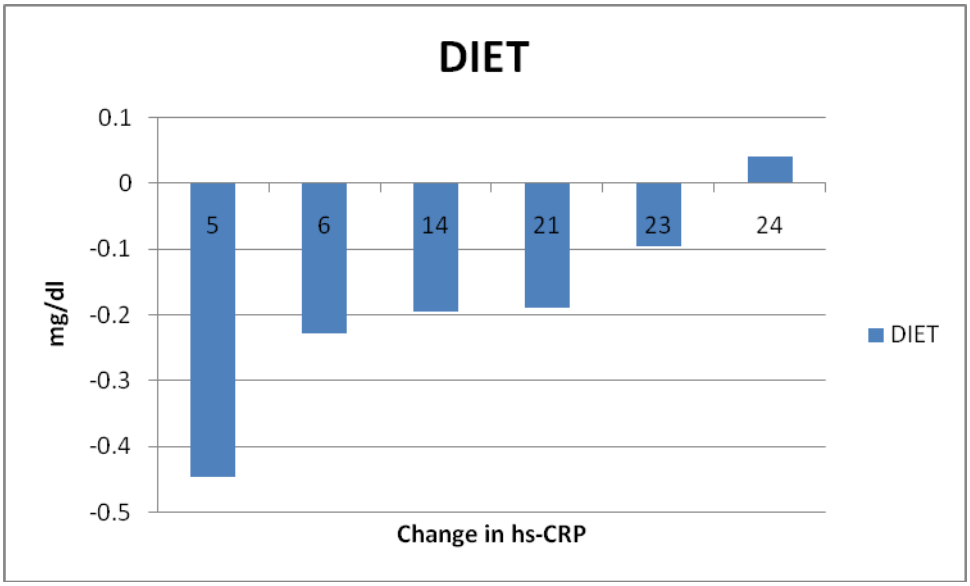
Signature of Physician Date

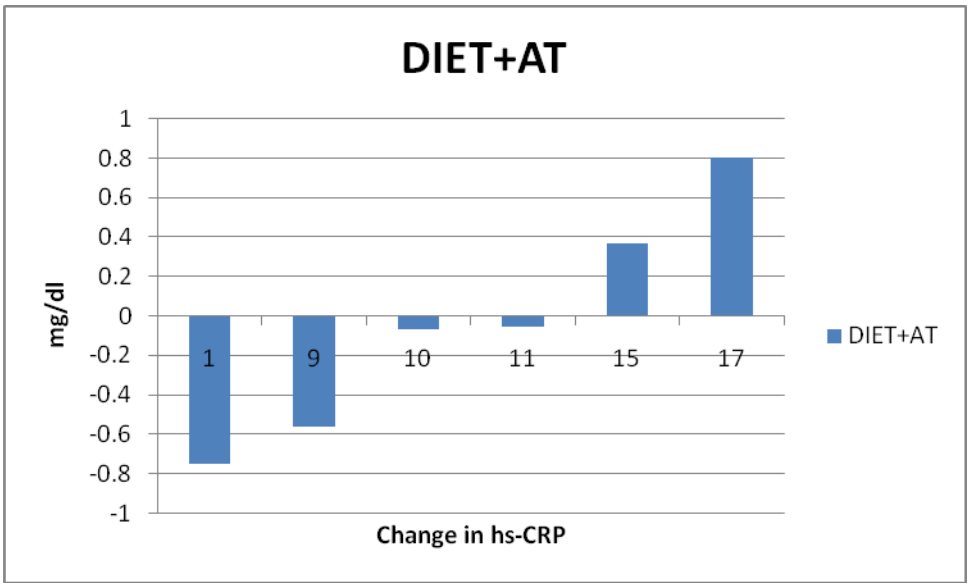
Please consider the following Inclusion and Exclusion Criteria as you evaluate whether your patient is capable of safely participating in the weight loss and exercise research study at the University of Pittsburgh.

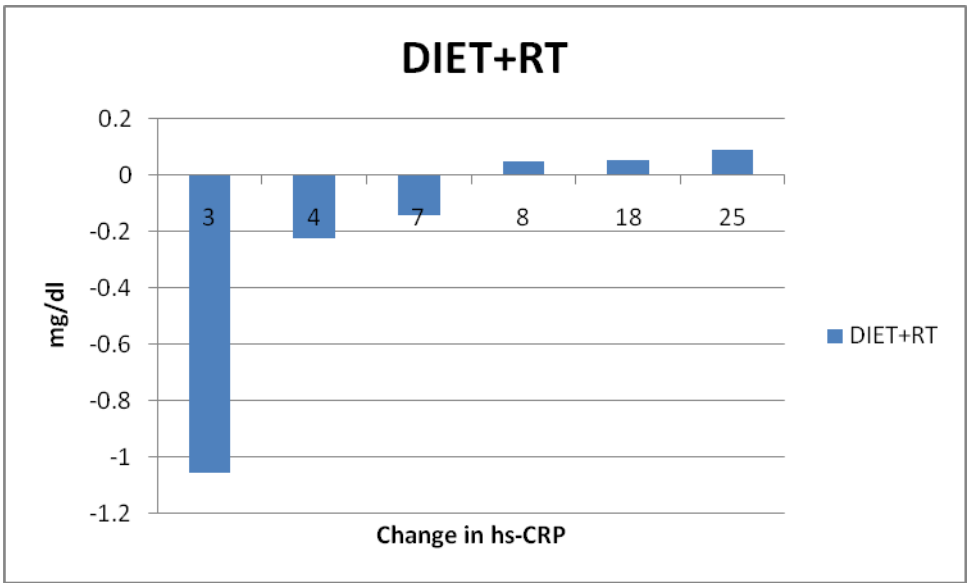
Inclusion Criteria:	Exclusion Criteria:
• Female • 30-55 years of age • BMI = 35.0-44.9 kg/m^2	• Currently pregnant, pregnant in the past 6 months, or planning on becoming pregnant in the next 6 months. • Regularly exercising for greater than 60 minutes per week. • Taking prescription or over-the-counter medications that affect body weight, metabolism, blood pressure, or heart rate, such as psychotropic medications (e.g. zoloft, prozac, paxil, xanax) and medications that have metabolic effects (e.g. synthroid, wellbutrin, metformin). • Having physical limitations that hinder or prevent exercise. • Currently being treated for coronary heart disease, diabetes mellitus, hypertension, or cancer. • Having a resting systolic blood pressure of > 150mmHg of diastolic blood pressure of > 100mmHg or currently taking any medications that affect blood pressure or heart rate (i.e. beta blockers). • Currently enrolled in an exercise or weight control study or participating in an exercise or weight control study in the past 6 months. • Have lost and not regained > 5% body weight in the past 6 months. • Currently being treated for any psychological problems or taking any psychotropic medications. • Abnormal kidney functions, as per current institutional standard for gadolinium adminstration. • Known allergy to IV gadolinium, or the discovery of an allergic reaction to IV gadolinium at baseline.

APPENDIX C

INDIVIDUAL CHANGES IN hs-CRP BY GROUP




DIET+AT
1
0.8
0.6
0.4
0.2
0
-0.2
-0.4
-0.6
-0.8
-1
mg/dl
1
9
10
11
15
17
Change in hs-CRP
DIET+AT


DIET+RT
0.2
0
-0.2
-0.4
-0.6
-0.8
-1
-1.2
mg/dl
3
4
7
8
18
25
Change in hs-CRP
DIET+RT

APPENDIX D

hs-CRP VALUES OF PARTICIPANTS (mg/dl)

Participant	Baseline hs CRP	Follow-up hsCRP
11	2.39	2.758
17	2.257	1.697
10	2.06	1.307
19	1.512	
2	1.442	
7	1.282	0.226
21	1.239	0.792
14	1.001	0.773
28	0.926	
4	0.738	0.791
9	0.682	1.486
6	0.619	0.43
18	0.6	0.377
23	0.582	0.386
24	0.58	0.62
5	0.576	0.48
15	0.532	0.465
3	0.496	0.351
12	0.403	
26	0.284	
25	0.194	0.242

8	0.192	0.283
16	0.184	
1	0.086	0.033

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