ABSTRACT

Title of Document: THE EFFECTS OF LOW-FAT DIET AND EXERCISE ON C-REACTIVE PROTEIN AND METABOLIC SYNDROME: FINDINGS FROM A RANDOMIZED CONTROLLED TRIAL

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Background: Low-fat diet (D) and exercise (E) are recommended for reducing cardiovascular disease risk. However, the independent and combined effects of D and E on C-reactive protein (CRP) and metabolic syndrome (MS) are unknown.

Purpose: The purpose of this dissertation was to examine the changes in CRP and MS between control (C), D, E and diet plus exercise (D+E).

Methods: Men (n=197) and postmenopausal women (n=180) with elevated low-density lipoprotein cholesterol and reduced high-density lipoprotein cholesterol, were randomized into a one-year trial with four groups: C, D, E or D+E (Stefanick et al., 1998). Weight loss was not an intervention focus. This secondary data analysis evaluated stored plasma samples for high-sensitivity CRP. MS prevalence was retrospectively found using the NCEP-ATP III definition. CRP change (ΔCRP) was examined between intervention groups using ANCOVA. Differences between groups

for MS at follow-up were retrospectively investigated using logistic regression. All analyses were stratified by gender and controlled for baseline values, body fat change and other appropriate covariates.

Results: In women, ΔCRP was different between D+E vs. C (-0.7 ± 0.33 mg/L, p = 0.04) and D+E vs. E (-0.9 ± 0.32 mg/L, p = 0.004). Women also had a decrease in CRP within D+E (Δ log CRP 0.2 ± 0.035 mg/L; p = 0.0002). After the intervention, ΔCRP did not differ for men between or within treatment groups. MS at follow-up was not different between C, D, E or D+E in either men or women. In women with MS, ΔCRP was different between D+E vs. C (-1.3 ± 0.43 mg/L; p = 0.006), D+E vs. E (-1.1 ± 0.44 mg/L; p = 0.02), and D vs. C (-1.2 ± 0.43 mg/L; p = 0.009). In women with MS, CRP decreased from baseline within D+E (Δ log CRP 0.2 ± 0.039 mg/L; p=0.0008). At follow-up, there were no differences between or within groups for ΔCRP in men with MS, or men without MS and women without MS.

Conclusion: D and D+E may be effective treatments for reducing CRP in women with MS. Further studies are needed to replicate results and clarify the influence of gender.

THE EFFECTS OF LOW-FAT DIET AND EXERCISE ON C-REACTIVE PROTEIN AND METABOLIC SYNDROME: FINDINGS FROM A RANDOMIZED CONTROLLED TRIAL

By

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Dedication

To my parents,
Milt and Kathy Camhi,
who gave me unwavering support throughout this whole process –
even though it took me 3000 miles from home.

To my grandmothers:
Bertha Soslow and Ann Camhi.
Although at 96 and 93 years old, they don't quite understand what Kinesiology is,
they always asked the most important question to keep me motivated...
"When are you going to be finished?".

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I would like to end with a quote that has inspired me and the other exercise physiology epidemiology ladies during the "marathon" PhD journey. These words are from a former University of Maryland Kinesiology student, Dr. Dana Phares:

I know a lot,
but I don't know everything,
and that's ok...
because what I do know,
is undoubtedly good enough!

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Chapter 1

Introduction

Lifestyle interventions such as low-fat diet and exercise can have positive effects on cardiometabolic risk factors. However, it is unclear how the individual and combined components of lifestyle interventions influence emerging cardiovascular risk factors such as chronic inflammation (C-reactive protein) and clustering of risk factors (metabolic syndrome). Associations exist between cardiovascular disease and elevated levels of C-reactive protein (CRP) (Ridker, 2003b; Ridker, 2003a; Ridker, Bassuk & Toth, 2003a) and metabolic syndrome status (Ford, 2004; Lakka et al., 2002). Thus, establishing the effects of lifestyle interventions on CRP and metabolic syndrome are important to manage the development and progression of cardiovascular disease.

Dissertation Purpose

The purpose of this dissertation was to systematically analyze an existing data set for the independent and combined effects of a low-fat diet and exercise on CRP and metabolic syndrome. The Diet for Elevated Risk Trial (DEER) was conducted in 1992 at Stanford University (Stefanick et al., 1998). This dissertation adds two measures: a new laboratory analysis for CRP and a retrospective analysis of metabolic syndrome.

First, a brief synopsis of background information is provided for CRP and metabolic syndrome, followed by the specific aims and hypotheses.

C-Reactive Protein

Chronic inflammation, a novel cardiovascular risk factor, is an increase in circulating pro-inflammatory cytokines such as C-reactive protein (CRP). CRP is an acute phase reactant which is released from the liver and is considered a downstream marker of chronic inflammation. CRP increases with aging (Rumley et al., 2006), body mass index (Ford, 1999) and is higher in women than in men (Ford, Giles, Myers, & Mannino, 2003; Ford, Giles, Mokdad, & Myers, 2004b). It is estimated that approximately 25% of adults have elevated levels of CRP (Pearson et al., 2003) which is linked to mortality (Harris et al., 1999) and cardiovascular disease (Ridker, 2003b; Ridker, 2003a; Ridker, Bassuk, & Toth, 2003a).

Comparisons between low-fat diet and diet plus exercise show that the combination results in larger changes in CRP than diet alone, although none of these studies included a control group for comparison (You et al., 2004; Esposito et al., 2003; Esposito et al., 2004). These randomized trials also induced concurrent weight loss (You et al., 2004; Esposito et al., 2003; Esposito et al., 2004). The release of CRP may be controlled by cytokines which can be stored and released from adipose tissue (Petersen et al., 2005). Therefore, the amount of body fat (Visser, Bouter, McQuillan, Wener, & Harris, 1999) and the change in body fat (Selvin, Paynter, & Erlinger, 2007) are directly related to the circulating levels of CRP. Thus, comparisons between the independent and combined effects of low-fat diet and exercise on CRP are difficult to distinguish from the influence of fat loss.

Another challenge with the present low-fat diet plus exercise interventions is that many only include a single gender, limiting the generalizability results (Dvorakova-

Lorenzova et al., 2006; Esposito et al., 2003; Roberts et al., 2006; Wegge, Roberts, Ngo, & Barnard, 2004; You, Berman, Ryan, & Nicklas, 2004). Since CRP baseline levels are higher in women, it is important to account for these differences (Ford et al., 2003; Ford et al., 2004b). Diet plus exercise interventions which include both genders, have not adjusted for the differences in CRP levels within their analyses (Bo et al., 2007; Esposito et al., 2004). Furthermore, it is possible that a higher CRP baseline value may alter the magnitude of change from lifestyle intervention.

Few randomized controlled trials have examined the independent responses of CRP to low-fat diet, exercise and diet plus exercise. Low-fat dietary interventions have shown reductions of CRP levels (Clifton, Keogh, Foster, & Noakes, 2005; Heilbronn, Noakes, & Clifton, 2001; O'Brien et al., 2005; Seshadri et al., 2004; Tchernof, Nolan, Sites, Ades, & Poehlman, 2002), though other studies do not show significant changes in CRP when compared to a control group (Erlinger, Miller, III, Charleston, & Appel, 2003). Exercise trials have also shown varying results for CRP, where some studies show significant reductions when compared to a control group (Mattusch, Dufaux, Heine, Mertens, & Rost, 2000; Milani, Lavie, & Mehra, 2004), while other studies have had no effect on CRP levels (Hammett et al., 2004; Huffman et al., 2006). Combined low-fat diet and exercise interventions have significantly reduced CRP levels (Bo et al., 2007; Roberts et al., 2006; Wegge et al., 2004), though control groups were not always included for comparison (Roberts et al., 2006;Wegge et al., 2004).

Specific Aim #1: To determine the effects of a low-fat diet and/or exercise intervention on change in CRP in men and women high cardiovascular risk.

Hypothesis 1: The low-fat diet plus exercise will significantly change CRP levels in both men and women when compared to the control group. Changes in CRP levels in the low-fat diet only or exercise only groups will not differ from the control group.

Metabolic Syndrome

Metabolic syndrome is a clustering of abnormal metabolic, lipid and non-lipid variables. The National Cholesterol Education Program (NCEP) has operationally defined metabolic syndrome as meeting at least three of the following criteria: 1) abdominal obesity, 2) elevated triglycerides, 3) low high density lipoprotein cholesterol (HDL), 4) hypertension and 5) high fasting glucose (2001).

The prevalence of metabolic syndrome has increased over the past ten years: 23% in 1994 (Ford, Giles, & Dietz, 2002), 27% by 1999 (Ford, Giles, & Mokdad, 2004a), and 35% in 2002 (Ford, 2005). Individuals with the presence of metabolic syndrome are two to three times more likely to die from cardiovascular disease (Ford, 2004; Lakka et al., 2002). Therefore, it is paramount to find effective treatments to reverse metabolic syndrome in order to curb the progression of cardiovascular disease.

The NCEP recommends reducing intake of saturated fat and cholesterol, increasing physical activity and achieving weight loss to reverse metabolic syndrome (2001). While these lifestyle modifications have shown benefits for individual cardiovascular risk factors (Grundy et al., 2004b), evidence for improving metabolic syndrome prevalence with low-fat diet and/or exercise has not been consistent.

Few randomized controlled trials have systematically examined the individual and combined effects of low-fat diet and exercise on metabolic syndrome. Diet plus exercise

demonstrates more success for reversing metabolic syndrome than diet or exercise alone, however these interventions also included concomitant weight loss (Anderssen, et al., 2007; Okura et al., 2007). Weight loss can improve many of the individual metabolic and lipid components of metabolic syndrome (Yu-Poth et al., 1999), making it difficult to identify the successful component(s) of the intervention. Another challenge within the current diet plus exercise research for metabolic syndrome is the absence of a control group for comparison, which may limit the interpretation of results (Roberts et al., 2006; Muzio et al., 2005; Esposito et al., 2004; Okura et al., 2007).

Studies which examine the independent effects for low-fat diet or exercise on metabolic syndrome prevalence have been limited. Interventions which included a low-fat diet have reduced the prevalence of metabolic syndrome, however, dietary protocols included multiple dietary alterations besides fat reduction with purposeful weight loss (Azadbakht et al., 2005; Marfella et al., 2004). These additional alterations may also influence and alter metabolic syndrome. Exercise interventions have shown success for reversal of metabolic syndrome (Johnson et al., 2007; Katzmarzyk et al., 2003; Milani & Lavie, 2003; Shubair et al., 2004), however, other studies did not show significant differences in metabolic syndrome prevalence when compared to a control group (Anderssen et al., 2007;Stewart et al., 2005). Combined low-fat diet plus exercise interventions which either controlled for weight loss (Esposito et al., 2004) or allowed ad-libitum intake (Roberts et al., 2006), found significant reductions in the prevalence of metabolic syndrome. However, none of these combined diet plus exercise studies incorporated a randomized controlled design.

Specific Aim #2: To determine the effects of a low-fat diet and/or exercise intervention on metabolic syndrome prevalence in men and women at high cardiovascular risk.

Hypothesis 2: The low-fat diet and diet plus exercise groups will lower metabolic syndrome prevalence compared to the control group. The exercise group will not result in a difference in metabolic syndrome prevalence compared to the control group.

C-Reactive Protein and Metabolic Syndrome

The process of atherosclerosis leading to cardiovascular disease has been hypothesized to be controlled through inflammation (Bhagat & Vallance, 1997; Libby, 2002; Ross, 1999). Individuals with metabolic syndrome also possess elevated levels of CRP (Ford, 2003; Pitsavos, Panagiotakos, Chrysohoou, Kavouras, & Stefanadis, 2005; Saltevo, Vanhala, Kautiainen, Kumpusalo, & Laakso, 2007). CRP and metabolic syndrome are each independent predictors of cardiovascular events (Rutter, Meigs, Sullivan, D'Agostino, Sr., & Wilson, 2004; Sattar et al., 2003) and the combination of both adds predictive and prognostic value to cardiovascular disease and type II diabetes risk (Ridker, Buring, Cook, & Rifai, 2003b; Sattar et al., 2003).

Few studies have compared the independent and combined effects of low-fat diet and exercise in individuals with metabolic syndrome. Many lifestyle interventions examine the effect of diet and exercise on individuals with hypertension, obesity or dyslipidemia, however these results may not be generalizable to individuals with metabolic syndrome who have multiple lipid and metabolic disorders. Thus, determining the most effective lifestyle treatment to lower CRP levels in individuals with metabolic syndrome without weight loss remains understudied. Combined diet plus exercise studies

show significant reductions in CRP in individuals with metabolic syndrome (Bo et al., 2007; Esposito et al., 2004; Roberts et al., 2006), even without purposeful weight loss (Bo et al., 2007; Roberts et al., 2006). However, these studies are either in a single gender (Roberts et al., 2006) or combine gender in the statistical analyses (Bo et al., 2007; Esposito et al., 2004). As previously mentioned, women have higher levels of CRP than their age-matched male counterparts (Ford et al., 2003; Ford et al., 2004b), and higher CRP values at baseline can result in larger decreases in CRP with both low-fat diet (Seshadri et al., 2004) and exercise (Goldhammer et al., 2005; Lakka et al., 2005).

Specific Aim #3: To explore the changes in CRP from low-fat diet and/or exercise in men and women with and without metabolic syndrome.

Hypothesis 3: Individuals with metabolic syndrome will show significant changes in CRP from baseline from low-fat diet, exercise and diet plus exercise.

Dissertation Organization

This dissertation is organized in seven chapters. In the next chapter, a literature review is presented for CRP and metabolic syndrome which focus on low-fat diet and exercise interventions. In Chapter 3, methodology of the original DEER study is presented. Chapter 4 presents the analysis for CRP in response to the independent and combined effects of low-fat diet and exercise. Chapter 5 describes metabolic syndrome changes with low-fat diet, exercise and diet plus exercise interventions. Chapter 6 explores the changes from low-fat diet and/or exercise for CRP in individuals with and without metabolic syndrome. Chapter 7 summarizes the three studies and gives recommendations for future research.

Chapter 2

Literature Review

Part I: C-Reactive Protein

Acute Inflammation and C-Reactive Protein

The immune system's response to an infection or injury involves a cascade of events to provide a natural defense. During this infection or tissue injury, various substances are released from the injured tissues locally and systemically around the body, which regulate the amplitude and duration of the immune response (van der Meide & Schellekens, 1996). The systemic reaction is termed the "acute phase response" which results in physiological, nutritional, biochemical and behavioral adaptations (Gabay & Kushner, 1999). During the acute phase response, specific circulating inflammatory markers, cytokines, increase at a rapid rate to more than 1000 fold (Ballou & Kushner, 1992; Gabay et al., 1999).

The first cytokine to be released during the acute phase response from infection is tumor necrosis factor (TNF-α), a pro-inflammatory cytokine. Elevated levels of circulating TNF-α signals an increase in the release of interleukin–6 (IL-6), which can act as either a pro- or anti-inflammatory cytokine (Petersen & Pedersen, 2005). IL-6 and TNF-α are tightly regulated together in a negative feedback loop. While TNF-α induces the release of IL-6, IL-6 can then inhibit the production of TNF-α. IL-6 is also the major regulator of C-reactive protein (CRP) release into the bloodstream.

CRP is an acute phase protein which has been defined as a chemical whose concentration increases or decreases by at least 25% during an inflammatory disorder

(Morley & Kushner, 1982). Thus, plasma CRP reflects the ongoing TNF-α production and acts as a "downstream" marker of inflammation. In humans, CRP can rapidly increase within 3-4 hours of exposure to bacteria or injury, peak within 24-72 hours and then rapidly fall to basal levels (Gewurz, Mold, Siegel, & Fiedel, 1982; Kushner & Feldmann, 1978). CRP values from 10-40 mg/L indicate mild inflammation while values 40-200 mg/L indicate acute inflammation and/or bacterial infection (Myers et al., 2004).

Chronic Inflammation and CRP

Regardless of the presence of an acute infection, humans have CRP present in the bloodstream. Chronic low-grade systemic inflammation refers to a condition in which there is a two to three fold increase in systemic concentrations of cytokines (Petersen et al., 2005).

Research has linked elevated low-grade basal inflammation (CRP) to development of metabolic syndrome (Das, 2002; Ridker et al., 2003b), progression of type II diabetes (Pradhan, Cook, Buring, Manson, & Ridker, 2003), increased atherosclerosis (Libby, 2002; Ross, 1999), increased coronary risk (Ridker & Morrow, 2003), increased cardiovascular disease events (Ridker, 2003b; Ridker, 2003a; Ridker et al., 2003a) and mortality (Harris et al., 1999). CRP may be a better predictor of first time cardiovascular events than traditional risk factors, such as LDL cholesterol (Ridker, Rifai, Rose, Buring, & Cook, 2002). Most Americans have CRP plasma levels under 3 mg/L which was historically regarded as clinically insignificant (Black, Kushner, & Samols, 2004). However, recently, the Centers for Disease Control (CDC) and American Heart Association (AHA) jointly re-categorized CRP as < 1.0 mg/L as low risk, average risk

1.0-3.0 mg/L; and high risk > 3.0 mg/L (Myers et al., 2004). It is estimated that approximately 25% of American adults have CRP levels classified as "high risk" (Pearson et al., 2003).

Quantification and Measurement for C-Reactive Protein

CRP is quantified with a standard fasting blood sample. The immunoturbidimetric method creates an antigen-antibody reaction which occurs between CRP in the sample and an anti-CRP antibody that has been sensitized to latex particles, and agglutination results. This antigen-antibody complex causes an increase in light scattering, which is detected spectrophotometrically, with the magnitude of the change being proportional to the concentration of CRP in the sample. Five-point calibration curves are standardized against the reference material CRM470. Immunoturbidimetric methods read the light scattered by antibody complexes. Since the photomultiplier tube is directly across from the light source, there is a decrease in the transmitted light due to scattering, reflectance and absorption. Values of density and absorption are read at 570 nm with a background subtract of 800 nm. This assay has a sensitivity of 0.03 mg/L. The day-to-day variability of the assay at concentrations of 0.91, 3.07 and 13.38 mg/L are 2.81, 1.61 and 1.1%, respectively.

This dissertation project utilizes a secondary data analysis which has blood plasma samples that have been frozen for approximately 15 years at -80°C without any freeze-thaw cycles. CRP is a stable protein with low temporal and diurnal variation and a long half life (Meier-Ewert et al., 2001; Ockene et al., 2001). CRP measurement and quantification is stable for seven freeze-thaw cycles (Aziz et al., 2003), even after 20 years of storage at -70 °C (Lewis, Callas, Jenny, & Tracy, 2001). Inflammatory cytokine

quantification may be limited for this secondary data analysis utilizing long-term stored blood samples due to short half-lives (May et al., 1992). Due to its stability over time, CRP may be a better clinical marker of inflammation when compared to other inflammatory cytokines such as TNF- α and IL-6 (Myers et al., 2004; Roberts, 2004). Thus, for the purposes of this dissertation project, CRP will be the major focus as a marker of inflammation.

Individual Characteristics and Lifestyle Influences on Levels of C-Reactive Protein

Quantification of CRP has allowed research to link elevated low-grade systemic chronic inflammation with various lifestyle factors. CRP has a positive linear relationship with aging (Rumley et al., 2006). Women tend to have approximately 35% higher levels of CRP (mean 2.5 mg/L) than men (mean 1.6 mg/L), even after controlling for oral contraception use (Ford et al., 2003; Ford et al., 2004b). Hormone replacement therapy (HRT) has shown 45-60% increases in CRP levels in some studies (Albert, Glynn, Buring, & Ridker, 2004; Cushman et al., 1999), while other studies show no significant differences in CRP levels with those on HRT (Smith, Dykes, Douglas, Krishnaswamy, & Berk, 1999; Hodis et al., 2008).

CRP has positive linear relationships with weight status (Ford, 1999) and body fat percentage (Lemieux et al., 2001). One of the hypothesized theories for the increased cytokine production of TNF-α and IL-6, which is present in low grade inflammation is from adipose tissue (Pedersen, 2005). The release of CRP is controlled by these inflammatory cytokines, which can be located and released from the adipose tissue (Petersen et al., 2005). Therefore, more body fat a person has, the higher the release of inflammatory cytokines and ultimately, the higher the release of CRP (Visser et al.,

1999). Approximately 30% of IL-6 is released into circulation at rest, directly from adipose tissue (Mohamed-Ali et al., 1997). Thus, an individual's body composition directly relates to the circulating magnitude of CRP.

CRP levels can be modified through lifestyle changes. Behaviors such as smoking increase levels of CRP (Frohlich et al., 2003), while moderate alcohol consumption has been known to lower levels of CRP (Albert, Glynn, & Ridker, 2003). Other lifestyle behaviors such as weight loss, physical activity, and dietary intake can also influence CRP levels. CRP levels are directly related to weight loss and the change in weight (Due, Toubro, Stender, Skov, & Astrup, 2005; Clifton et al., 2005). For each kilogram of weight loss, the mean change in CRP level is estimated to be -0.13 mg/L (Selvin et al., 2007).

Complex relationships exist between CRP and physical activity, fitness and dietary changes. First, theorized physiological mechanisms are discussed in an attempt to explain how CRP levels are modified through low-fat diet and increased physical activity. Next, evidence is presented from intervention research which evaluates the changes in CRP in response to low-fat diet and/or increased physical activity.

Dietary Mechanism for Reducing CRP Levels

The exact physiological mechanism relating dietary fat intake to CRP levels is poorly understood. Increased intake of dietary fat can induce a pro-inflammatory profile increasing circulating levels of cytokines such as IL-6 and TNF-α (Nappo et al., 2002). Consumption of n-3 polyunsaturated fatty acids, a type of fat common in low-fat foods, can inhibit cytokine release directly from the endothelium in a vascular model of atherosclerosis (De, Liao, & Libby, 2000). The lower levels of cytokines may result in

lower circulating CRP levels. Low-fat foods may simultaneously change macronutrient intake and quality (Jenkins et al., 2005). Individuals who consume low-fat diets also tend to increase intake of fruits, vegetables and whole grains (Jenkins et al., 2005). Increased fruit and vegetable consumption can induce anti-inflammatory effects which may also lower CRP levels (Middleton E Jr, 1998).

Meals that cause a rise in insulin and epinephrine output are associated with higher levels of CRP (Liu et al., 2002). IL-6 release is also hypothesized to be stimulated by the amount of insulin and catecholamine in the bloodstream (McCarty, 2005). Thus, meals that limit the increase in postprandial glucose may also reduce levels of cytokines and CRP in the bloodstream (Clifton, 2003). In fact, consumption of meals which limit the rise in insulin and glucose, such as low glycemic diets, reduce CRP levels (Wolever et al., 2008).

Low-Fat Dietary Interventions and CRP

Epidemiological research has uncovered positive relationships between CRP and total lipid, saturated fat, and cholesterol intake (Bertran et al., 2005; Fredrikson et al., 2004). Low-fat dietary interventions decrease CRP levels as high as 32% (Clifton et al., 2005; Heilbronn et al., 2001; O'Brien et al., 2005; Seshadri et al., 2004; Tchernof et al., 2002) though the change in CRP is not always significant (Cardillo, Seshadri, & Iqbal, 2006; Desroches et al., 2006; Jenkins et al., 2005; Koren et al., 2006).

Inconsistencies with the results of low-fat diets on CRP may be due to small sample sizes (Koren et al., 2006; Jenkins et al., 2005; Tchernof et al., 2002) or interventions in a single gender (Desroches et al., 2006, Heilbronn et al., 2001; O'Brien et al., 2005; Tchernof et al., 2002). Importantly, none of the above mentioned studies had

a control group for comparison. One study which included a control group did not find changes in CRP between the low-fat group relative to the control group, providing evidence for a non-significant effect of low-fat diet on CRP (Erlinger et al., 2003).

Several low-fat diet interventions involved only obese individuals limiting the generalizability to normal and overweight individuals due to their higher overall CRP levels (Cardillo et al., 2006; Heilbronn et al., 2001; O'Brien et al., 2005; Seshadri et al., 2004; Tchernof et al., 2002). In order to account for the differences in CRP by weight status, many studies adjust for weight status in their statistical analysis by using body mass index (BMI). Some research has found the relationship of physical activity and CRP independent of obesity as measured by BMI (Abramson & Vaccarino, 2002; Albert, Glynn, & Ridker, 2004; Geffken et al., 2001; King, Carek, Mainous, III, & Pearson, 2003; Rawson et al., 2003; Reuben, Judd-Hamilton, Harris, & Seeman, 2003; Wannamethee et al., 2002), while other studies which adjust for BMI also attenuated this relationship (Pischon, Hankinson, Hotamisligil, Rifai, & Rimm, 2003; Rawson et al., 2003; Verdaet et al., 2004; Elosua et al., 2005). More accurate assessments for estimating body fat, utilizing percent body fat or visceral fat, found that CRP levels are not independent of adipose tissue (Colbert et al., 2004; Hammett et al., 2004; Manns, Williams, Snow, & Wander, 2003). Thus, the relationship between physical activity and CRP may be dependent on an individual's body fat.

Many dietary protocols include concomitant weight loss making it difficult to assess whether the low-fat diet or weight loss was the stimulus to change CRP (Clifton et al., 2005; Heilbronn et al., 2001; Koren et al., 2006; O'Brien et al., 2005; Seshadri et al., 2004; Tchernof et al., 2002). Weight reductions of 10% or more may induce CRP

changes which eliminates the ability to detect the independent effect of low-fat intake (Heilbronn et al., 2001; Tchernof et al., 2002). When weight loss was unintentional through isocaloric or ad libitum intake, no significant changes in CRP were found (Desroches et al., 2006; Koren et al. 2006; Jenkins et al., 2005).

Therefore, in order to examine the independent effects of low-fat diet on CRP levels, future studies need randomized controlled trials, larger sample size and isocaloric intake. Studies also need to account for the individual's baseline body fat to eliminate the known influence on CRP. Possible solutions include either including a more diverse distribution of body fat within the sample, adjusting the analysis by body fat, or stratifying the analysis by categories of weight status.

Physical Activity Mechanisms for Reducing CRP

Physical activity may modify inflammation through the IL-6 pathway. IL-6 is released during a bout of exercise from various tissues including adipose tissue, skeletal muscle, mononuclear cells, and brain (Pedersen, 2006; Shephard, 2002). Acutely, the increase in IL-6 increases CRP levels approximately 8-12 hours after the exercise bout (Petersen et al., 2005) and can remain elevated for 16 hours after exhaustive exercise (Castell et al., 1997).

Despite the increase in IL-6 and CRP after an acute bout of exercise, IL-6 also acts to stimulate the release of anti-inflammatory agents (Pedersen, 2006; Shephard, 2002). Increase in IL-6 levels during the acute phase initiates also initiates a cascade response involving the secretion of anti-inflammatory markers such as interleukin-10 (IL-10) and interleukin-1 receptor antagonist (IL-1ra) which downregulate the release of TNF-α (Pedersen, 2006; Petersen et al., 2005). The decrease in TNF-α may also inhibit

the release of IL-6, and ultimately, the release of CRP from the liver. This negative feedback loop suggests that although the acute effect increases CRP, the chronic influence of exercise may decrease CRP. It is hypothesized that the mechanism behind the decrease in inflammation with chronic activity is due to one or more of the following reasons 1) reduced adiposity, 2) reduced macrophage accumulation in adipose tissue, 3) increase in parasympathetic system due to training and/or 4) improved acute stress sensitivity from repeated exercise bouts and increased anti-oxidant activity (Woods, Vieira, & Keylock, 2006).

Physical Activity Interventions and CRP

Epidemiological research has established an association of lower CRP levels in physically active men and women (Ford, 2002; Koenig et al., 1999; Panagiotakos, Pitsavos, Chrysohoou, Kavouras, & Stefanadis, 2005; Pitsavos et al., 2003), elevated cardiovascular risk adults (Pitsavos, Panagiotakos, Chrysohoou, Kavouras, & Stefanadis, 2005), and older adults (Colbert et al., 2004; Geffken et al., 2001; Reuben et al., 2003; Taaffe, Harris, Ferrucci, Rowe, & Seeman, 2000). Individuals with high cardiorespiratory fitness had 57-80% lower CRP values than their low fit counterparts in both men (Church et al., 2002; Kuo, Yen, Chen, Yu, & Bean, 2007) and women (Kuo et al., 2007; LaMonte, Ainsworth, & Durstine, 2005).

While the association between physical activity/fitness and CRP in cross sectional studies is established, the effect of increasing physical activity levels and its effects on CRP levels has been variable. Physical activity interventions significantly decreased CRP levels (Goldhammer et al., 2005; Kohut et al., 2006; Mattusch et al., 2000; Milani et al., 2004; Oberbach et al., 2006; Obisesan et al., 2004; Okita et al., 2004; Smith et al.,

1999; Tisi, Hulse, Chulakadabba, Gosling, & Shearman, 1997). The range for reductions for CRP through increasing physical activity and/or fitness are estimated to be between 19-77% (Plaisance & Grandjean, 2006). However, CRP differences with increasing physical activity have not always been statistically significant (Hammett et al., 2004; Huffman et al., 2006; Marcell, McAuley, Traustadottir, & Reaven, 2005; Rauramaa et al., 2004; Nicklas et al., 2004).

Many of these studies involved individuals with existing chronic diseases such as insulin resistance, type II diabetes or cardiovascular disease where the CRP baseline levels tend to be elevated (Goldhammer et al., 2005; Kohut et al., 2006; Milani et al., 2004; Oberbach et al., 2006; Tisi et al., 1997). Previous research has shown that only individuals with initially high CRP levels are able to significantly reduce CRP with increased physical activity/ fitness (Goldhammer et al., 2005; Lakka et al., 2005). This may indicate that individuals with existing chronic disease, who have elevated CRP levels, may decrease their CRP levels to a greater extent than those who are healthy.

Inconsistent effects for CRP from physical activity are found across gender. Some studies show consistent effects between genders for the changes in CRP (Hammett et al., 2004; Huffman et al., 2006; Lakka et al., 2005), while others show significantly greater change among men (Nicklas et al 2004). Studies may include both men and women in their sample, and adjust their statistical analysis for gender (Goldhammer et al., 2005; Kohut et al., 2006; Marcell et al., 2005; Milani et al., 2004; Oberbach et al., 2006; Obisesan et al., 2004). However, since men and women have different baseline levels of CRP, they also may have a different response to exercise training. Thus independent

statistical analyses by gender may reveal distinct responses in the CRP to increased physical activity.

As previously mentioned, general weight loss can directly decrease CRP levels. However, weight loss specific from physical activity has shown inconsistent results between the relationship between physical activity and CRP. Changes in CRP were independent from physical activity induced weight loss in one study (You et al., 2004), whereas another study attributed the weight loss as the major contributor to CRP change over the change in physical activity (Haffner et al., 2005).

Most importantly, a number of interventions did not have control groups, limiting the inferences that can be made concerning the effect of the physical activity intervention on CRP levels (Lakka et al., 2005; Okita et al., 2004). Interventions with a control group show inconsistent CRP results, where some studies show significant reductions (Mattusch et al., 2000; Milani et al., 2004), while others have had no effect on CRP (Hammett et al., 2004; Huffman et al., 2006). Varying results for CRP in controlled trials may be due to an alternative CRP measurement technique (Mattusch et al., 2000), non-randomized groups (Milani et al., 2004) or high variability in the CRP values (Hammett et al., 2004).

In summary, to provide convincing evidence for the effects of physical activity interventions on CRP levels in both men and women, several issues need to be addressed: inclusion of randomized controlled design, controlling for weight loss in the statistical analysis and stratification of gender.

Combined Lifestyle Interventions on CRP

The physiological mechanisms theorized to be responsible for reductions in CRP from low-fat diet are distinct from mechanisms for increasing physical activity. Although not confirmed in research, it can be hypothesized that the combination of diet plus exercise may have an additive effect on lowering CRP levels, though other multidisciplinary interventions show sub-additive effects on traditional cardiovascular risk factors (Appel et al., 2003).

Multidisciplinary interventions which combine exercise and low-fat diet had positive reductions of CRP levels in both men and women (Dvorakova-Lorenzova et al., 2006; Esposito et al., 2003; Haffner et al., 2005; Roberts et al., 2006; Wegge et al., 2004). However, the effect of combined lifestyle intervention on lowering CRP has not shown consistency between genders, where one study shows similar reductions in CRP (Haffner et al., 2005) and another shows a greater reduction in men (Nicklas et al., 2004). Furthermore, most combined diet and exercise interventions were limited to a single gender (Dvorakova-Lorenzova et al., 2006; Esposito et al., 2006; Roberts et al., 2006; You, Berman, Ryan, & Nicklas, 2004) and obese only participants (Esposito et al., 2003; Roberts et al., 2006; You et al., 2004) limiting the generalizability of the results.

Combined dietary and exercise interventions without restricted caloric intake or intentional weight loss significantly reduced CRP levels between 30-45% (Bo et al., 2007; Roberts et al., 2006; Wegge et al., 2004). However, several of these interventions were in a single gender (Roberts et al., 2006;Wegge et al., 2004), did not adjust for gender (Bo et al., 2007) or did not include a control group for comparison (Roberts et al., 2006;Wegge et al., 2004). Several controlled trials also included hypocaloric intake to

induce weight loss which may have lowered levels of CRP beyond the contributions of low-fat diet and exercise alone (Haffner et al., 2005; Nicklas et al., 2004). Weight loss, when combined with exercise showed greater reductions in CRP than exercise alone, making it difficult to distinguish whether it was weight loss, diet or exercise which caused the changes in CRP (Nicklas et al., 2004). Reductions in CRP from the diet plus exercise group were independent of the change in body composition (You et al., 2004; Milani et al., 2004), however more controlled studies are needed which account for the weight loss within the analysis.

Comparisons Between Low-Fat Diet, Exercise and Diet plus Exercise for CRP

A major limitation in the current diet plus exercise intervention research include the lack of controlled randomized design to compare CRP changes between control, diet, exercise and diet plus exercise. Many studies examined the combination of diet plus exercise, making it difficult to discern the independent contributions of each (Bo et al., 2007; Dvorakova-Lorenzova et al., 2006; Esposito et al., 2003; Haffner et al., 2005; Roberts et al., 2006). Diet plus exercise has shown larger changes in CRP than diet alone, although none of these studies included a control group (You et al., 2004; Esposito et al., 2003; Esposito et al., 2004). A randomized controlled trial which compared diet, exercise and diet plus exercise to a control group, found significant treatment effects in the diet only and diet plus exercise groups (Nicklas et al., 2004). However, Nicklas et al., also incorporated resistance training, which can activates IL-6 to be released from the muscle tissues altering both the inflammatory and anti-inflammatory response (Petersen et al., 2005). In addition, all of randomized controlled trials had concurrent weight loss which may have inflated the changes in CRP, masking the independent effects from

exercise or low-fat diet. Therefore, systematic evaluation for the independent and combined effects of low-fat diet and increased physical activity on CRP levels needs to be examined.

In summary, the current research for the effects of increased physical activity and diet on CRP levels has not methodically determined the independent and combined contributions of each component in men and women, without the contribution of concurrent weight loss.

Part II: Metabolic Syndrome

Metabolic syndrome is a constellation of elevated metabolic, lipid and non-lipid variables that tend to cluster in an individual. The National Cholesterol Education Program Adult Treatment Panel III (NCEP ATP III) developed a definition to identify metabolic syndrome in which an individual must have three of the following: 1) waist circumference over 102 cm men; 88 cm women, 2) triglycerides over 150 mg/dL, 3) high density lipoprotein cholesterol (HDL) under 40 mg/dL for men; 50 mg/dL for women, 4) blood pressure over 130/85 mmHg and 5) fasting glucose over 100 mg/dL (2001; Grundy, Brewer, Jr., Cleeman, Smith, Jr., & Lenfant, 2004a).

The exact mechanisms responsible for the pathogenesis of metabolic syndrome remain unknown. Early perspectives for the manifestation of metabolic syndrome involved insulin resistance (Reaven, 1988) and obesity (Grundy, 2004). Recent theories attempting to explain the etiology of metabolic syndrome include chronic systemic inflammation, endothelial dysfunction, sympatho-adrenal irregularities, gene environment interactions, and lifestyle (Fowler, Moussouttas, & Mancini, 2005).

Despite the unknown mechanisms, metabolic syndrome is intricately linked with the development of cardiovascular disease. Individuals with metabolic syndrome have more advanced signs of atherosclerosis including plaque build-up in the lumen, greater intima-media thickness and increased stenosis of the arterial wall (Bonora et al., 2003; McNeill et al., 2004; Wallenfeldt, Hulthe, & Fagerberg, 2005). Adults with metabolic syndrome also show greater progression of atherosclerosis over time (Bonora et al., 2003; Wallenfeldt et al., 2005). Metabolic syndrome is associated with an increase in cardiovascular disease events and mortality in both men and women (Ford, 2004; Lakka et al., 2002; Scuteri, Najjar, Morrell, & Lakatta, 2005). Metabolic syndrome may be able to predict cardiovascular disease events, mortality and morbidity better than any of its individual risk factors (Isomaa et al., 2001; Malik et al., 2004). Thus, understanding the correlates of metabolic syndrome and finding appropriate treatments is paramount in order to curb the progression and development of cardiovascular disease.

The prevalence of metabolic syndrome has increased over the past ten years. The National Health and Nutrition Examination 1988-1994 Survey found prevalence was approximately 23% (Ford, Giles, & Dietz, 2002), which increased significantly in both men and women to 27% in 1999 (Ford, Giles, & Mokdad, 2004), and 35% in 2002 (Ford, 2005).

Prevalence of metabolic syndrome increases with aging, where it is six times more prevalent among elderly than in young adults (Ford, Giles, & Dietz, 2002). Metabolic syndrome prevalence is equal in men and women (Ford et al., 2003; Park et al., 2003), however, prevalence increases during menopause (Carr, 2003). Postmenopausal women on hormone replacement therapy (HRT) show reduced levels of

the individual components of metabolic syndrome (Carr, 2003; Dallongeville et al., 1995; Salpeter et al., 2006). Finally, weight status is a major contributor to metabolic syndrome status. As weight status increases from normal weight to obesity, prevalence of metabolic syndrome increases from 5% to 57% (Park et al., 2003).

For management of the metabolic syndrome, the NCEP recommends the incorporation of positive lifestyle changes including the reduction in saturated fat and cholesterol, increasing physical activity and weight loss (2001). Adults with metabolic syndrome have higher total dietary fat intake (Finley et al., 2006) and low-fat diet can reduce the individual components of metabolic syndrome (Yu-Poth et al., 1999). However, few studies have explored the influence of low-fat diet on the presence of metabolic syndrome, the condition in which an individual has clustering of the individual cardiovascular risk factors. Men and women who are physically active are less likely to have or develop metabolic syndrome (Laaksonen et al., 2002; Irwin et al., 2002). Thus, lower intake of dietary fat and higher levels of physical activity are associated with a lower prevalence of metabolic syndrome. However, the influence of increasing physical activity and/or incorporating a low-fat diet on metabolic syndrome status is more complex. The following literature review for metabolic syndrome briefly summarizes physiological mechanisms for the reduction in metabolic syndrome from low-fat and physical activity. Then, evidence for reversal of metabolic syndrome from low-fat diet and physical activity is presented.

Low-Fat Dietary Mechanisms for Reversing Metabolic Syndrome

High fat diets can induce metabolic syndrome in animals (Barnard, Faria, Menges, & Martin, 1993), but can be reversed through incorporation of a low-fat diet

(Roberts, Vaziri, Liang, & Barnard, 2001). Although the exact physiological mechanism to explain how fat reduction reverses the metabolic syndrome is unknown, it is hypothesized to be through weight loss and/or concurrent changes in dietary macronutrient quality intake (Feldeisen & Tucker, 2007).

Low-fat diets can result in weight loss, even without intentionally lowering overall caloric intake (Astrup, Grunwald, Melanson, Saris, & Hill, 2000). Weight loss can improve the overall cardiovascular risk profile (Yu-Poth et al., 1999) which may result in reversal of metabolic syndrome.

Carbohydrate sources within low-fat diets may also be fiber-rich, which limits the increased postprandial glucose and insulin levels (Barnard, Scialli, Turner-McGrievy, Lanou, & Glass, 2005). Since carbohydrate and lipid metabolism are intricately linked, decreasing the hyperinsulinemic response from a meal could lower plasma levels of glucose and lipoproteins. Thus, a low-fat diet could ultimately decrease the presence of metabolic syndrome. Evidence from a meta-analysis of low-fat diets promoting complex carbohydrate intake shows improvements for the cardiovascular risk factor profile (Yu-Poth et al., 1999), possibly resulting in reversal of metabolic syndrome.

Low-Fat Diet Interventions and Metabolic Syndrome

The NCEP Step I and Step II diets were created to lower an individual's risk of having cardiovascular related events and reduce the presence of abnormal cardiovascular risk factors (1993). The major components of the NCEP diet are reduction of dietary fat intake, whereby the Step II diet has more stringent restrictions. Table 2.1 outlines the program details.

Table 2.1. National Cholesterol Education (NCEP) Program Dietary Guidelines (1993).

DIET PROGRAM	SPECIFIC GUIDELINES
NCEP Step I	• Reduction of saturated fat < 10% • Reduction of total cholesterol < 300 mg/day
NCEP Step II	• Reduction of total fat < 30% of total caloric intake • Reduction of saturated fat < 7% of total caloric intake • Reduction of dietary cholesterol < 200 mg/day • Reduction of total caloric intake for weight loss if applicable

In comparison to the NCEP Step I diet, the NCEP Step II diet demonstrates greater reductions in triglycerides, low density lipoprotein (LDL), and the ratio between total cholesterol and high density lipoprotein (HDL) (Yu-Poth et al., 1999).

A reduced fat diet has shown resolution of the metabolic syndrome (Muzio et al., 2005), though this intervention did not include a control group. Low-fat dietary patterns, such as the DASH diet (low-fat dairy, increased fruits and vegetables, reduced sodium) have also shown success for reducing metabolic syndrome in comparison to a control condition (Azadbakht, Mirmiran, Esmaillzadeh, Azizi, & Azizi, 2005). However, since the DASH diet has several dietary changes beyond incorporating low-fat foods, it is difficult to discern whether it is a single entity or the combination of a few components that make this diet successful in reducing presence of metabolic syndrome.

A major challenge in the existing literature for metabolic syndrome and low-fat diet, is that many dietary studies involve concomitant weight loss (Muzio et al., 2005; Azadbakht et al., 2005). Weight loss alone can independently improve many of the components of metabolic syndrome (Yu-Poth et al., 1999). Due to the influential impact of weight loss on several metabolic and cardiac risk factors, it is difficult to discern the contributing effects of weight loss and the individual impact of the low-fat diet.

Clearly, randomized controlled trials are needed to evaluate the effects of low-fat diet on metabolic syndrome, independent from weight loss.

Physical Activity Mechanisms to Reverse Metabolic Syndrome

It is well established that physical activity has two major effects for prevention of cardiovascular disease: 1) it can effect cardiovascular disease through reductions in morbidity and mortality (Paffenbarger, Jr., Hyde, Hsieh, & Wing, 1986; Paffenbarger, Jr., Hyde, Wing, & Hsieh, 1986; Paffenbarger, Jr. et al., 1993) and 2) it can positively effect the biological cardiovascular risk factors associated with the disease (Grundy et al., 2004b). However, the exact mechanisms by which increases in physical activity result in the reduction of metabolic syndrome remain largely unexplored. It is hypothesized that the reduction in metabolic syndrome from physical activity may be due to favorable changes in each of the individual components: triglycerides, HDL cholesterol, blood pressure, glucose and abdominal obesity, which in combination, result in decreased metabolic syndrome.

Theories to explain the decrease in plasma triglyceride concentrations with exercise training may be through alterations in lipid metabolism. Triglycerides are broken down into free fatty acids that can be used for energy through fatty acid oxidation. Exercise training enhances free fatty acid oxidation and gluconeogenesis during rest and exercise, which can lower the presence of triglycerides in the blood (Martin, III, 1996; Bergman et al., 2000). HDL cholesterol levels increase with overall energy expenditure possibly through an intricate relationship with lipid metabolism. Increases in lipoprotein lipase activity also increase HDL formation while simultaneously reduce intramuscular triglycerides stores and decreases plasma triglycerides (Thompson et al., 2001). Changes in blood pressure from increased physical activity are theorized to be from the adaptations in the sympathetic nervous system resulting in decreases in catecholamines,

total peripheral resistance, as well as alterations in the release of vasodilators and vasoconstrictors (Pescatello et al., 2004). Improvements in glucose function from chronic exercise stem from increased GLUT4 transporters, capillary density, and muscle fiber conversion to type IIa and enzymes for fatty acid oxidation and gluconeogenesis (Houmard et al., 1991; Ivy, 1997). These alterations increase glucose uptake into the muscle tissue which improves insulin resistance and utilization of glucose for energy. There is also evidence to suggest that increased physical activity decreases abdominal obesity even in the absence of weight loss (Kay & Fiatarone Singh, 2006). Reduced visceral fat is associated with improved insulin and glucose homeostasis (Ross, Freeman, Hudson, & Janssen, 2002). Increasing energy expenditure through increased physical activity can also result in weight loss which can indirectly but favorably change the individual cardiovascular risk factors (Dattilo & Kris-Etherton, 1992; Leenen et al., 1993; Chobanian et al., 2003; Yu-Poth et al., 1999).

In summary, increased physical activity can improve each of the individual components of metabolic syndrome, which may result in a reversal of metabolic syndrome status.

Physical Activity Interventions and Metabolic Syndrome

For every 3.3 individuals with metabolic syndrome who are treated with exercise, one participant successfully reverses the presence of metabolic syndrome (Katzmarzyk et al., 2003). Physical activity interventions show reductions in magnitude between 15-37% for metabolic syndrome (Johnson et al., 2007; Katzmarzyk et al., 2003; Milani & Lavie, 2003; Shubair, Kodis, McKelvie, Arthur, & Sharma, 2004) although this difference in

metabolic syndrome prevalence has not always been significant (Anderssen, Carroll, Urdal, & Holme, 2007; Stewart et al., 2005).

Physical activity has reduced the prevalence of metabolic syndrome in studies which combine men and women (Katzmarzyk et al., 2003; Shubair et al., 2004). One exercise intervention did not find differences between genders for the response of metabolic syndrome to exercise (Johnson et al., 2007). However, results from cross sectional studies which explore the relationships between physical activity and gender are not consistent. In one study, when men and women are separated for the statistical analysis, an inverse association between physical activity and metabolic syndrome was only significant in men (Zhu, St-Onge, Heshka, & Heymsfield, 2004; Liu et al., 2006), whereas another studies found it only significant in women (Pitsavos et al., 2005). Only one study separated genders for statistical analysis, and found similar reductions in metabolic syndrome for both men and women (Shubair et al., 2004). Thus, it appears the relationship between physical activity and metabolic syndrome among genders may need further study.

Other explanations for the inconsistent results may be due to weight loss. Longitudinal studies indicate that after adjusting for changes in weight over time, the relationship between physical activity and metabolic syndrome is no longer significant (Carnethon et al., 2004). This suggests that the changes in metabolic syndrome from physical activity may be explained by changes in body weight. Meta-analyses suggests that long term physical activity, even in the absence of weight loss can improve cardiovascular risk factors, however, weight loss may provide further benefits (Carroll &

Dudfield, 2004; Yu-Poth et al., 1999). Thus weight loss may contribute to decreased metabolic syndrome in addition to the increased physical activity and fitness.

Many physical activity interventions do not include a control group for comparison (Katzmarzyk et al., 2003; Stewart et al., 2005). When an exercise group was directly compared to a control group post intervention, results were inconsistent, whereby decreases in metabolic syndrome prevalence were found in one study (Johnson et al., 2007) and no differences in metabolic syndrome were found in another (Anderssen et al., 2007).

In summary, future studies for physical activity and their effects on metabolic syndrome should include more randomized controlled trials, analyze men and women separately, and control for change in weight in the statistical analysis to eliminate the effects of weight loss.

Combined Lifestyle Interventions Effects on Metabolic Syndrome

The combination of diet and exercise has improved several of the individual cardiovascular risk factors (Dengel, Hagberg, Pratley, Rogus, & Goldberg, 1998). Diet plus exercise has also been successful in reducing the prevalence of metabolic syndrome (Bo et al., 2007; Esposito et al., 2004; Muzio et al., 2005; Orchard et al., 2005; Roberts et al., 2006).

Diet plus exercise interventions involving the low-fat Mediterranean diet has reduced the presence of metabolic syndrome (Esposito et al., 2003; Esposito et al., 2004) though none of these interventions were randomized controlled trials.

Many of the combined lifestyle interventions involved concomitant weight loss in addition to changing diet and physical activity habits (Bo et al., 2007; Esposito et al.,

2004; Muzio et al., 2005; Orchard et al., 2005; Roberts et al., 2006). The magnitude of the reduction in the individual cardiovascular risk factors appears to be dependent on the amount of weight loss (Katzel, Bleecker, Rogus, & Goldberg, 1997; Watkins et al., 2003; Dansinger, Gleason, Griffith, Selker, & Schaefer, 2005; Muzio, Mondazzi, Sommariva, & Branchi, 2005).

Diet plus exercise interventions which examined metabolic syndrome as the outcome and either controlled for weight loss or allowed ad-libitum intake, still found significant reductions in metabolic syndrome status (Roberts et al., 2006; Esposito et al., 2004). However, none of the protocols which did not induce purposeful weight loss were randomized controlled trials.

Comparisons Between Low-Fat Diet, Exercise and Diet plus Exercise on Metabolic Syndrome

Understanding the independent and combined effects for diet and exercise is not able to be determined from previous research. The combination of diet and exercise improves the individual cardiovascular risk factors for metabolic syndrome better than diet alone (Yu-Poth et al., 1999) though this result has not been consistent (Stefanick et al., 1998). Not many intervention studies have systematically studied comparisons between the diet and exercise and control conditions for reductions in metabolic syndrome. Diet plus exercise had more success for reversing metabolic syndrome than diet or exercise alone (Anderssen et al., 2007; Okura et al., 2007). Anderssen et al., found that the combination of diet plus exercise reduced metabolic syndrome status 67% and also proved to be a better treatment than either diet or exercise alone, however, this study included purposeful weight loss. Okura and colleagues found a 3.68 odds of decreasing metabolic syndrome with diet plus exercise over diet alone, though there was

no control group for comparison (Okura et al., 2007). Many other existing diet plus exercise interventions also lacked a true control group for comparison (Roberts et al., 2006; Muzio et al., 2005; Esposito et al., 2004; Okura et al., 2007).

In conclusion, to examine the independent and combined contributions of diet plus exercise in men and women without the influence of the change in weight, randomized control designs need to statistically control for weight changes in the analysis.

Part III: C-Reactive Protein and Metabolic Syndrome

Individuals with the presence of metabolic syndrome also possess elevated levels of CRP (Ford, 2003; Pitsavos et al., 2005; Saltevo et al., 2007). For every 1 pg/mL of CRP, there is a 37% increased risk of having metabolic syndrome (Hassinen et al., 2006). As risk factors for metabolic syndrome increase, CRP has been shown to add predictive and prognostic value to the risk of cardiovascular events, cardiovascular disease and type II diabetes (Ridker et al., 2003b; Sattar et al., 2003). CRP and metabolic syndrome are each independent predictors of cardiovascular events (Rutter et al., 2004; Sattar et al., 2003). Chronic inflammation has been suggested to be an actual feature of metabolic syndrome (Festa et al., 2000), and some recommend the addition of CRP to the clinical definition for identifying metabolic syndrome (Haffner, 2006).

Physiological Connection Between Inflammation and Metabolic Syndrome

Despite evidence for the deleterious connection between CRP and metabolic syndrome, it is not known whether inflammation is the cause or effect of metabolic syndrome (Fernandez-Real & Ricart, 2003). The etiology and pathogenesis of metabolic

syndrome has two theories which intimately link with the process of inflammation: 1) insulin resistance and 2) obesity.

The release of TNF-α, one regulator of CRP release, can cause insulin resistance through the following pathways: inhibition of the GLUT4 transporter (Petersen et al., 2005), and reduction in tyrosine, but not serine, phosphorylation of the insulin receptor (Goodyear & Kahn, 1998). Ultimately , this results in a decrease in insulin released by the pancreas and an increase in glucose in the bloodstream (Petersen et al., 2005). This increase in circulating cytokines can also decrease insulin's natural anti-inflammatory properties (Dandona, Aljada, & Bandyopadhyay, 2004).

The other physiological relationship between the inflammation and metabolic syndrome is through the amount of adipose tissue (Esposito & Giugliano, 2004). The primary site for production and release of cytokines, such as TNF- α and IL-6, is directly from the adipose tissue (Petersen et al., 2005). Since TNF- α and IL-6 control CRP release from the liver, an increase in adipose mass would increase CRP levels. A positive relationship exists between higher BMI, fat mass, waist girth and visceral fat with CRP levels in adults (Ford, 1999; Lemieux et al., 2001). Central obesity is considered the major determinant of CRP levels in those with the metabolic syndrome (Santos, Lopes, Guimaraes, & Barros, 2005). Increased cytokine activity is also linked to abnormal lipid metabolism from excess adipose tissue, resulting in increased circulation of triglycerides and free-fatty acids (Guilherme, Virbasius, Puri, & Czech, 2008). Increases in lipids within the bloodstream could also lead to metabolic syndrome. Thus, obesity, can increase cytokine production, CRP and increase the individual components of the metabolic syndrome.

While it is not known whether obesity and/or insulin resistance is responsible for the pathogenesis of the metabolic syndrome, inflammation appears to be involved with both possible mechanisms.

Lifestyle Effects on CRP Levels in Individuals with Metabolic Syndrome

The influence of an individual's metabolic syndrome status may attenuate or intensify the effects of diet and/or physical activity on CRP levels. Evidence of CRP changes in healthy men and women show positive effects from low-fat diet (Clifton et al., 2005; Heilbronn et al., 2001; O'Brien et al., 2005; Tchernof et al., 2002), exercise (Mattusch et al., 2000; Obisesan et al., 2004; Okita et al., 2004) and diet plus exercise (Dvorakova-Lorenzova et al., 2006; Esposito et al., 2003; Marfella et al., 2004; Nicklas et al., 2004; You et al., 2004). However, only a few studies have examined the effects of lifestyle modification in men and women with metabolic syndrome.

CRP Changes from Low-Fat Diet in Metabolic Syndrome

Fat intake explains some of the variability in CRP levels in persons with dyslipidemia, however, the same relationship does not exist in healthy individuals (Ghayour-Mobarhan, Yaghootkar, Lanham-New, Lamb, & Ferns, 2007). This cross-sectional research suggests a different effect of lowering fat intake in high risk versus healthy individuals.

Previous research has found low-fat diet was not successful in reducing CRP levels in hyperlipidemic subjects (Jenkins et al., 2005). However, Seshadri et al., found significant changes in CRP with low-fat diet only when stratifying individuals by those who had high baseline values for CRP (Seshadri et al., 2004). Neither Jenkins et al., or Seshadri et al., examined the effect of low-fat diet on CRP in individuals with metabolic

syndrome. In addition, neither of these studies were randomized controlled trials. The response of a low-fat diet may be different in individuals with metabolic syndrome due to their multiple lipid and metabolic abnormalities and higher CRP levels.

Future studies need to examine low-fat diet in individuals with the metabolic syndrome using randomized controlled trials.

CRP Changes from Physical Activity in Metabolic Syndrome

Cross sectional research demonstrates that individuals with metabolic syndrome and high cardiorespiratory fitness have lower levels of CRP (Aronson et al., 2004a). No physical activity interventions were identified that examine the changes in CRP in individuals with metabolic syndrome, however, several studies examine adults at increased cardiovascular risk. Exercise training studies found significant reductions in CRP in adults with either normal glucose tolerance, insulin resistance or type II diabetes (Oberbach et al., 2006). Oberbach et al., did observe a greater magnitude of CRP change in individuals with type II diabetes versus those with insulin resistance and normal glucose tolerance, however, neither of these groups were compared to a control group (2006). In contrast, dyslipidemic adults did not show reductions in CRP in an exercise training controlled trial (Huffman et al., 2006). While both of these studies involved elevated cardiovascular risk individuals, the presence metabolic syndrome status was not assessed.

Exercise interventions reveal that only individuals with initially high CRP levels (whether healthy or with established chronic disease) significantly change CRP levels after increasing physical activity and fitness (Goldhammer et al., 2005; Lakka et al., 2005), though neither of these studies were randomized controlled trials which examined

individuals with metabolic syndrome. However, this research does imply individuals with metabolic syndrome may successfully lower CRP levels with exercise training due to higher baseline CRP values.

Future randomized controlled exercise training trials should examine the changes in CRP specifically in individuals with metabolic syndrome.

CRP Changes from Combined Lifestyle Interventions in Metabolic Syndrome

Combined diet plus exercise studies have had significant reductions in CRP in individuals with metabolic syndrome (Bo et al., 2007; Esposito et al., 2004; Roberts et al., 2006). While Bo et al., did examine the CRP changes with a randomized controlled trial, weight loss also occurred which may have further influenced the changes. Another diet plus exercise intervention cites weight loss as the more important contributor to the reduction in CRP than change in physical activity (Haffner et al., 2005). One diet plus exercise study adjusted their statistical analysis for weight loss and significant improvements were still found in CRP adults with metabolic syndrome (Esposito et al., 2004).

Although studies that examined CRP changes without purposeful weight loss were not identified for individuals with metabolic syndrome, interventions in dyslipidemic adults revealed significant CRP reductions in men and women (Roberts et al., 2006; Wegge et al., 2004). However, dyslipidemia is only part of the metabolic syndrome and the clustering of multiple risk factors in metabolic syndrome, may alter CRP in response to diet plus exercise.

Diet plus exercise interventions often combine men and women in the statistical analysis but do not account for possible gender differences (Bo et al., 2007; Esposito et

al., 2004). As CRP levels do differ by gender, these analyses are unable to account for the possible gender interactions with diet plus exercise, and may have influenced the results (Ford et al., 2003; Ford et al., 2004b). Separate gender analysis has been performed in insulin resistant men and women, and revealed similar magnitudes of CRP change (Haffner et al., 2005), however these results are not necessarily generalizable to individuals with metabolic syndrome who have multiple lipid and metabolic disorders.

In conclusion, future studies in individuals with metabolic syndrome need to incorporate weight randomized controlled trials that allow men and women to be analyzed independently for the changes of CRP. Furthermore, systematic study for the independent and combined effects for low-fat diet and increased physical activity in adults with metabolic syndrome are warranted.

Chapter 3

Methods

This dissertation project is a secondary data analysis. This methods section presents a detailed account of the original methodology and protocol for the Diet and Exercise for Elevated Risk Trial (DEER) study, which is the data set used for this work. The new laboratory analysis for CRP and retrospective analysis of metabolic syndrome are detailed in Chapters 4 and 5, respectively.

DEER began in 1992 as a year-long, single-center randomized controlled clinical trial. DEER was performed at Stanford University as a project within the Stanford Medical School's Stanford Center for Research in Disease Prevention. Funding was provided by National Heart, Lung and Blood Institute. The Principal Investigator for the study was Dr. Peter D. Wood, with Co-Principal Investigators Dr. Marcia L. Stefanick and William L. Haskell. The original results of the intervention trial were published in 1998 (Stefanick et al., 1998).

DEER's primary objective was to investigate the effects of diet and exercise interventions for men and postmenopausal women with elevated LDL cholesterol and low HDL cholesterol. The specific study objectives are outlined in Table 3.1.

Table 3.1. DEER Study Objectives.

Primary Objective	Examine the effects of diet, exercise, alone or in combination on the changes in HDL cholesterol
Secondary Objective	Describe the differences of physiological factors between the groups concerning: • Cardiovascular Risk Factors: Total cholesterol, LDL, HDL_2, HDL_3, apolipoproteins A-I, apolipoprotein B • Glucose Metabolism Factors: Fasting and two hour post-load plasma glucose and insulin • Body Composition Variables: BMI, percent body fat and WHR • Cardiovascular Variables: Resting heart rate, systolic and diastolic blood pressure • Aerobic Physical Fitness Variables: Maximal oxygen uptake, time to exhaustion on treadmill • Dietary Intake Components • Physical Activity Levels

Subject Recruitment

Beginning in the year 1992**,** postmenopausal women between the ages of 45-64 years and men between the ages of 30-64 years were recruited from the surrounding area of Stanford University in Palo Alto, California. A variety of mass media methods were utilized to recruit including: worksite contacts, posted flyers, handouts, brochures to area physician offices and clinics, newsletters, mailings to past participants, Stanford Blood Bank, Peninsula Blood Bank and paid advertisements. Table 3.2 presents the eligibility criteria. Table 3.3 presents the exclusion criteria.

Table 3.2. Eligibility criteria for DEER.

	MEN	**WOMEN**
Age (yrs)	30-64	45-64
HDL cholesterol (mg/dL)	< 45	<60
LDL cholesterol (mg/dL)	126-189	126-209
Triglycerides (mg/dL)	<500	<500
Glucose, fasting (mg/dL)	<140	<140
Blood pressure (mmHg)	<160/95	<160/95
BMI (kg/m^2)	≤ 34	≤ 32

Table 3.3. Exclusion criteria for DEER

EXCLUSION CRITERIA	SPECIFIC DETAILS
History of Heart Disease	Myocardial infarction, coronary artery bypass graft (CABG), percutaneous transluminal coronary angioplasty (PTCA), current use of cardiac medications, current use of anti-hypertensive medications
Abnormal Response to Symptom- Limited Treadmill Exercise Test	Angina, ST depression > 1mm, complex arrhythmias, inadequate systolic blood pressure response (failure to increase with exercise)
Insulin Dependent Diabetes Mellitus	Current use of insulin or other medications for diabetes, fasting glucose > 140 mg/L on repeat test with first test > 140 mg/L, 2 hour glucose > 200 mg/L on repeat test, first test > 200 mg/L
Neuromuscular/Orthopaedic Disability	Any condition that would limit moderate intensity physical activity, especially walking or jogging
Other Medical Conditions	Any other medical condition that may influence survival during the next 18 months significantly decrease exercise capacity or modify lipoprotein metabolism such as cancer diagnosed within five years, other than skin cancer, severe obstructive pulmonary disease (COPD), etc.
Use of Any Lipid Medication	As prescribed by physician or niacin > 500 mg/day or fish oils in doses > 2 capsules/day
Not Euthyroid Low Hematocrit Judgment of a Physician	On stable thyroid medication (for at least three months) < 30% of hemoglobin < 9.5 gm/dL
Smoking	Smoking more than nine cigarettes/day (self-report)
Alcohol Consumption	Consuming more than four alcoholic drinks per day (self-report)
Commuting Status	Lives or works beyond easy access of Stanford University or plans to move out of the area within the next 18 months
Adherence & Participation	Unwilling to attend 10 weekly nutrition classes and make changes in current eating habits Unwilling to add a minimum of three 30 minute sessions of aerobic activity per week to current activity Refusal to sign informed consent At the discretion of the clinic staff
Menopausal Status	Bilaterial oophorectomy before age 40 or hysterectomy less than 2 months before recruitment Last menstrual period less than 12 months prior (if uterus is present) Unwilling to make no changes in current Hormone Replacement Therapy for 18 months

Enrollment Screening & Sequence of Events

Subjects underwent telephone screening where exclusionary criteria were identified. Interested participants were then scheduled for various screening tests including laboratory blood analysis, dietary screening, exercise habits and physical examination. Four clinic visits and five telephone interviews were necessary to collect the baseline data.

DEER Methods and Measurement

All measures were taken at baseline prior to randomization and then repeated at one year follow-up. DEER measurement staff were blinded to the treatment of the subjects. All data were double key entered to ensure accurate data entry. Table 3.4 summarizes all measures taken during baseline and follow-up.

Table 3.4. Measurements for DEER at Baseline and Follow-up.

ASSESSMENT	SPECIFIC MEASURES
Demographics	Ethnicity Age Socioeconomic Status
Cardiovascular Risk Factors	Total Cholesterol HDL LDL Triglycerides Blood Pressure Resting Heart Rate
Body Composition	Height Weight BMI Hydrostatic Weighing
Diet Assessment	24 Hour Dietary Recall
Psychological Assessment	Stages of Change Profile of Mood States
Physical Activity Status	Physical Activity Diaries General Exercise Habits Godin Leisure Time Physical Activity Questionnaire
Physical Fitness	Maximal Oxygen Consumption
General Health	Medications Lifestyle Questionnaire Reproductive History

Lifestyle Behavior Measurement

Dietary assessment

Dietary intake assessment included five unannounced 24 hour dietary recalls. The questionnaire was administered over the phone with Nutrient Data Systems (NDS), a computer assisted interviewing and data entry program developed by the University of Minnesota Nutrition Coordinating Center. Diet recalls captured one weekend day and four weekdays on non-consecutive days. Nutrient intake was calculated with Food database software Version 5a & 6a. Data included mean from the four weekdays and one weekend recall.

Physical activity assessment

Physical activity was assessed with the Godin Leisure Time Exercise Questionnaire (Godin) (Godin & Shephard, 1985). The Godin instrument has been shown to be valid and reliable against maximal oxygen consumption testing in men and women (Godin et al., 1985; Jacobs, Jr., Ainsworth, Hartman, & Leon, 1993). The questionnaire asks "Considering a seven day period (a week), how many times on average do you do the following kinds of exercises for more than 15 minutes during your free time?". Activities assessed include 1) strenuous exercise (heart beats rapidly), 2) moderate exercise (not exhausting) and 3) mild exercise (minimal effort). Total weekly 15 minute bouts for strenuous, moderate and mild exercise were multiplied by 3, 5 and 9, respectively to convert to MET-weighted units. The total MET-weighted units are then summed to identify a physical activity score.

Physical fitness assessment

Physical fitness was measured by oxygen consumption from a graded exercise treadmill test using a semi-automated metabolic analysis system (Savin, Haskell, Schroeder, & Stinson, 1980). Oxygen uptake was measured every 30 seconds during the test. Speed started at 2 miles per hour at 2.5% grade for 3 minutes. The stages progressed to 3 miles an hour with an increase in grade 2.5% every 3 minutes. Subjects exercised to volitional fatigue and maximal oxygen consumption (VO_2max) was an average of the highest two measures during the final minutes of exercise.

Physiological Measures

Body composition measurement

Body weight was measured with a standard medical beam balance scale. Height was measured using a Harpenden stadiometer. Body mass index (BMI) was calculated by dividing the subject's body weight in kilograms into their height squared (kg/m^2).

Waist circumference was taken at the narrowest circumference of the torso when viewed from the front. Waist circumference measures were taken three times and averaged.

Skinfolds were taken using the three site method as identified by Jackson and Pollack (Jackson & Pollock, 1978; Jackson, Pollock, & Ward, 1980). Men were measured at the chest (diagonal fold at the midpoint between axillary line and nipple), abdomen (vertical fold one cm to the right of the umbilicus), and the thigh (vertical fold at the midpoint between inguinal fold and the superior patella). Women were measured at the thigh (identical to male site), suprailiac (diagonal fold superior to the iliac crest), and triceps (vertical fold midway between the olecranon process and the acromion

process). Three measurements were taken and averaged. Jackson-Pollock gender specific formulas were applied to calculate body density (Jackson et al., 1978; Jackson et al., 1980) and then the Siri equation was applied to calculate percent body fat (Siri, 1961).

Cardiovascular parameters

Resting heart rate, systolic and diastolic blood pressure were taken in duplicate after five minutes of quiet rest in the morning. A standard mercury sphygmomanometer and stethoscope was utilized to estimate blood pressure from the brachial artery. First and fifth phase Korotkoff were noted as systolic and diastolic blood pressure readings in millimeters of mercury (Fortmann, Haskell, & Wood, 1988). The two systolic and diastolic blood pressure readings were averaged.

Prior to the venous blood collection, subjects were asked to fast with no food or drink, except water, for at least 12 hours. Smoking was not allowed during the hour prior to blood collection. Participants also abstained from alcohol consumption and vigorous activity for at least 24 hours prior to blood collection. Blood samples were taken in the morning on two different visits at baseline. All collected blood was mixed with 1.5 mg/mL of EDTA. Standard methods were used: serum was allowed to clot for 30-60 minutes, centrifuged, put on ice, and then transferred to the freezer where aliquots of plasma were stored at -80° C.

Both fasting blood samples were analyzed for lipoproteins and then averaged. Total cholesterol and triglycerides were measured using enzymatic procedures (Allain, Poon, Chan, Richmond, & Fu, 1974; Sampson, Demers, & Krieg, 1975). High density lipoprotein (HDL) was measured using dextran sulfate – magnesium precipitation

(Warnick, Benderson, & Albers, 1982) as well as enzymatic measurement of non-precipitated cholesterol (Allain et al., 1974). Very low density lipoprotein (VLDL) was calculated as triglycerides divided by five (Friedewald, Levy, & Fredrickson, 1972). If triglyceride levels were over 400 mg/dL enzymatic methods were used to measure VLDL according to Friedewald methods (Friedewald et al., 1972). Low density lipoprotein (LDL) was calculated as total cholesterol minus the sum of HDL + VLDL (Friedewald et al., 1972).

Figure 3.1 describes the flow of recruitment and measurement.

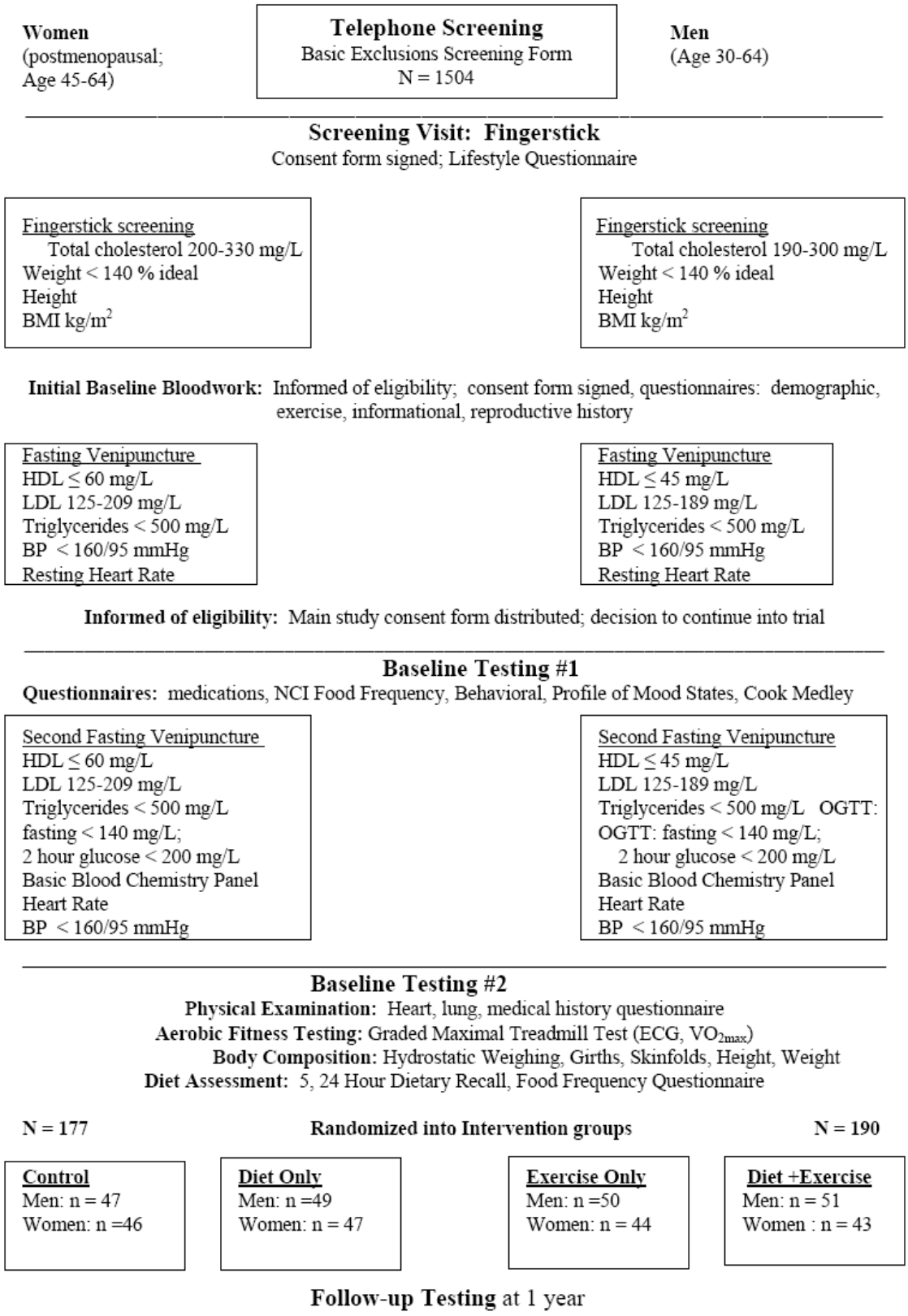


Figure 3.1. DEER Recruitment and Measurement.

Randomization

Randomization was done by the Efron procedure in which subjects were assigned to one of four groups: control, low-fat diet, exercise, diet plus exercise. The Efron procedure uses a computer algorithm to weight the probability of group assignment to balance individual groups for sample size, average HDL cholesterol and LDL cholesterol (Efron, 1971).

The final number of participants for men and women within each intervention group are presented in Table 3.5. DEER enrolled nine cohorts to complete data collection.

Table 3.5. Deer Subject Sample Size For Each Intervention Arm.

INTERVENTION ARM	MEN	WOMEN	**TOTAL**
Control	47	46	**93**
Diet	49	47	**96**
Exercise	50	44	**94**
Diet + Exercise	51	43	**94**
TOTAL	**197**	**180**	**377**

DEER Intervention Program Details

Control group

The control group was instructed to maintain their usual diet and exercise habits until tests were completed one year post randomization. The diet and/or exercise treatments were offered to all persons in the control group as a "delayed" intervention.

Dietary Intervention

Participants followed the dietary goals set by the National Cholesterol Education Program (NCEP) Step II Guidelines (1993). Components of the guidelines are outlined below in Table 3.6. It is important to note that the NCEP Step II Guidelines which focused on cholesterol dietary changes were emphasized, not total caloric reduction or weight loss.

Table 3.6. National Cholesterol Education (NCEP) Program Dietary Guidelines (1993).

	SPECIFIC GUIDELINES
NCEP Step II	Reduction of total fat to < 30% of calories Reduction of saturated fat to < 7% of calories Reduction of dietary cholesterol < 200 mg/day Encouragement to decrease total fat and saturated fat below recommended levels *Reduction of total calories (if needed) to achieve ideal body weight** *Weight reduction as applicable**

* Not an intervention target for DEER

Post randomization visit

The 12 week adoption phase began with an individualized appointment for each subject with a dietitian. The main goals of the visit were to establish a baseline dietary intake history and weight history. The information was integrated into individualized dietary recommendations to meet the NCEP dietary goals.

Major intervention phase

Eight weekly group sessions comprised the major intervention phase for the dietary treatment arm. Dietary class content was based on the NCEP Step II Guidelines which included information concerning the rationale and behavioral approaches to

positively change dietary habits. The dietary class content used a six-step approach for behavioral change included 1) identifying the problem, 2) making a commitment and building confidence, 3) increasing awareness, 4) developing and implementing an action plan, 5) evaluating the action plan and 6) maintaining changes. The one hour nutrition classes were held for approximately 15 mixed gender participants. Each session included a short lecture and discussion, practical demonstration, or hands-on activity with homework to complete before the next class. Participants could bring a family member or guest to promote adherence. Nutrition class topics and titles are listed below in Table 3.7.

Table 3.7. Nutrition Class Topics for Diet Intervention.

SESSION NUMBER	NUTRITION SESSION CLASS TITLE
I	Getting Started. Where are we going and how are we going to get there? Fat Demonstration
II	Meat, Fish, and Poultry…Choosing and Using the Best for Health
III	Workshop of Using Less Meat
IV	The Dairy Case…Finding your "Whey"
V	Fats, Oils, Salad Dressing, and Nuts
VI	Let's Do Lunch. Strategies for Managing Breakfast, Lunch and Snacks
VII	More Strategies…For Dinners and Desserts
VIII	Getting Ready for Minor Intervention: Making More Dietary Changes and Maintaining the Ones We've Already Made

Each participant received The New American Diet System as a textbook for the study (Connor & Connor W.E., 1986). Participants also received fat gram booklets to assist fat gram counting.

Minor intervention phase

Following the major intervention phase, participants were contacted every other month via individual appointments, group sessions, telephone calls and/or mailings. The

major goal of the minor intervention phase was to prevent relapse and encourage other positive dietary behavioral changes over the course of the following six to eight months. Individuals were given contact information of dietary interventionists for questions and were able to make private appointments as needed.

Once all measures were completed at follow-up, all subjects in the diet only group were given the option to participate in the exercise program.

Exercise Intervention

Post randomization visit

Immediately following randomization, individuals assigned to the exercise only group met with a member of the exercise intervention team. Physical activity and exercise history was obtained to individualize the exercise intervention materials. Participants were taught how to monitor their pulse for monitoring intensity, and received an individualized exercise prescription for their appropriate training range based on the results from their maximal graded treadmill test.

Participants in the exercise group received the following books to act as guides for the intervention, The Stanford Heart Disease Prevention Project Walking Kit or Jogging Kit, <u>The Exercise Book For People Who Do Not Exercise</u>, and/or a handout entitled, "The How Do I Begin? Exercise Workshop: A Complete Guide to Fitness Master". These materials were originally created for the Stanford Weight Control Project. Individuals who were already active were told to continue their current programs and add more minutes to their existing routines, not replace their current routines with the DEER program recommendations.

Adoption phase

The initial six weeks of exercise training consisted of three days a week of one hour supervised aerobic sessions by exercise intervention staff. The exercise adoption program consisted of three days a week of 45-60 minute exercise sessions for both men and women together.

Major intervention phase

After completion of the adoption phase, participants engaged in supervised training sessions led by trained exercise intervention staff. Intensity recommendations were to achieve 60-85% maximum heart rate for 20 minutes three times a week for the first six weeks. Participants increased duration over the course of a year. Any individuals who were already active upon randomization were asked to add 20 minutes three times a week to their existing physical activity programs.

Encouragement was given for activities outside of the exercise sessions one day a week including biking, swimming, dancing etc to engage participants in four days a week of physical activity. Exercise participants were also encouraged to further increase their activity by using the stairs, walking instead of driving and increase recreational opportunities. The exercise program progression is outlined below in Table 3.8.

It is important to note that weight loss was not a component of the exercise program.

Table 3.8. DEER Exercise Intervention Details.

EXERCISE COMPONENT	EXERCISE PROGRAM DETAILS
Exercise Adoption Phase	**Duration:** Initial 6 weeks **Type:** Aerobic training **Frequency:** 3 days/week
Main Intervention Exercise Phase	Supervised training sessions **Duration:** 3 months **Type:** Walking or jogging **Frequency:** 3 days/week **Intensity:** 60-85% maximum heart rate **Time:** 20 minutes for the first 6 weeks; Increased duration throughout program to 45-60 minutes Note: Already active participants added 20 more minutes to existing exercise routine 3x/week Encouragement for Recreational Exercise **Frequency:** 1 day/week **Type:** biking, swimming, dancing, etc Encouragement for Lifestyle habits **Type:** taking the stairs, parking further away, walking for transportation
Minor Exercise Intervention Phase	**Duration:** 7-8 months Individualized counseling session for identifying future exercise goals Either supervised training sessions or adopted home program **Duration:** 10-12 miles per week **Type:** Brisk walking/jogging/running

Attendance at supervised exercise sessions was monitored by the exercise intervention staff and intervention and counseling was given when appropriate.

Minor intervention phase

An individualized meeting was scheduled at the completion of the major intervention phase to identify exercise goals and develop future exercise plans. Participants continued with three days a week of supervised activity or were encouraged to adopt a home program for the following 7-8 months. Weekly exercise programs consisted of a minimum of 10 miles of brisk walking, jogging, or running each week. Monthly special group exercise activities were held including races, par courses, special track games, introducing new routes/trails etc. A "Mileage Plus" program was initiated

to promote adherence. Participants who accumulated the appropriate mileage received small gifts. Newsletters providing training tips, injury prevention and local activities were provided throughout the minor intervention phase.

Once all measures for the intervention were completed at follow-up, all subjects in the exercise only group were given the option to participate in the diet intervention program.

Diet Plus Exercise Intervention

Participants randomized into the diet plus exercise group received both interventions as individual treatments. Nutritionists made no reference to physical activity and the exercise intervention group made no references to dietary changes. Classes were scheduled in a coordinated manner to promote adherence for the diet plus exercise group. All dietary classes and exercise sessions were held separately for diet, exercise and diet plus exercise participants.

Promotion of Adherence

Special events and rewards were integrated into the dietary and exercise treatment arms to further promote adherence and attendance. Participants who displayed poor adherence to either dietary or exercise interventions were contacted and given private sessions to discuss strategies to prevent or minimize the problems. Six attempts were made to contact participants who had major adherence issues. Individuals who did not communicate with the intervention staff were mailed a letter to reiterate the goals of the DEER study and to emphasize the importance of one year follow-up measures.

Chapter 4

The Effects of Low-Fat Diet and/or Exercise on C-Reactive Protein: Results from a Randomized Controlled Trial

Introduction

Chronic inflammation, a novel cardiovascular risk factor, is an increase in circulating pro-inflammatory cytokines such as C-reactive protein (CRP). CRP is an acute phase reactant which is released from the liver and is considered a downstream marker of chronic inflammation. CRP increases with aging (Rumley et al., 2006), body mass index (Ford, 1999) and is 35% higher in women than in men (Ford et al., 2003; Ford et al., 2004b). It is estimated that approximately 25% of adults have elevated levels of CRP (Pearson et al., 2003) which is linked to mortality (Harris et al., 1999), cardiovascular disease (Ridker, 2003b; Ridker, 2003a; Ridker et al., 2003a), type II diabetes (Pradhan et al., 2003) and metabolic syndrome (Das, 2002; Ridker et al., 2003b).

The incorporation of positive lifestyle changes such as a low-fat diet and exercise has reduced cardiovascular risk and improved overall health (Blair et al., 1989; Paffenbarger, Jr. et al., 1993; Pate et al., 1995; Yu-Poth et al., 1999; Elmer et al., 2006). Lower CRP levels are associated with lower consumption of total fat, saturated fat and cholesterol intake (Bertran et al., 2005; Fredrikson et al., 2004). Lower CRP levels are also correlated with higher physical activity in both healthy adults (Ford, 2002; Koenig et al., 1999; Panagiotakos et al., 2005; Pitsavos et al., 2003) and in adults with high cardiovascular risk (Colbert et al., 2004; Geffken et al., 2001; Pitsavos et al., 2005; Reuben et al., 2003; Taaffe et al., 2000). Individuals who have high levels of

cardiorespiratory fitness also demonstrate lower levels of CRP (Aronson et al., 2004b; Church et al., 2002; Kuo et al., 2007; LaMonte et al., 2002).

Despite the robust cross sectional relationships between CRP and positive lifestyle habits, low-fat diet and exercise interventions have not convincingly lowered CRP. Few randomized controlled trials have attempted to examine the independent and combined responses of CRP to diet, exercise and diet plus exercise. The independent effects of low-fat diet and/or exercise on CRP change have been inconsistent. Low-fat dietary interventions have shown reductions of CRP levels as high as 32% (Clifton et al., 2005; Heilbronn et al., 2001; O'Brien et al., 2005; Seshadri et al., 2004; Tchernof et al., 2002), however, these changes in CRP were not significant in comparison to a control group in another study (Erlinger et al., 2003). Exercise trials have shown varying results for changes in CRP, where some studies show significant reductions in comparison to a control group (Mattusch et al., 2000; Milani et al., 2004), while other studies show no effect on CRP levels (Hammett et al., 2004; Huffman et al., 2006).

Combined low-fat diet and exercise interventions have significantly reduced CRP levels between 30-45% (Bo et al., 2007; Roberts et al., 2006; Wegge et al., 2004), though control groups were not always included for comparison (Roberts et al., 2006;Wegge et al., 2004). In addition, many lifestyle interventions only examine the combination of diet plus exercise, making it difficult to discern the independent contributions of each (Bo et al., 2007; Dvorakova-Lorenzova et al., 2006; Esposito et al., 2003; Haffner et al., 2005; Roberts et al., 2006).

Comparisons between low-fat diet and diet plus exercise have shown that the combination results in larger changes in CRP than diet alone, although none of these

studies included a control group (You et al., 2004; Esposito et al., 2003; Esposito et al., 2004). These randomized trials also induced concurrent weight loss which can increase the magnitude of changes in CRP (You et al., 2004; Esposito et al., 2003; Esposito et al., 2004). The release of CRP is controlled, in part, by cytokines which can be stored and released from adipose tissue (Petersen et al., 2005). Therefore, the amount of body fat (Visser et al., 1999) and the change in body fat (Selvin et al., 2007) are directly related to the circulating levels of CRP. Thus, comparisons between the independent and combined effects of low-fat diet and exercise on CRP are difficult to distinguish from the influence of fat loss.

Another challenge with the present low-fat diet plus exercise interventions is that many only include a single gender, limiting the generalizability of the results (Dvorakova-Lorenzova et al., 2006; Esposito et al., 2003; Roberts et al., 2006; You et al., 2004; Wegge et al., 2004). Since CRP baseline levels are consistently higher in women, it is important to account for this difference within the analysis (Ford et al., 2003; Ford et al., 2004b). Diet plus exercise interventions which include both genders, have not accounted for the differences in CRP levels between men and women in their analysis (Bo et al., 2007; Esposito et al., 2004). Furthermore, it is possible that due to the higher CRP baseline value among women, the magnitude of change for CRP from lifestyle interventions may differ by gender.

The purpose of this study was to examine the changes in CRP, separately in men and women, between control, low-fat diet, exercise and diet plus exercise from a randomized controlled trial. We hypothesized that diet plus exercise would be the most efficacious for reducing CRP levels in both men and women.

Methods

The Diet and Exercise for Elevated Risk Trial (DEER) was a year-long, single-center randomized controlled clinical trial. DEER began in 1992 at Stanford Medical School's Center for Research in Disease Prevention. The original objective was to examine the response of lipoproteins to a low-fat diet and/or exercise in individuals with elevated cardiovascular disease risk. This project utilizes the existing data set and stored blood samples to retrospectively examine the effect of the diet and exercise programs for the change in CRP.

Specific eligibility criteria for men were: age between 30-64 years, high density lipoprotein cholesterol (HDL) < 45 mg/dL, low density lipoprotein cholesterol (LDL) 126-189 mg/dL and body mass index (BMI) $\leq$ 34 kg/m^2. Eligibility criteria for women were: age between 45-64 years, postmenopausal, HDL < 60 mg/dL, LDL 126-209 mg/dL and BMI $\leq$ 32 kg/m^2. In addition, men and women had to have blood pressure below 160/95 mmHg, triglycerides under 500 mg/dL, fasting glucose under 140 mg/dL and a normal maximal exercise treadmill test. Exclusion criteria included history of heart disease, abnormal response to symptom-limited treadmill exercise test, insulin dependent diabetes mellitus, neuromuscular/orthopaedic disability, use of lipid medication, non-euthyroid, low hematocrit, excessive smoking or alcohol consumption, inability to attend sessions, or by judgment of a physician.

Recruitment was from the Stanford University area in Palo Alto, California. Recruiting methods consisted of mass media including: worksite contacts, posted flyers, handouts, brochures to area physician offices and clinics, newsletters, mailings and paid advertisements.

Measurement

Participant eligibility was assessed with telephone screening, blood analysis, dietary analysis, exercise history and physical examination. Baseline measures were taken prior to randomization and then repeated at the end of the one year intervention. DEER clinic staff performing testing and measurement at baseline and follow-up were blinded to the participants' intervention group assignment.

Body composition

Body weight was measured with a standard medical beam balance scale. Height was measured using a Harpenden stadiometer. BMI was calculated by dividing the participant's body weight in kilograms into their height squared (kg/m^2). BMI categories were determined using the guidelines presented by the National Institutes of Health (NIH) (1998). Waist circumference was taken at the narrowest circumference of the torso when viewed from the front. Calculation of body density and body composition were made using skinfold measurements. Skinfold measures were made in triplicate on the right side of the body and averaged. For males, the locations of the skinfolds were chest, abdomen, thigh. For women, the locations were triceps, suprailiac and thigh. Body density was estimated using generalized equations (Jackson et al., 1978; Jackson et al., 1980) and percent body fat was calculated using the Siri equation (Siri, 1961).

Blood collection

Prior to venous blood collection, participants were asked to abstain from any smoking for the hour prior, fast with no food or drink (except water) for at least 12 hours, as well as refrain from alcohol consumption and vigorous physical activity for at least 24 hours. Blood samples were taken in the morning on two different visits at baseline. All

collected blood was mixed with 1.5 mg/mL of EDTA. Standard methods were used for the blood samples: serum clotted for 30-60 minutes, centrifuged, put on ice, and then plasma was transferred to the freezer for storage at -80° C.

C-Reactive Protein

Plasma high sensitivity CRP concentrations were determined from randomly choosing one of the two stored plasma samples. Immunoturbidimetric assay was performed on the Hitachi 917 analyzer (Roche Diagnostics - Indianapolis, IN), using reagents and calibrators from DiaSorin (Stillwater, MN). The immunoturbidimetric method creates an antigen-antibody reaction between CRP and an anti-CRP antibody that has been sensitized to latex particles, resulting in agglutination. This antigen-antibody complex causes an increase in light scattering, which is detected spectrophotometrically, with the magnitude of the change being proportional to the concentration of CRP in the sample. Since the photomultiplier tube is directly across from the light source, there is a decrease in the transmitted light due to scattering, reflectance and absorption. Values of density and absorption are read at 570 nm with a background subtract of 800 nm. Five-point calibration curves were constructed and standardized against CRM470. This assay has a sensitivity of 0.03 mg/L. The day-to-day variability of the assay at concentrations of 0.91, 3.07 and 13.38 mg/L are 2.81, 1.61 and 1.1%, respectively.

Dietary intake

Dietary intake assessment included five unannounced 24 hour dietary recalls. Recalls captured one weekend day and four weekdays on non-consecutive days. Data were collected utilizing Nutrient Data Systems (NDS), a computer assisted telephone interview developed by the University of Minnesota Nutrition Coordinating Center.

Nutrient intake was calculated as the mean of the five data collections, with use of Food database software Version 5a & 6a.

Physical activity

Physical activity was assessed with the Godin Leisure Time Exercise Questionnaire (Godin) (Godin et al., 1985). The Godin questionnaire is valid and reliable against maximal oxygen consumption (Godin et al., 1985; Jacobs, Jr. et al., 1993). The questionnaire specifically asks "Considering a seven day period (a week), how many times on average do you do the following kinds of exercises for more than 15 minutes during your free time?". Intensities assessed include: 1) strenuous exercise (heart beats rapidly), 2) moderate exercise (not exhausting) and 3) mild exercise (minimal effort). Total weekly 15 minute bouts for strenuous, moderate and mild exercise were multiplied by 3, 5 and 9, respectively to convert to MET-weighted units. The total MET-weighted units were summed to identify a physical activity score.

Physical fitness

Physical fitness was measured from a graded exercise treadmill test using a semi-automated metabolic system (Savin et al., 1980). Oxygen uptake was measured every 30 seconds during the test. Speed started at two miles per hour at 2.5% grade for three minutes. The stages progressed to three miles an hour with an increase in grade 2.5% every three minutes. Subjects exercised to volitional fatigue and maximal oxygen consumption (VO_2max) was the average of the highest two oxygen measures during the final minutes of exercise.

Randomization

Eligible subjects were randomized into intervention groups by the Efron procedure. This procedure is based on a computer algorithm which balances groups for sample size, HDL and LDL cholesterol measures (Efron, 1971). The four intervention groups were: 1) control, 2) diet, 3) exercise and 4) diet plus exercise.

Control

The control group was instructed to maintain their usual lifestyle habits for the duration of the intervention.

Low-fat diet

The dietary goals of the DEER were based on the National Cholesterol Education Program (NCEP) Step II Guidelines (1993): 1) reduce total fat to less than 30% of total calories, 2) reduce saturated fat to less 7% of total calories and 3) reduce dietary cholesterol to less than 200 mg/day. Each participant met with a dietician to individualize dietary recommendations and attended eight group sessions about the NCEP Step II Guidelines. After completion of the group dietary sessions, participants were contacted every other month by individual appointments, group sessions, telephone calls and/or mailings.

Exercise

Exercise participants met with a member of the exercise intervention team to individualize the exercise prescription based on the results of the maximal exercise test. All exercise participants began with six weeks of aerobics sessions three days a week for one hour. After the adoption phase, participants were instructed to perform 20 minutes three times a week at 60-85% maximum heart rate, increasing duration over the course of

the year to 45-60 total minutes. Any individuals who were already active upon randomization were asked to add 20 minutes three times a week to their existing physical activity programs. Participants had the option of continuing supervised training or adopting a home program for the remaining eight months. The typical exercise program involved a minimum of ten miles per week of brisk walking, jogging, or running.

Diet plus exercise

Participants in the diet plus exercise group received both the diet and exercise interventions as individual treatments. Project staff involved with nutrition made no reference to physical activity and the exercise intervention staff made no references to diet. Diet plus exercise had separate nutrition and exercise sessions from the other groups to prevent contamination.

It is important to note that the diet, exercise and diet plus exercise groups did not emphasize weight loss as an intervention goal.

Statistical Analysis

All statistical analyses were performed by using SAS software version 9.1 (SAS Institute, Cary, NC). Participants with CRP levels at baseline or follow-up over 10 mg/L were removed from the analysis to eliminate the acute effects of infection (n=9) (Myers et al., 2004). Values were rounded to one decimal point to standardize CRP reporting (Myers et al., 2004). Since the DEER demographic eligibility criteria varied for men and women, all analyses were stratified by gender.

An analysis of covariance (ANCOVA) was used to test the effects of low-fat diet and exercise on CRP changes between intervention groups. Change was calculated as a difference between the follow-up value and the baseline value. Differences for the

change in CRP between intervention groups were compared for: 1) control versus diet, 2) control versus exercise, 3) control versus diet plus exercise, 4) diet versus diet plus exercise and 5) exercise versus diet plus exercise. An α level of 0.01 was adopted to control for type I error and to account for the multiple statistical comparisons. ANCOVA analyses controlled baseline CRP, cohort, baseline body fat percentage, change in body fat, cigarettes per day, alcoholic drinks per day and age. For women, hormone replacement therapy (HRT) status was also included as a covariate. Analyses controlled for the change in body fat in an attempt to eliminate the influence on the change in CRP. Models were also analyzed without including the change in body fat as a covariate.

Repeated measures generalized linear modeling assessed changes in CRP within each intervention group between baseline and follow-up with adjustments for the same covariates as previously described. CRP values at baseline and follow-up did not have a normal distribution, and were skewed to the right of the mean. Thus to satisfy the normality assumptions for ANCOVA, a log transformation was made. Results for within group differences were quantified as Δ log CRP and were considered statistically significant if $p < 0.05$.

The CDC/AHA established categories to identify individuals at increased risk for cardiovascular disease based on their level of CRP: low risk (less than 1 mg/L), average risk (1-3 mg/L), and high risk (above 3.0 mg/L) (Myers et al., 2004). Logistic regression analyzed the change in distribution for high risk individuals between intervention groups, controlling for baseline CRP category and the previously mentioned covariates.

Results

Of the total 377 DEER participants who were randomized for the study, 278 participants (74%, 149 men and 129 women) were analyzed for changes in CRP. Participants were not included in the analysis due to incomplete data which was assumed to be missing at random.

Men and women had an average age of 49.0 ± 8.83 and 57.6 ± 5.05 years, respectively. Participants were mostly Caucasian (~85%), non-smokers (~ 98%) and consumed less than one alcoholic drink per day (~ 95%). Men and women were highly educated: 61% of men and 42% of women had a college degree or greater. The mean BMI for men was approximately 26 kg/m^2 and 26% were normal weight, 58% were overweight and 16% were obese. In women, mean BMI was approximately 26 kg/m^2, and 37% were normal weight, 49% were overweight and 13% were obese. Approximately 45% of women were on HRT. Table 4.1 shows the baseline study variables by intervention groups for men and women.

Data from the original DEER study indicate adherence to the assigned treatment groups for men and women: the change in fitness (VO_2max) from baseline was significantly greater in the exercise group and diet plus exercise groups relative to controls. Furthermore, the changes in total fat, saturated fat and cholesterol were significantly different within the diet group and diet plus exercise groups relative to the control group, in both men and women (Table 4.2). There was little loss to follow-up for data collection, and retention was 98% and 96% in men and women, respectively (Stefanick et al., 1998).

Table 4.1 Baseline Characteristics in Men and Women (mean ± SD).

Men (n=149)	**Control**	**Diet**	**Exercise**	**Diet plus Exercise**
N	33	39	35	42
Age	48.8 ± 9.79	49.3 ± 9.17	49.5 ± 8.67	48.5 ± 8.12
Caucasian (%)	91	79.5	91	81
Non-Smokers (%)	97	100	100	100
≤1 Alcoholic Drink per day (%)	88	92	91	95
Waist Circumference (cm)	95.3 ± 9.39	95.9 ± 10.15	95.4 ± 7.85	94.8 ± 8.52
Body Fat (%)	21.1 ± 3.60	21.3 ± 4.48	22.3 ± 4.96	21.6 ± 4.13
BMI (kg/m^2)	26.7 ± 3.19	26.9 ± 3.10	26.9 ± 2.64	26.6 ± 2.63
CRP (mg/L)	1.4 ± 1.47	1.0 ± 1.16	1.3 ± 1.26	1.4 ± 1.34
High CRP (>3mg/L) n(%)	3(9)	2(5)	5(14)	5(12)
Women (n=129)	**Control**	**Diet**	**Exercise**	**Diet plus Exercise**
N	34	32	30	33
Age	58.4 ± 4.84	57.9 ± 5.36	57.1 ± 5.36	56.8 ± 4.80
Caucasian (%)	85	91	89	90
Non-Smokers (%)	100	100	100	91
≤1 Alcoholic Drink per day (%)	94	97	90	100
Waist Circumference (cm)	85.4 ± 11.60	85.3 ± 7.83	83.3 ± 7.11	83.7 ± 9.19
Body Fat (%)	31.7 ± 5.73	31.8 ± 4.77	31.6 ± 5.30	32.8 ± 5.25
BMI (kg/m^2)	26.0 ± 3.88	26.6 ± 2.82	25.6 ± 2.51	26.3 ± 3.42
CRP (mg/L)	2.2 ± 2.19	1.9 ± 1.60	1.8 ± 1.55	1.9 ±1.75
High CRP (>3mg/L) n(%)	9(25)	7(21)	5(16)	5(14)

CRP: C-reactive protein; BMI: body mass index.

Table 4.2. Baseline Values and Changes in Body Fat, Physical Activity, Physical Fitness, Dietary Fat, Saturated Fat and Cholesterol Intake.[+]

Men	**Baseline**	**Δ Control**	**Δ Diet**	**Δ Exercise**	**Δ Diet plus Exercise**
Body Fat (%)	21.6 ± 0.35	0.5 ± 0.59	-1.5 ± 0.55[*]	-0.5 ± 0.67	-2.0 ± 0.51[*]
Physical Activity Score	27.8 ± 1.69	-4.4 ± 3.46	1.9 ± 3.25	13.3 ± 3.02[*]	21.3 ± 4.53[*]
VO_2max (mL/kg/min)	37.7 ± 0.56	-0.9 ± 0.60	1.0 ± 0.61	1.9 ± 0.77[*]	4.8 ± 0.77[*]
Fat (% of total intake)	30.5 ± 0.59	-1.1 ± 1.13	-8.7 ± 1.35[*]	-0.9 ± 0.99	-8.3 ± 0.93[*]
Saturated Fat (% of total intake)	10.0 ± 0.26	-0.13 ± 0.45	-3.8 ± 0.52[*]	-0.3 ± 0.38	-3.9 ± 0.42[*]
Cholesterol intake (mg)	261.4 ± 10.41	-15.4 ± 22.84	-115.5 ± 20.79[*]	-5.2 ± 15.90	-110.3 ± 17.24[*]
Women	**Baseline**	**Δ Control**	**Δ Diet**	**Δ Exercise**	**Δ Diet plus Exercise**
Body Fat (%)	32.1 ± 0.46	1.4 ± 0.62	-1.4 ± 0.77[*]	0.09 ± 0.70	-2.2 ± 0.79[*]
Physical Activity Score	23.0 ± 2.11	14.1 ± 7.30	2.3 ± 2.91	17.0 ± 6.53	12.5 ± 6.30
VO_2max (mL/kg/min)	26.0 ± 0.41	-1.0 ± 0.45	0.5 ± 0.77[*]	2.2 ± 0.59[*]	3.6 ± 0.64[*]
Fat (% of total intake)	28.1 ± 0.63	-0.1 ± 1.12	-5.9 ± 1.31[*]	1.4 ± 1.05	-8.5 ± 1.07[*]
Saturated Fat (% of total intake)	9.0 ± 0.25	0.1 ± 0.46	-2.3 ± 0.52[*]	0.3 ± 0.54	-3.2 ± 0.42[*]
Cholesterol intake (mg)	174.6 ± 7.23	7.4 ± 15.34	-67.2 ± 12.58[*]	17.3 ± 16.53	-67.7 ± 14.34[*]

Adapted from Stefanick et al., 1998

[+] Means ± standard error

[*] $p < 0.05$ for the change in the variable between intervention group and control group

(Change in body fat, physical activity and VO_2max adjusted for age and baseline values; Fat, saturated fat and cholesterol adjusted for baseline values)

CRP Changes Between Intervention Groups

There were no baseline CRP differences between control, diet, exercise or diet plus exercise groups in either men or women. CRP changes from baseline to follow-up between control, diet, exercise and diet plus exercise groups were not different in men ($p = 0.99$) (Figure 4.1). CRP changes were significantly different between intervention groups in women ($p = 0.036$). Reductions in CRP in women were significantly greater when comparing diet plus exercise to the control group (-0.7 ± 0.33 mg/L, $p = 0.04$) and exercise group (-0.9 ± 0.32 mg/L, $p = 0.004$) (Figure 4.2).

CRP Changes Within Intervention Groups

In men, CRP did not change from baseline to follow-up within the control, exercise, diet or diet plus exercise groups. In women, only the diet plus exercise group had a difference in CRP from baseline to follow-up (Δ log CRP 0.2 ± 0.035 mg/L; $p = 0.0002$).

Change Between Intervention Groups for CRP Category

Approximately 10% of men and 19% of women were classified in the high CRP risk category (> 3 mg/L) at baseline. At follow-up, approximately 6% of men and 17% of women remained classified as high CRP risk. Logistic regression for the high risk category at follow-up did not reveal differences for the distribution of individuals between intervention groups either in men ($p = 0.74$) or women ($p = 0.38$).

No differences in statistical significance were noted after removing the change in body fat as a covariate either between or within intervention groups in men or women.

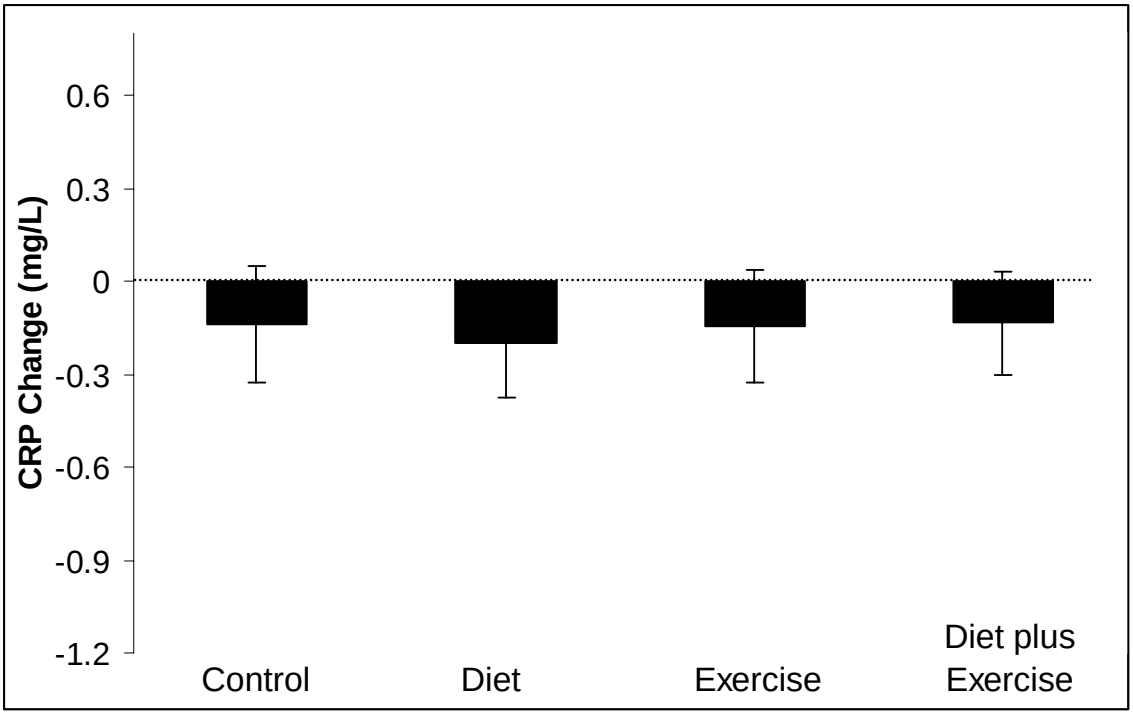
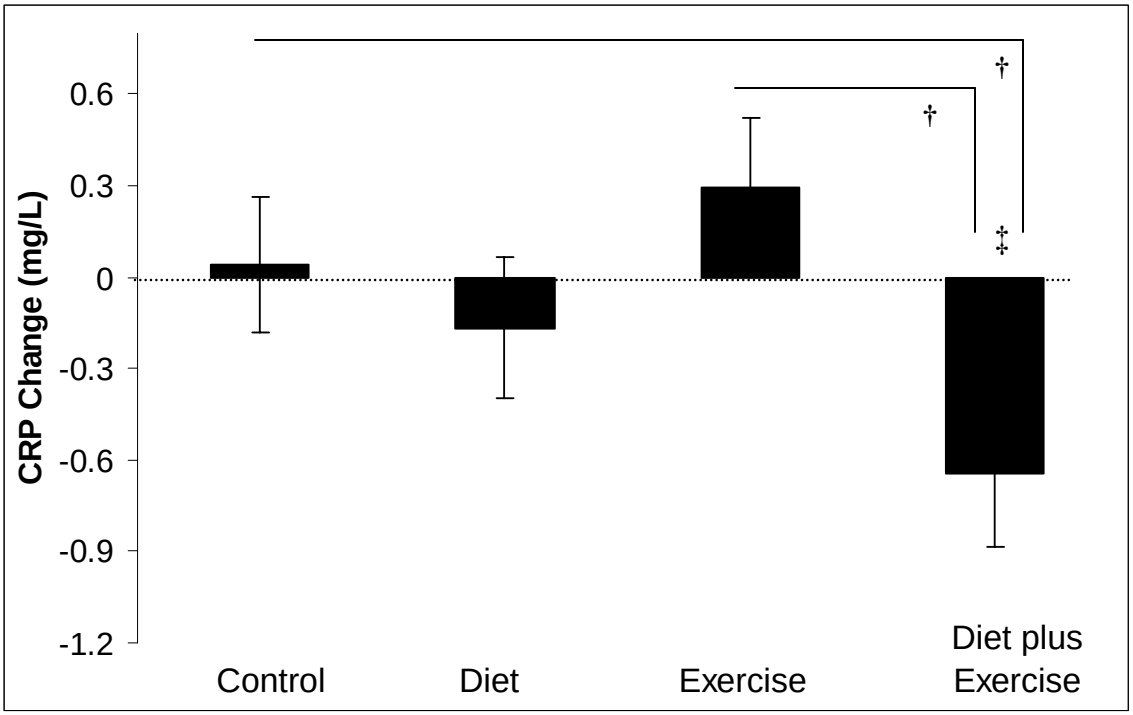


Figure 4.1. CRP change in men between groups (n = 149). Adjusted changes in CRP (mg/L) ± standard error are presented. ANCOVA statistical comparisons between intervention groups adjusted for the following covariates: baseline CRP, baseline body fat percentage, change in body fat, cohort, cigarettes per day, alcoholic drinks per day and age. In men, no differences existed between or within control, diet, exercise or diet plus exercise groups.



† significant difference for CRP change between intervention groups
‡ significant change in CRP from baseline to follow-up within intervention group

Figure 4.2. CRP change in women between groups (n = 129). Adjusted changes in CRP (mg/L) ± standard error are presented. ANCOVA statistical comparisons between intervention groups adjusted for the following covariates: baseline CRP, baseline body fat percentage, change in body fat, cohort, cigarettes per day, alcoholic drinks per day, age and hormonal therapy status. In women, differences for the change in CRP were found between the control versus diet plus exercise group ($p = 0.04$); exercise versus diet plus exercise group ($p = 0.0043$). In women, CRP changes from baseline to follow-up were significant within the diet plus exercise group ($p = 0.0002$).

Discussion

In postmenopausal women, CRP was significantly different from low-fat diet plus exercise in comparison to both the control and exercise only groups. In contrast, men did not show significant differences for the CRP change between control, diet, exercise or diet plus exercise.

These findings for women revealed that a low-fat diet plus exercise intervention was more efficacious than exercise alone, which was also confirmed in other research (Nicklas et al., 2004). In contrast to our findings, previous research indicated that diet plus exercise was more effective at reducing CRP than diet alone (You et al., 2004; Esposito et al., 2003; Esposito et al., 2004). However, none of these studies included a control group for comparison, and weight loss was also induced through hypocaloric intake (You et al., 2004; Esposito et al., 2003; Esposito et al., 2004). The change in CRP is directly related to weight loss (Due et al., 2005) and the change in weight (Clifton et al., 2005). For each one kilogram of diet or exercise induced weight loss, CRP is reduced -0.13 mg/L (Selvin et al., 2007). Therefore, from previous research, it is difficult to discern the exact stimulus for CRP change: weight loss, low-fat diet and/or exercise. Our intervention did not include purposeful weight loss and an adjustment for the changes in body fat were also made. Results from our study suggest that changes in CRP are no different between diet plus exercise and diet when compared to control.

Reductions for CRP for women between diet plus exercise and control were comparable to other low-fat diet plus exercise programs in which weight loss was unintentional (Bo et al., 2007). However, Bo et al., included both men and women together in the same analysis without adjustment for gender despite known differences in

CRP levels at baseline (2007). Thus, our findings in women suggest that CRP levels can be reduced from the incorporation of a low-fat diet combined with exercise, even while controlling for the change in body fat.

Men in the DEER trial did not significantly reduce their CRP levels in the diet plus exercise group when compared to a control group. Contrary to our findings, a diet plus exercise intervention in men significantly reduced CRP levels (Roberts et al., 2006). However the Roberts et al., study involved a three week residential diet and exercise program which may not be entirely generalizable to the DEER community dwelling one year intervention (2006). The Roberts et al., dietary protocol involved fat restriction to 12-15% of total intake, which is a more stringent reduction than the DEER goal of less than 30% total fat intake. Roberts et al., also included only obese men, and did not adjust CRP values for baseline weight status or the possible change in weight (2006). The release of CRP is controlled, in part, by TNF-α and IL-6, which can be located and released from the adipose tissue (Petersen et al., 2005). Thus, the more body fat a person has, the higher the release of CRP (Visser et al., 1999). Therefore, it is imperative to control for body fat in order to eliminate the effect of excess adipose tissue on CRP levels. Most importantly, Roberts et al., did not have a control group for comparison (2006). Our results imply that when controlling for baseline body fat, and possible changes in body fat, low-fat diet plus exercise may not decrease CRP in men when compared to a control group.

The current study did not find significant independent effects of low-fat diet alone or exercise alone in comparison to a control group, in either men or women. Other low-fat diet interventions, which did not attempt to induce concurrent weight loss, either

through isocaloric or ad-libitum consumption, agree with our findings (Koren et al., 2006; Desroches et al., 2006; Jenkins et al., 2005), though none of these were compared to a control condition. Our null findings for exercise versus control are also confirmed with other training interventions (Hammett et al., 2004; Huffman et al., 2006; Marcell et al., 2005; Rauramaa et al., 2004; Smith et al., 1999). Thus, our results suggest that the independent effects of both low-fat diet and exercise are not effective for changing CRP levels in men and women compared to a control group.

Possible explanations for the lack of effect either between or within interventions groups for changes in CRP may be due to a lower than expected mean value for CRP. Since CRP and dyslipidemia are positively associated (Medina-Urrutia et al., 2008; Wisse, 2004), we expected CRP values above the United States population median (Ford et al., 2003; Ford et al., 2004). Other studies with dyslipidemic subjects have had similar mean values to those we hypothesized (Festa et al., 2000). However, the DEER means were 1.3 ± 1.30 mg/L for men and 1.9 ± 1.73 mg/L in women, which were lower than the age and gender matched national median values. This discrepancy in CRP could be due to the DEER participants average levels of body fat (2006). It is possible that although the subjects had dyslipidemia, the subjects were mostly normal weight and overweight, which could have resulted in the lower baseline CRP levels. The lower CRP mean may have not provided enough power to detect differences between groups for the change in CRP.

We did not directly compare men and women for CRP response to lifestyle intervention in the current study. Due to eligibility criteria for the enrollment of the present study, women were an average of ten years older than the male participants. As

previously mentioned, CRP levels are higher in women and also increase with aging (Ford et al., 2003; Ford et al., 2004; Rumley et al., 2006). Therefore, we performed our statistical analysis separately within the genders, to avoid bias from age and/or gender. Stratified gender analyses make it challenging to distinguish whether the reduction in CRP from diet plus exercise in women was due to gender, age, or higher overall CRP levels. Future studies should continue to analyze men and women separately and explore possible biological mechanisms to explain the possible gender discrepancy for the response of CRP to lifestyle intervention.

Several physiological mechanisms exist to explain the changes in CRP from low-fat diet and exercise. Low-fat foods may simultaneously change macronutrient intake and quality (Jenkins et al., 2005). IL-6, which controls the release of CRP, is hypothesized to also be stimulated by the amount of insulin in the bloodstream (McCarty, 2005). Meals that limit postprandial glucose and hyperinsulinemia, such as fruits, vegetables and whole grains, may reduce levels of CRP in the bloodstream (Clifton et al., 2003). Increased fruit and vegetable consumption can also induce an anti-inflammatory response which may also lower CRP levels (Middleton E Jr, 1998). During a bout of exercise, IL-6 is released from muscle tissue. An increase in IL-6 levels initiates the secretion of anti-inflammatory markers such as interleukin-10 (IL-10) and interleukin-1 receptor antagonist (IL-1ra) which downregulate the pro-inflammatory effects of TNF-α (Pedersen, 2006; Petersen et al., 2005). This negative feedback loop suggests that chronic exercise decreases CRP levels. While the mechanisms that describe low-fat diet and physical activity and their relationship to CRP reduction appear to be independent, one could theorize that physiological effects could be additive. However, the exact

physiological mechanism relating the decrease in CRP from diet plus exercise is poorly understood.

Since most of our subjects were Caucasian, highly educated, and highly motivated, our results may be limited to persons with similar characteristics. Furthermore, all of the women were postmenopausal which also reduces the generalizability.

Despite the limitations in the present study, this report eliminated many of shortcomings from previous research. Our study included a randomized controlled design which examined the independent and combined effects of low-fat diet and exercise. Our analysis also adjusted for baseline body fat and changes in body fat, which have known influences on the level of circulating CRP. Finally, our separate analyses in men and women removed the possible gender bias.

In conclusion, CRP levels decreased for women in the diet plus exercise group compared to exercise and control groups. These results suggest that CRP levels can be successfully reduced with the combination of a low-fat diet plus exercise in women.

Chapter 5

The Effects of a Low-Fat Diet and Exercise on Metabolic Syndrome: Evidence from a Randomized Controlled Trial

Introduction

Metabolic syndrome is a clustering of abnormal metabolic, lipid and non-lipid variables. The National Cholesterol Education Program (NCEP) has operationally defined metabolic syndrome as meeting at least three of the following criteria: 1) abdominal obesity, 2) elevated triglycerides, 3) low high density lipoprotein cholesterol (HDL), 4) hypertension and 5) high fasting glucose (2001).

The prevalence of metabolic syndrome has increased over the past ten years. Data from the 1988-1994 National Health and Nutrition Examination Survey estimated the prevalence of metabolic syndrome to be approximately 23% (Ford et al., 2002), which increased to 27% by 1999 (Ford et al., 2004a). Metabolic syndrome prevalence among Americans from the 2002 National Health and Nutrition Survey (NHANES) was 35% (Ford, 2005). The presence of metabolic syndrome may predict cardiovascular disease mortality and morbidity better than any of its individual components (Isomaa et al., 2001). In fact, individuals with the presence of metabolic syndrome are two to three times more likely to die from cardiovascular disease (Ford, 2004; Lakka et al., 2002). Therefore, it is paramount to find effective treatments to reverse metabolic syndrome in order to curb the progression of cardiovascular disease.

Recommendations for treatment for reversing metabolic syndrome from the NCEP Adult Treatment Panel emphasize changing negative lifestyle behaviors such as

reducing intake of saturated fat and cholesterol, increasing physical activity and achieving weight loss (2001). While these lifestyle modifications have shown benefits for individual risk factors (Grundy et al., 2004b), evidence for improving metabolic syndrome prevalence with low-fat diet and/or physical activity without intentional weight loss has not been consistent.

Few randomized controlled trials have systematically examined the individual and combined effects of low-fat diet and exercise on metabolic syndrome. Diet plus exercise demonstrates more success for reversing metabolic syndrome than diet or exercise alone (Anderssen, et al., 2007; Okura et al., 2007), however these interventions also included concomitant weight loss. Weight loss can improve many of the individual metabolic and lipid components of metabolic syndrome (Yu-Poth et al., 1999), making it difficult to identify the successful component(s) of the intervention. Another challenge within the current lifestyle research for metabolic syndrome is the absence of a control group for comparison, which may limit the interpretation of results (Roberts et al., 2006; Muzio et al., 2005; Esposito et al., 2004; Okura et al., 2007).

Interventions that examined the independent effects of low-fat diet or exercise on metabolic syndrome status have also been limited. Low-fat dietary patterns, such as Mediterranean and DASH, have reduced metabolic syndrome prevalence (Azadbakht et al., 2005; Marfella et al., 2004), however, these low-fat diets also included purposeful weight loss. Furthermore, both the Mediterranean and DASH diets have multiple dietary alterations beyond reducing fat intake, which also may decrease the presence of metabolic syndrome. While exercise interventions have reduced the presence of metabolic syndrome (Katzmarzyk et al., 2003; Milani & Lavie, 2003; Shubair et al.,

2004), studies which included a control group for comparison have been inconsistent (Anderssen et al., 2007; Johnson et al., 2007; Stewart et al., 2005). Combined diet plus exercise interventions which either controlled for weight loss in the statistical analysis (Esposito et al., 2004) or allowed ad-libitum intake (Roberts et al., 2006), found significant reductions in the prevalence of metabolic syndrome. However, none of these combined diet plus exercise studies incorporated a randomized controlled design.

The purpose of this study was to examine the effects of low-fat diet and exercise on metabolic syndrome prevalence with a randomized controlled trial. Based on the previous literature, we hypothesized that exercise alone will show no change in metabolic syndrome prevalence when compared to a control. We also hypothesized that low-fat diet and diet plus exercise will show significant decreases in metabolic syndrome prevalence in comparison to a control condition.

Methods

The Diet and Exercise for Elevated Risk Trial (DEER) began in 1992 as a year-long, single-center randomized controlled clinical trial within the Stanford Medical School's Center for Research in Disease Prevention. The original primary objective was to examine the effects of low-fat diet and exercise, alone and together, on plasma lipoproteins in men and postmenopausal women (Stefanick et al., 1998). The present secondary data analysis retrospectively determined the effects of low-fat diet and exercise on metabolic syndrome status.

Specific eligibility criteria for men were: age 30-64 years, HDL < 45 mg/dL, low density lipoprotein cholesterol (LDL) 126-189 mg/dL and body mass index (BMI) ≤ 34

kg/m^2. Eligibility criteria for women were: postmenopausal, age 45-64 years, HDL < 60 mg/dL, LDL 126-209 mg/dL and BMI ≤ 32 kg/m^2. In addition, men and women had blood pressure below 160/95 mmHg, triglycerides under 500 mg/dL, fasting glucose under 140 mg/dL and a normal maximal exercise treadmill test. Exclusion criteria included history of heart disease, abnormal response to symptom-limited treadmill exercise test, insulin dependent diabetes mellitus, neuromuscular/orthopaedic disability, use of lipid medication, non-euthyroid, low hematocrit, excessive smoking or alcohol consumption, inability to attend sessions or by judgment of a physician.

A variety of mass media was utilized for recruitment from the community surrounding Stanford University in Palo Alto, California. Recruiting strategies included worksite contacts, posted flyers, handouts, brochures to area physician offices and clinics, newsletters, mailings and paid advertisements.

Measurement

Participants underwent telephone screening and a number of tests including laboratory blood analysis, dietary screening, exercise habits and physical examination. All measures were taken at baseline prior to randomization and then repeated at one year follow-up. Clinic staff who performed the measures were blinded to participants' treatment status.

Body composition

Body weight was measured with a standard medical beam balance scale. Height was measured using a Harpenden stadiometer. BMI was calculated by dividing the participant's body weight in kilograms by height squared (kg/m^2). BMI categories were determined using the guidelines presented by the National Institutes of Health (NIH)

(1998). Waist circumference was taken in triplet and average from the narrowest circumference of the torso when viewed from the front. Estimation of body composition was made utilizing skinfold measurements. All skinfold measures were taken three times on the right side of the body and averaged. For males, the locations of the skinfolds were chest, abdomen, thigh. For women, the skinfold locations were triceps, suprailiac and thigh. Body density was estimated using generalized equations (Jackson et al., 1978; Jackson et al., 1980). Percent body fat was calculated using the Siri equation (Siri, 1961).

Cardiovascular risk factors

A mercury sphygmomanometer estimated blood pressure from the brachial artery. Averages of two readings of the first and fifth phase Korotkoff were noted as systolic and diastolic blood pressure readings in millimeters of mercury (Fortmann et al., 1988).

For the venous blood collection, participants were asked to refrain from smoking for the hour prior, fast with no food or drink (except water) for at least 12 hours, and abstain from alcohol consumption and vigorous activity for at least 24 hours. Blood samples were taken in the morning on two different visits at baseline. All collected blood was mixed with 1.5 mg/mL of EDTA. Standard methods were used: serum was allowed to clot for 30-60 minutes, centrifuged, put on ice, and then plasma was transferred to the freezer at -80° C.

Both fasting blood samples were analyzed for lipoproteins. Total cholesterol and triglycerides were measured using enzymatic procedures (Allain et al., 1974; Sampson et al., 1975). HDL was measured using dextran sulfate – magnesium precipitation (Warnick et al., 1982) as well as enzymatic measurement of non-precipitated cholesterol (Allain et al., 1974). Very low density lipoprotein (VLDL) was calculated as

triglycerides divided by five (Friedewald et al., 1972). If triglyceride levels were over 400 mg/dL enzymatic methods were used to measure VLDL according to Friedewald methods (Friedewald et al., 1972). LDL was calculated as total cholesterol minus the sum of HDL + VLDL (Friedewald et al., 1972). Lipoprotein values were averaged between the two fasting blood samples.

Metabolic syndrome definition

Metabolic syndrome prevalence was determined using the NCEP's Adult Treatment Panel III definition (NCEP-ATPIII) (2001). Clinical identification of the metabolic syndrome involves an individual having at least three or more abnormal cardiovascular risk factors including abdominal obesity, dyslipidemia, hypertension and abnormal glucose metabolism. Details of the definition are provided in Table 5.1.

Table 5.1. Clinical Identification of the Metabolic Syndrome NCEP-ATP III

Diagnosis is established when ≥ 3 of the following risk factors are present:

RISK FACTOR	**DEFINING LEVEL FOR MEN**	**DEFINING LEVEL FOR WOMEN**
Abdominal Obesity Waist Circumference	> 102 cm	> 88 cm
Dyslipidemia Triglycerides	≥ 150 mg/dL	≥ 150 mg/dL
High Density Lipoprotein	< 40 mg/dL	< 50 mg/dL
Hypertension Blood Pressure	≥ 130 / ≥ 85 mmHg	≥ 130 / ≥ 85 mmHg
Metabolism Fasting Glucose	≥ 100 mg/dL*	≥ 100 mg/dL*

*This definition is updated to reflect the current American Diabetes Association fasting glucose level of ≥ 100 mg/dL (Genuth et al., 2003).

Dietary intake

Five unannounced 24 hour dietary recalls were administered using Nutrient Data Systems (NDS), a computer assisted telephone interview developed by the University of Minnesota Nutrition Coordinating Center. Interviews included one weekend day and four weekdays on non-consecutive days. Nutrient intake was calculated as the mean of the five recalls with use of food database software Version 5a & 6a.

Physical activity

Physical activity was assessed with the Godin Leisure Time Exercise Questionnaire (Godin) (Godin et al., 1985). The Godin questionnaire is valid and reliable against maximal oxygen consumption testing (Godin et al., 1985; Jacobs, Jr. et al., 1993). The specific questionnaire asks "Considering a seven day period (a week), how many times on average do you do the following kinds of exercises for more than 15 minutes during your free time?". Intensities assessed include: 1) strenuous exercise (heart beats rapidly), 2) moderate exercise (not exhausting), and 3) mild exercise (minimal effort). Total weekly 15 minute bouts for strenuous, moderate and mild exercise are multiplied by 3, 5 and 9, respectively to convert to MET-weighted units. The total MET-weighted units are then summed to identify a physical activity score.

Physical fitness

A maximal graded exercise test was administered to measure physical fitness by oxygen consumption. A semi-automated metabolic analysis system measured oxygen consumption every 30 seconds (Savin et al., 1980). Speed started at two miles per hour at 2.5% grade for three minutes. The stages progressed to three miles an hour with an increase in grade 2.5% every three minutes. Subjects exercised to volitional fatigue and

maximal oxygen consumption (VO_2max) was the average of the highest two measures during the final minutes of exercise.

Randomization

Individuals were randomized into four intervention groups: 1) control, 2) diet, 3) exercise and 4) diet plus exercise. Randomization was done by the Efron procedure in which participants were assigned to one of the four groups by a computer algorithm which balanced groups for sample size, average HDL cholesterol, and LDL cholesterol (Efron, 1971).

Control

The control group was asked to maintain their usual diet and exercise habits until tests were completed one year after randomization.

Low-fat diet

Participants were instucted to achieve the dietary goals set by the NCEP Step II Guidelines (1993): 1) reduce total fat to less than 30% of total calories, 2) reduce saturated fat to less 7% of total calories and 3) reduce dietary cholesterol to less than 200 mg/day. Each participant met with a dietitian to establish individualized dietary recommendations and attended eight weekly group sessions. After completion of group sessions, participants were contacted every other month via individual appointments, group sessions, telephone calls and/or mailings.

Exercise

Individuals in the exercise group met with a member of the exercise intervention team in order to individualize the exercise prescription. The adoption phase for exercise training consisted of six weeks of three days a week of one hour supervised aerobics

training. After the adoption period, participants began with 20 minutes duration, three times a week and proceeded to increase duration to 45-60 minutes total over the course of a year. Initial intensity recommendations were to achieve 60-85% maximum heart rate which was determined from the maximal graded treadmill test. Any individuals who were already active upon randomization were asked to add 20 minutes three times a week to their existing physical activity programs. After three months, participants continued with supervised activity or were encouraged to adopt a home program for the remaining 7-8 months. Weekly exercise programs consisted of a minimum of ten miles of brisk walking, jogging or running each week.

Diet plus exercise

Participants randomized into the diet plus exercise group received both interventions as individual treatments. Dietitians made no reference to physical activity and the exercise interventionists made no references to dietary changes. The diet plus exercise group had separate nutrition and exercise sessions from the other groups to prevent contamination.

It is important to note that the diet, exercise and diet plus exercise groups did not emphasize weight loss as an intervention goal.

Statistical Analysis

All statistical analyses were performed by using SAS software version 9.1 (SAS Institute, Cary, NC). Since enrollment criteria for cardiovascular risk factors were different for men and women, all analyses were done separately within gender.

Metabolic syndrome

Logistic regression was used to compare between intervention groups for metabolic syndrome prevalence at follow-up. Specific comparisons were between: 1) control versus diet, 2) control versus exercise, 3) control versus diet plus exercise, 4) diet versus diet plus exercise and 5) exercise versus diet plus exercise. A conservative α level of 0.01 was chosen to protect against type I error. All models controlled for baseline metabolic syndrome prevalence, age and change in body fat percent. For women, hormone replacement therapy (HRT) was added as a covariate. All statistical models for metabolic syndrome were adjusted for the change in percentage body fat in an attempt to eliminate its effect on metabolic syndrome status. All models were also analyzed without the adjustment for the change in body fat to note any changes in significance.

Repeated measures generalized linear modeling assessed within intervention group prevalence for metabolic syndrome from baseline to follow-up. The binary distribution was adjusted for age and change in body fat percent. Results were considered statistically significant if $p < 0.05$.

Finally, intervention groups were combined within gender to explore whether the changes in individual cardiometabolic risk factors predicted the presence of metabolic syndrome at follow-up. Specifically, logistic regression examined the changes in the following predictors for metabolic syndrome: fasting glucose, HDL, waist circumference, systolic and diastolic blood pressure, and triglycerides. Each analysis controlled for the covariates as described previously. Among individuals with baseline metabolic syndrome, t-tests were conducted to determine differences for the mean values of the

individual cardiometabolic risk factors in those who reversed or maintained metabolic syndrome at follow-up.

Number of abnormal risk factors

The number of abnormal risk factors was defined as the mean number of criteria from the metabolic syndrome definition that each participant met. For example, if a participant had blood pressure above the threshold for the metabolic syndrome definition, but did not meet any of the other criteria, then the number of abnormal risk factors would be 1. Change was calculated as the difference between the follow-up and baseline values for the number of abnormal risk factors. Differences between the intervention groups for the change in the number of abnormal risk factors were compared using general linear modeling. Statistical comparisons were made between intervention groups as described previously, also adopting a conservative α level of 0.01 to protect against type I error. Repeated measures generalized linear modeling assessed within intervention group changes for the number of abnormal risk factors from baseline to follow-up. Results were considered statistically significant if $p < 0.05$. All models with the number of abnormal risk factors as the outcome were controlled for baseline number of abnormal risk factors, cohort, age, change in body fat percent and HRT (as appropriate).

Results

Of the total 377 DEER participants who were randomized for the study, 325 participants (86%, 179 men and 146 women) had sufficient data for determining metabolic syndrome prevalence at both baseline and follow-up. The average age in years for men and women was 48.6 ± 8.70 and 57.8 ± 5.08, respectively. Participants were mostly Caucasian (~85%) and 61% of men and 42% of women had completed a college

degree. Men (Table 5.2) and women (Table 5.3) had elevated LDL cholesterol, low HDL cholesterol, normal blood pressure and normal fasting glucose levels. Approximately 46% of women were on HRT. The mean BMI was approximately 26 kg/m^2 for both men and women. BMI classifications in men were 28% normal weight, 58 % overweight and 15% obese. BMI classifications in women were 37% were normal weight, 48% overweight and 14% obese. Baseline characteristics and demographics for men are presented in Table 5.2 and for women in Table 5.3.

Data from the original DEER study indicate adherence to the assigned treatment groups for men and women: fitness (VO_2max) from baseline was significantly greater in the exercise group and diet plus exercise groups relative to controls. Changes in total fat, saturated fat and cholesterol were significantly different within the diet group and diet plus exercise groups relative to the control group, in both men and women (Table 5.4). There was little loss to follow-up for data collection, and retention was 98% and 96% in men and women, respectively (Stefanick et al., 1998).

Table 5.2. Baseline Characteristics in Men (mean ± SD).

Men (n=179)	**Control**	**Diet**	**Exercise**	**Diet plus Exercise**
N(%)	44(25)	47(26)	42(23)	46(26)
LDL (mg/dL)	158.0 ± 17.72	156.5 ± 18.70	155.0 ± 16.37	156.5 ± 17.18
HDL (mg/dL)	34.2 ± 5.32	35.3 ± 5.24	35.1 ± 4.26	34.6 ± 4.65
Systolic Blood Pressure (mmHg)	112.5 ± 10.40	113.2 ± 11.73	113.1 ± 12.64	113.2 ± 11.25
Diastolic Blood Pressure (mmHg)	76.0 ± 7.62	75.4 ± 8.40	75.7 ± 6.34	75.4 ± 8.34
Triglycerides (mg/dL)	175.0 ± 87.18	183.5 ± 94.43	178.9 ± 63.37	160.3 ± 63.66
Fasting Glucose (mg/dL)	93.0 ± 9.01	96.9 ± 6.46	97.9 ± 10.54	95.3 ± 8.05
Waist Circumference (cm)	94.9 ± 8.74	95.3 ± 10.04	94.9 ± 7.81	95.4 ± 8.51
Percent Body Fat (%)	21.0 ± 3.77	21.4 ± 4.45	22.0 ± 4.88	22.0 ± 4.07
BMI (kg/m^2)	26.7 ± 2.89	26.8 ± 3.10	26.7 ± 2.65	26.8 ± 2.63
Metabolic Syndrome n(%)	9(20)	15(32)	13(31)	16(35)
Number of Abnormal Risk Factors	1.8 ± 1.11	2.1 ± 1.12	2.0 ± 0.99	2.2 ± 1.03

Key: LDL: low density lipoprotein cholesterol; HDL: high density lipoprotein cholesterol

Table 5.3. Baseline Characteristics in Women (mean ± SD).

Women (n=146)	**Control**	**Diet**	**Exercise**	**Diet plus Exercise**
N(%)	37(25)	40(27)	33(23)	36(25)
LDL (mg/dL)	163.9 ± 17.46	163.9 ± 23.93	168.6 ± 23.98	165.0 ± 21.43
HDL (mg/dL)	44.9 ± 7.06	45.1 ± 7.33	44.8 ± 7.17	45.3 ± 6.85
Systolic Blood Pressure (mmHg)	113.3 ± 12.59	116.1 ± 15.67	114.0 ± 13.49	112.5 ± 12.40
Diastolic Blood Pressure (mmHg)	72.2 ± 7.67	73.7 ± 8.77	71.5 ± 7.91	71.7 ± 7.83
Triglycerides (mg/dL)	161.2 ± 68.1	155.3 ± 78.58	159.6 ± 77.92	150.7 ± 70.9
Fasting Glucose (mg/dL)	92.9 ± 8.76	92.8 ± 7.40	94.2 ± 10.52	93.9 ± 10.63
Waist Circumference (cm)	85.1 ± 11.65	84.9 ± 7.68	83.5 ± 9.33	83.7 ± 9.19
Percent Body Fat (%)	31.6 ± 5.71	31.9 ± 4.69	33.1 ± 5.21	32.8 ± 5.25
BMI (kg/m^2)	25.9 ± 3.94	26.5 ± 2.92	26.4 ± 3.42	26.3 ± 3.42
Metabolic Syndrome n(%)	10(27)	12(30)	11(33)	14(39)
Number of Abnormal Risk Factors	1.9 ± 1.17	1.9 ±1 .22	2.0 ± 1.12	1.9 ± 1.20

Key: LDL: low density lipoprotein cholesterol; HDL: high density lipoprotein cholesterol

Table 5.4. Baseline Values and Changes in Body Fat, Physical Activity, Physical Fitness, Dietary Fat, Saturated Fat and Cholesterol.[+]

Men	**Baseline**	**Δ Control**	**Δ Diet**	**Δ Exercise**	**Δ Diet plus Exercise**
Body Fat (%)	21.6 ± 0.32	0.6 ± 0.49	-1.6 ± 0.48[*]	-0.6 ± 0.58	-1.9 ± 0.48[*]
Physical Activity Score	28.1 ± 1.51	-3.7 ± 2.79	4.4 ± 3.62	13.3 ± 3.23[*]	21.7 ± 4.17[*]
VO_2max (mL/kg/min)	37.6 ± 0.51	-0.8 ± 0.53	0.4 ± 0.58	2.0 ± 0.67[*]	4.8 ± 0.71[*]
Fat (% of total intake)	30.4 ± 0.53	-0.7 ± 0.90	-8.2 ± 1.19[*]	-0.7 ± 0.90	-8.4 ± 0.86[*]
Saturated Fat (% of total intake)	10.1 ± 0.23	-0.02 ± 0.37	-3.6 ± 0.46[*]	-0.2 ± 0.41	-3.9 ± 0.39[*]
Cholesterol intake (mg)	257.2 ± 9.11	-7.0 ± 18.65	-106.3 ± 19.09[*]	-9.2 ± 15.22	-111.6 ±15.82[*]
Women	**Baseline**	**Δ Control**	**Δ Diet**	**Δ Exercise**	**Δ Diet plus Exercise**
Body Fat (%)	32.2 ± 0.42	1.2 ± 0.64	-1.1 ± 0.63[*]	0.1 ± 0.67	-1.9 ± 0.73[*]
Physical Activity Score	23.3 ± 1.93	12.4 ± 6.53	1.2 ± 2.48	16.0 ± 5.97	13.6 ± 6.02
VO_2max (mL/kg/min)	26.1 ± 0.40	-0.9 ± 0.41	0.2 ± 0.68	2.4 ± 0.63[*]	3.5 ± 0.68[*]
Fat (% of total intake)	28.2 ± 0.58	0.3 ± 1.05	-5.8 ± 1.13[*]	1.6 ± 1.14	-8.4 ± 1.01[*]
Saturated Fat (% of total intake)	8.9 ± 0.23	0.1 ± 0.43	-2.4 ± 0.44[*]	0.5 ± 0.56	-3.1 ± 0.39[*]
Cholesterol intake (mg)	176.3 ± 6.75	12.2 ± 14.66	-74.4 ± 10.92[*]	22.2 ± 16.5	-57.0 ± 11.84[*]

Adapted from Stefanick et al., 1998

[+] Means ± standard error

[*] $p < 0.05$ for the change in the variable between intervention group and control group

(Change in body fat, physical activity and VO_2max adjusted for age and baseline values; Fat, saturated fat and cholesterol adjusted for baseline values)

Metabolic Syndrome Prevalence

At baseline, prevalence of metabolic syndrome was 30% and 32% in men and women, respectively. In men at follow-up, the prevalence of metabolic syndrome was 20% in the controls, 17% in the diet group, 14% in the exercise group and 15% in the diet plus exercise group. For women at follow-up, the prevalence of metabolic syndrome at follow-up was 27% in the controls, 8% in the diet group, 21% in the exercise group and 22% in the diet plus exercise group.

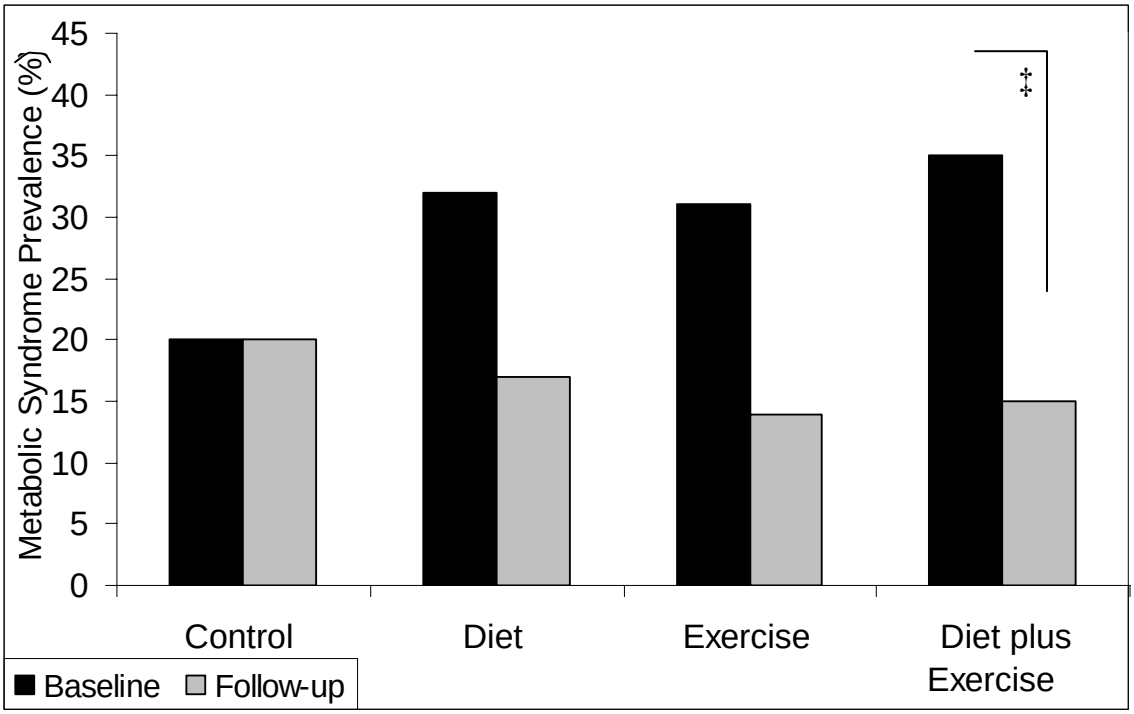
Differences in metabolic syndrome prevalence between intervention groups

Comparisons for the odds of having metabolic syndrome at follow-up between intervention groups revealed no differences in men ($p = 0.87$) (Figure 5.1) or women ($p = 0.12$) (Figure 5.2). The difference in the log odds for having metabolic syndrome between diet and control groups approached statistical significance in women (OR: 2.0, 95% CI: 0.37-3.59; $p = 0.0157$).

Differences in metabolic syndrome prevalence within intervention groups

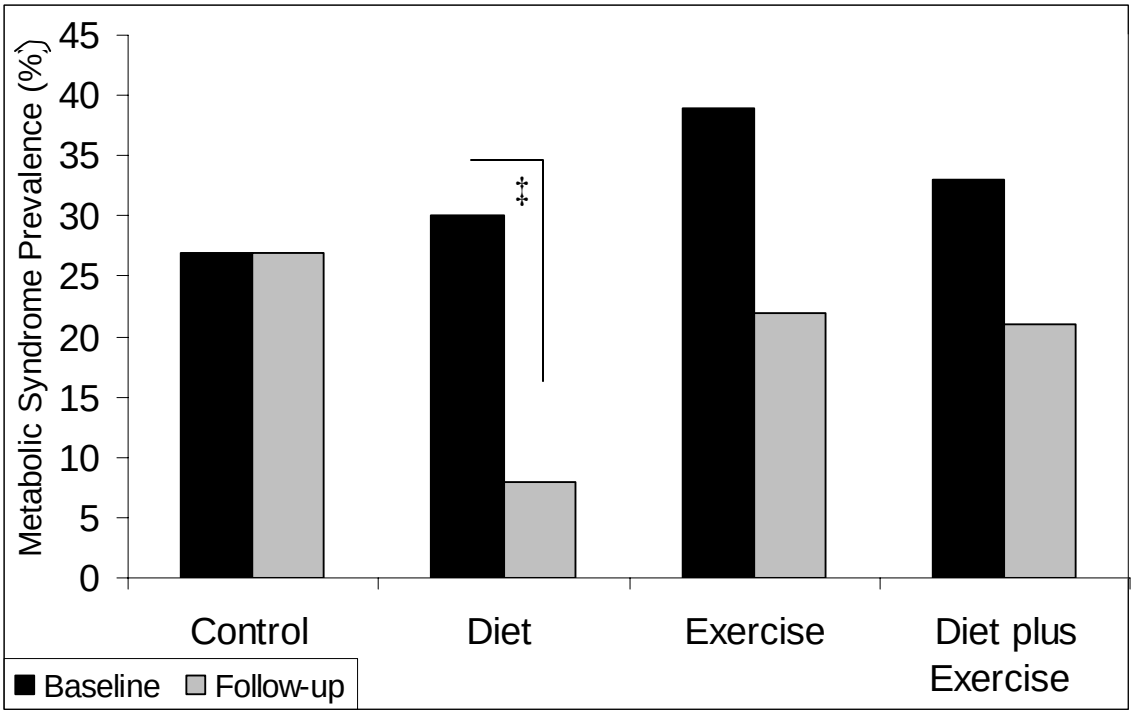
Differences in the odds ratio for metabolic syndrome from baseline to follow-up were found within the diet plus exercise groups in men (OR: 3.5, 95% CI: 1.14-10.56; $p = 0.029$) and the diet only group in women (OR: 5.9, 95% CI: 1.38-24.84; $p = 0.018$). Thus, for men in the diet plus exercise group, the odds of having metabolic syndrome at baseline, was 3.5 times of that at follow-up.

No changes in significance were noted after removing body fat change as a covariate either between or within intervention groups for metabolic syndrome prevalence.



‡ significant difference in metabolic syndrome within intervention group from baseline to follow-up

Figure 5.1. Changes in metabolic syndrome prevalence in men (n = 179). Unadjusted prevalence (%) at baseline and follow-up is shown. Logistic regression was utilized to compare metabolic syndrome prevalence at follow-up between intervention groups, controlling for baseline metabolic syndrome prevalence, change in body fat and age. No differences between control, diet, exercise and diet plus exercise were found for metabolic syndrome prevalence at follow-up in men. The diet plus exercise group had significant decreases in metabolic syndrome prevalence from baseline to follow-up ($p = 0.029$).



‡ significant difference in metabolic syndrome within intervention group from baseline to follow-up

Figure 5.2. Changes in metabolic syndrome prevalence in women (n = 146). Unadjusted prevalence (%) at baseline and follow-up is shown. Logistic regression compared metabolic syndrome prevalence at follow-up between intervention groups while adjusting for baseline metabolic syndrome prevalence, change in body fat, HRT and age. No differences were found between control, diet, exercise and diet plus exercise for metabolic syndrome prevalence at follow-up in women. The diet group had a significant decrease in metabolic syndrome prevalence from baseline to follow-up ($p = 0.018$).

Predictors of Metabolic Syndrome

Since no differences between intervention groups existed for metabolic syndrome prevalence at follow-up, intervention groups were combined by gender to examine possible predictors for metabolic syndrome at follow-up. The change in glucose, systolic blood pressure and diastolic blood pressure predicted the presence of metabolic syndrome at follow-up in men (Table 5.5). For women, the change in waist circumference, systolic blood pressure, diastolic blood pressure, triglycerides and glucose predicted the presence of metabolic syndrome at follow-up (Table 5.5). For a one unit change in a specific predictor, the odds for the presence of metabolic syndrome are expected to change by the odds ratio estimate. For example, in men, the odds ratio of metabolic syndrome at follow-up was predicted by the change in glucose as 1.1 (CI: 1.03-1.14), which indicates that there is increase in the odds of having the metabolic syndrome for each mg/dL increment change in glucose levels.

Table 5.5. Cardiometabolic Predictors of Metabolic Syndrome at Follow-up for Men and Women.

Men	OR	95% CI	p-value
Total Men (n=179)			
Change in Waist Circumference (cm)	1.1	0.99-1.20	0.08
Change in Triglycerides (mg/dL)	1.0	1.00-1.01	0.08
Change in HDL cholesterol (mg/dL)	0.9	0.85-1.02	0.11
Change in Systolic Blood Pressure (mmHg)	1.1	1.01-1.12	**0.02**
Change in Diastolic Blood Pressure (mmHg)	1.1	1.03-1.17	**0.005**
Change in Glucose (mg/dL)	1.1	1.03-1.14	**0.001**
Women	OR	95% CI	p-value
Total Women (n=146)			
Change in Waist Circumference (cm)	1.2	1.05-1.31	**0.005**
Change in Triglycerides (mg/dL)	1.0	1.01-1.02	**0.002**
Change in HDL cholesterol (mg/dL)	1.0	0.94-1.04	0.33
Change in Systolic Blood Pressure (mmHg)	1.1	1.01-1.09	**0.01**
Change in Diastolic Blood Pressure (mmHg)	1.1	1.04-1.21	**0.002**
Change in Glucose (mg/dL)	1.1	1.01-1.14	**0.03**

Abbreviations: CI: Confidence Intervals; HDL: high density lipoprotein

In men who had metabolic syndrome at baseline, significant differences for the changes in triglycerides, HDL, diastolic blood pressure and fasting glucose were found between those who maintained versus reversed metabolic syndrome status (Table 5.6). In women, significant differences in the mean values existed between those who reversed or maintained metabolic syndrome status for waist circumference, triglycerides, and diastolic blood pressure (Table 5.6).

Table 5.6. Differences in Cardiometabolic Risk Factors Between Individuals Who Maintained or Reversed Metabolic Syndrome.

	Men		Women	
	Mean Change[+]	**p-value***	**Mean Change**[+]	**p-value***
Waist Circumference (cm)				
Total	-3.3 ± 0.35		-3.1 ± 0.42	
Reverse	-5.6 ± 0.77		-5.3 ± 0.72	
Maintain	-2.8 ± 1.28	0.06	0.4 ± 0.88	**<0.0001**
Triglycerides (mg/dL)				
Total	-2.5 ± 6.04		-5.7 ± 5.52	
Reverse	-45.2 ± 14.28		-46.9 ± 12.40	
Maintain	21.0 ± 19.77	**0.009**	16.1 ± 16.41	**0.003**
HDL cholesterol (mg/dL)				
Total	0.3 ± 0.38		1.5 ± 0.64	
Reverse	3.0 ± 1.01		2.4 ± 1.67	
Maintain	-0.3 ± 0.87	**0.02**	1.3 ± 1.15	0.58
Systolic Blood Pressure (mmHg)				
Total	-0.5 ± 0.64		-2.1 ± 1.04	
Reverse	-3.6 ± 2.54		-4.6 ± 3.16	
Maintain	0.2 ± 2.54	0.19	2.0 ± 2.29	0.11
Diastolic Blood Pressure (mmHg)				
Total	-0.4 ± 0.54		-1.8 ± 0.59	
Reverse	-4.0 ± 1.15		-5.1 ± 1.58	
Maintain	0.5 ± 1.84	**0.04**	3.5 ± 1.44	**0.0003**
Fasting Glucose (mg/dL)				
Total	-6.5 ± 0.68		-6.3 ± 0.93	
Reverse	-11.9 ±1.44		-9.7 ± 1.41	
Maintain	-5.8 ±2.44	**0.03**	0.05± 4.73	0.059

[+] unadjusted means ± SE

[*] p-value: t-tests between mean values for individual cardiometabolic risk factors for reverse versus maintain

Total: men n =179; women n = 146

Reverse: Baseline metabolic syndrome, Follow-up no metabolic syndrome (men n = 35; women n = 26)

Maintain: Baseline metabolic syndrome, Follow-up metabolic syndrome (men n = 18; women n = 21)

Number of Abnormal Cardiometabolic Risk Factors

No differences were found between intervention groups for the number of abnormal risk factors at baseline in either men or women. Figures 5.3 and 5.4 display the adjusted changes in the number of abnormal cardiometabolic risk factors in men and women for each intervention group.

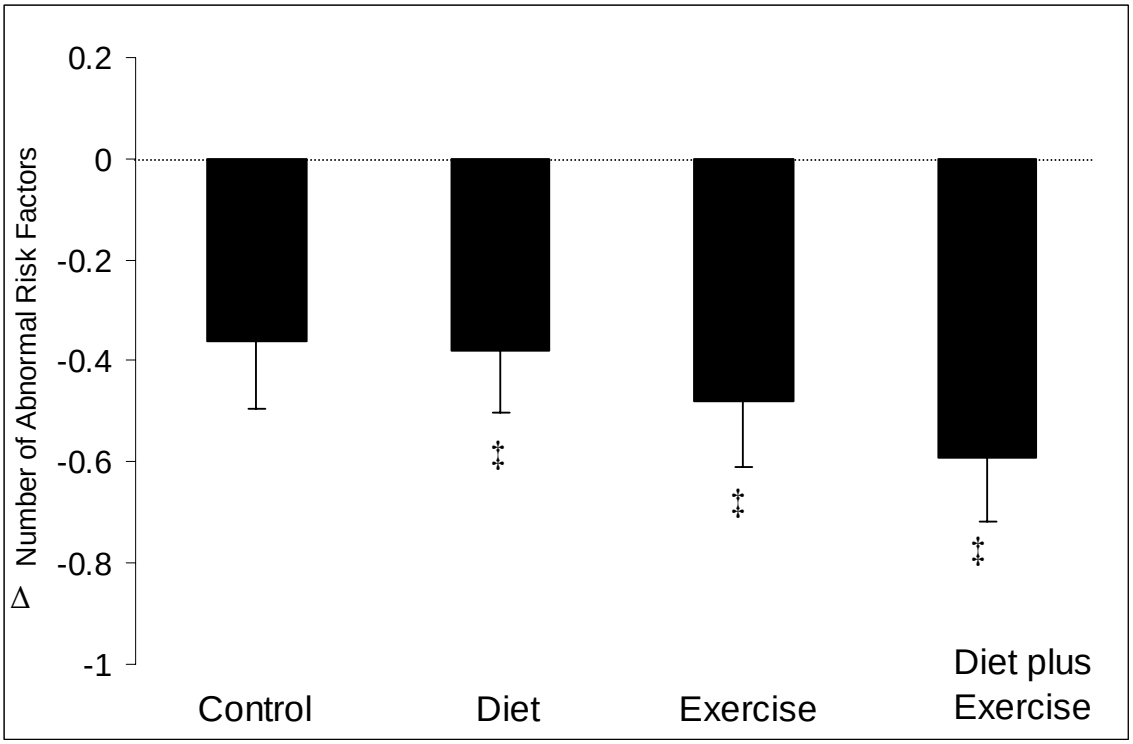
Changes in cardiometabolic risk factors between intervention groups

There were no differences for the change in abnormal risk factors between control, diet, exercise or diet plus exercise groups in men ($p = 0.53$) or women ($p = 0.42$).

Changes in cardiometabolic risk factors within intervention groups

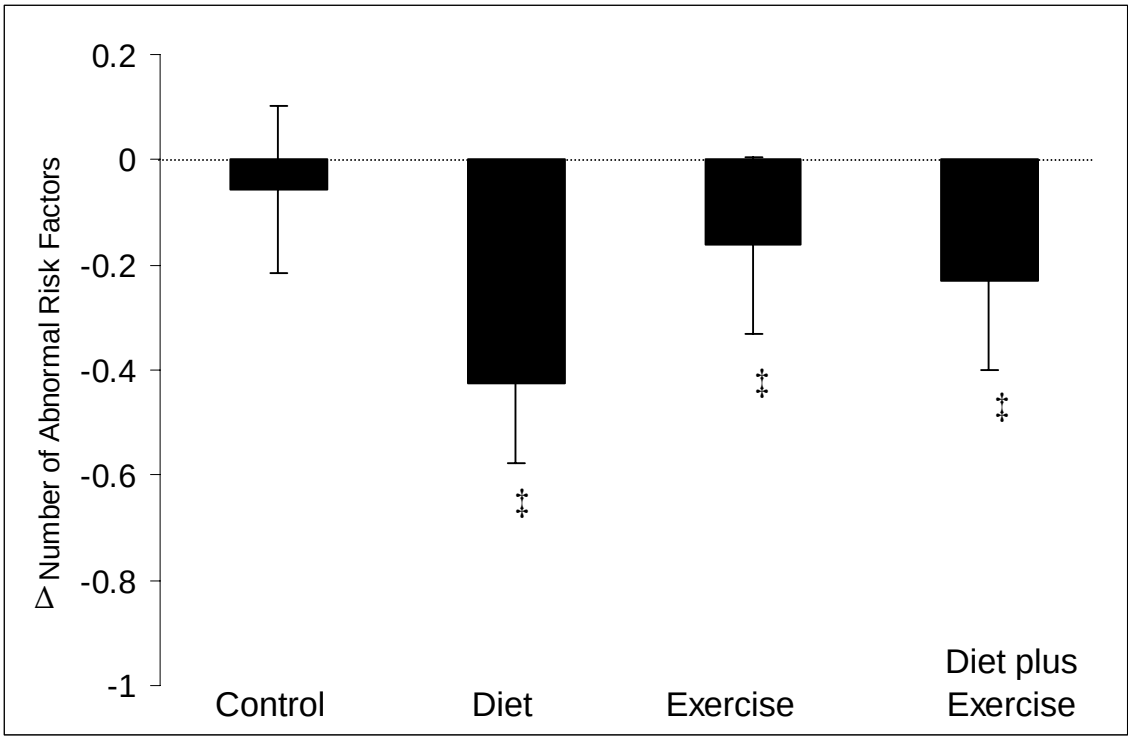
In men, decreases in the number of abnormal risk factors between baseline and follow-up were found for the diet group (-0.4 ± 0.19; $p = 0.02$), exercise group (-0.4 ± 0.15; $p = 0.006$) and diet plus exercise group (-0.7 ± 0.13; $p < 0.0001$). In women, decreases in the number of abnormal risk factors were significant for diet group (-0.6 ± 0.22; $p = 0.006$), exercise (-0.4 ± 0.11; $p = 0.003$) and diet plus exercise (-0.4 ± 0.20; $p = 0.045$).

No changes in significance were noted when removing body fat change as a covariate, either between or within intervention groups, for the number of abnormal cardiometabolic risk factors in men or women.



‡significant change in the number of abnormal risk factors within intervention group between baseline and follow-up

Figure 5.3. Change in the number of abnormal risk factors in men (n = 179). Adjusted means for the change ± standard error are shown. Comparisons between groups adjusted for baseline number of risk factors, change in body fat, cohort and age. No differences were found for the change in abnormal risk factors between control, diet, exercise and diet plus exercise groups in men. Decreases within intervention group from baseline were found within the diet group ($p = 0.02$), exercise group ($p = 0.006$) and diet plus exercise group ($p < 0.0001$).



‡significant change in the number of abnormal risk factors within intervention group between baseline and follow-up

Figure 5.4. Change in the number of abnormal risk factors in women (n = 146). Adjusted means ± standard error are shown. Comparisons between intervention groups controlled for baseline number of abnormal risk factors, change in body fat, cohort, hormone therapy status and age. No differences were found between control, diet, exercise or diet plus exercise for the change in the number of risk factors. Decreases within intervention group from baseline were significant within diet ($p = 0.01$), exercise ($p = 0.003$) and diet plus exercise ($p = 0.045$).

Discussion

To our knowledge, our study is the first to systematically compare the independent and combined effects of low-fat diet and exercise on metabolic syndrome prevalence from a randomized controlled trial. A one-year lifestyle intervention did not result in any significant differences for metabolic syndrome prevalence between the low-fat diet, exercise and diet plus exercise in either men or postmenopausal women relative to control. Despite the null findings for between intervention group differences, there were large net reductions for metabolic syndrome prevalence within groups for men (diet plus exercise: -57%) and women (diet: -76%). Furthermore, decreases in the number of abnormal risk factors were found within low-fat diet, exercise and diet plus exercise from baseline to follow-up, though none of these changes were significantly different from the control group. These results suggest that in comparison to a control group, low-fat diet, exercise and diet plus exercise are ineffective for lowering metabolic syndrome in men or women.

The low-fat diet and exercise program in the current study did not appear to have additive effects as hypothesized. Despite our own null findings, several studies found that diet plus exercise was more successful at reversing metabolic syndrome status over diet alone (Anderssen et al., 2007; Okura et al., 2007) or exercise alone (Anderssen et al., 2007). Importantly, Okura and colleagues did not have a control condition to compare the effects of diet alone or diet plus exercise (2007). Other discrepancies between our findings and Andersen et al., (2007) and Okura et al., (2007) may be due to their recruitment of only obese individuals, limiting generalizability to DEER's mostly normal weight and overweight men and women. Furthermore, both Okura et al., (2007) and

Anderssen et al., (2007) also restricted energy intake to promote weight loss. Weight loss can also favorably change the individual components of metabolic syndrome (Dansinger et al., 2005; Yu-Poth et al., 1999), whereby the greater the weight loss, the larger the magnitude of change in cardiovascular risk factors (Muzio et al., 2005). Our intervention did not promote weight loss, and weight loss was controlled for in the statistical analysis. Thus, our results from a diet plus exercise randomized controlled trial suggest that changing lifestyle in normal weight/overweight individuals, without an emphasis on weight loss may be insufficient for reversing metabolic syndrome status.

Another explanation for our findings may be due to the baseline characteristics of our study participants. One study suggests that diet plus exercise interventions are more successful for reducing metabolic syndrome prevalence in adults with abdominal obesity and hypertension compared with individuals with lipid abnormalities (Orchard et al., 2005). Our group of men and women were dyslipidemic at baseline, but did not exceed the abdominal obesity threshold or hypertension criteria for defining the metabolic syndrome. Thus, lifestyle intervention may have been less effective in resolving the metabolic syndrome in this specific group of participants.

Our non-significant findings for the independent effects of exercise for reducing metabolic syndrome prevalence for men and women are confirmed in other controlled studies (Stewart et al., 2005; Anderssen et al., 2007). However, our findings for the independent effects of low-fat diet on metabolic syndrome are not in agreement with previous research. Low-fat diet interventions have significantly decreased the presence of metabolic syndrome (Muzio et al., 2005), even in comparison to a control group (Azadbakht et al., 2005). However, Azadbakht et al., also induced weight loss and

included the DASH diet which modifies multiple dietary components beyond reducing fat intake (2005). Our findings suggest that reducing only fat intake, while controlling for the change in body fat, is not efficacious for reducing metabolic syndrome in men or women.

Previous interventions for low-fat diet plus exercise resulted in a decrease in metabolic syndrome prevalence (Esposito et al., 2004; Muzio et al., 2005; Orchard et al., 2005; Roberts et al., 2006). However, many of the combined diet plus exercise studies lacked a true control condition for comparison (Esposito et al., 2004; Muzio et al., 2005; Roberts et al., 2006), included significant weight loss (Esposito et al., 2004; Muzio et al., 2005; Orchard et al., 2005), or incorporated several dietary changes besides reducing fat intake (Esposito et al., 2003; Esposito et al., 2004). Our low-fat diet plus exercise intervention changed only dietary fat intake and controlled for weight loss, however no significant improvements in metabolic syndrome prevalence were found when compared to a control condition in either men or women.

A possible explanation for the lack of independent effects of diet, exercise and diet plus exercise versus the control group may be the nature of the control group. Eligibility criteria did not include sedentary individuals and the control group was not screened for high physical activity or low-fat dietary intake. While the control group did not adopt a dietary or fitness protocol, they were asked to continue their current daily routines. Therefore, this control group was not necessarily a sedentary, unfit or higher dietary fat consuming group. For example, as reported in Stefanick et al (1998), the total fat intake for men at baseline was approximately 30% and for women was 28%, which was already very close to the intervention goal of less than 30%. Therefore, the control

group was essentially meeting the dietary requirements for the intervention and there may not have been a sufficient change between controls and intervention groups to provide statistical power to detect differences between intervention groups.

Regardless of the addition of low-fat diet and/or exercise, metabolic syndrome status at follow-up was predicted by the change in blood pressure and glucose in men and women. Changes in waist circumference, and triglycerides were also factors for women. Bo and colleagues demonstrated that the changes in metabolic syndrome in adults are mostly related to the changes in triglycerides, glucose and waist circumference (2007). While the mechanism causing metabolic syndrome remains unknown, insulin resistance and adiposity are thought to be the main components (Eckel, Grundy, & Zimmet, 2005). Predictors of metabolic syndrome at follow-up for both men and women included surrogate measures of adiposity and insulin resistance.

Although the sample was considered at high cardiovascular risk due to their dyslipidemia, only approximately 30% had metabolic syndrome at baseline. It was hypothesized that metabolic syndrome would be closer to 55%, as evidenced in other studies of high cardiovascular risk adults (Orchard et al., 2005). The lower prevalence most likely reduced statistical power to detect significant differences between intervention groups for metabolic syndrome.

Men and women in this study were analyzed separately in order to account for the gender differences in recruitment and eligibility criteria concerning age and cardiovascular risk factors. Since metabolic syndrome is directly assessed from these variables, we felt that it was more appropriate to stratify genders for analyses. It is possible that separating the genders reduced power to explore between intervention group

differences. An additional statistical analysis was done which utilized the entire sample of men and women while controlling for age and gender. Gender was not significant in the overall model, which agrees with previous research for the response to exercise (Katzmarzyk et al., 2003) and diet plus exercise (Bo et al., 2007). Even with the larger sample size, there were no significant differences in metabolic syndrome prevalence at follow-up between control, diet, exercise and diet plus exercise.

Women in the current study were all postmenopausal, which places these women at an increased risk for metabolic syndrome (Park et al., 2003). While postmenopausal status does eliminate certain hormonal differences from men, it also reduces the generalizability of results of this study to premenopausal women. Also, it is important to consider that the sample is mostly Caucasian, highly educated, highly motivated and had high retention to measurement at follow-up, which further limits the generalizability of our findings.

Future work is needed to identify the ideal combination of diet and physical activity to successfully reduce metabolic syndrome in men and women. Low-fat dietary patterns such as the DASH and Mediterranean diet need to confirm findings for reversal of metabolic syndrome status without purposeful weight loss. Larger doses of physical activity have resulted in significant reductions in metabolic syndrome prevalence in elevated cardiovascular risk subjects (Johnson et al., 2007), and future studies should continue to examine the appropriate threshold of physical activity, also in combination with low-fat diet or dietary pattern.

In conclusion, no differences in metabolic syndrome were found in either men or women between a control group and a low-fat diet, exercise and diet plus exercise program.

Chapter 6

Changes in C-Reactive Protein from Low-Fat Diet and/or Exercise in Men and Women With and Without Metabolic Syndrome: Results from a Randomized Controlled Trial

Introduction

The process of atherosclerosis leading to cardiovascular disease has been hypothesized to be controlled through inflammation (Bhagat et al., 1997; Libby, 2002; Ross, 1999). Chronic inflammation is characterized by elevated levels of C-reactive protein (CRP), an acute phase reactant released from the liver in response to various cytokines such as interleukin-6 (IL-6) and tumor necrosis factor (TNF-α). CRP is also theorized to be an actual feature of metabolic syndrome, precursor to cardiovascular disease involving a clustering of multiple metabolic and lipid abnormalities (Festa et al., 2000).

Individuals with metabolic syndrome also possess elevated levels of inflammatory cytokines and CRP (Ford, 2003; Pitsavos et al., 2005; Saltevo et al., 2007). For every 1 pg/mL of CRP, there is a 37% increased risk of having metabolic syndrome (Hassinen et al., 2006). CRP and metabolic syndrome are each independent predictors of cardiovascular events (Rutter et al., 2004; Sattar et al., 2003) and the combination of both adds predictive and prognostic value to the estimation of cardiovascular disease and type II diabetes risk (Ridker et al., 2003b; Sattar et al., 2003).

Prevalence of metabolic syndrome among Americans is approximately 35% (Ford, 2005) while 25% of adults have elevated CRP levels (Pearson et al., 2003). For

management of metabolic syndrome, The National Cholesterol Education Program (NCEP) recommends weight loss, reduction in dietary fat intake and increased physical activity (2001). Diet or exercise induced weight loss can independently improve the individual components of metabolic syndrome (Yu-Poth et al., 1999) and CRP levels (Clifton et al., 2005; Due et al., 2005; Selvin et al., 2007). However, determining the most effective lifestyle treatment to lower CRP levels in individuals with metabolic syndrome without the contribution of weight loss is unknown. Few studies have compared the independent and combined effects of low-fat diet and exercise in individuals with metabolic syndrome.

Combined diet plus exercise studies show significant reductions in CRP in individuals with metabolic syndrome (Bo et al., 2007; Esposito et al., 2004; Roberts et al., 2006), even when weight loss was not an intervention goal (Bo et al., 2007; Roberts et al., 2006). However, these studies are either in a single gender (Roberts et al., 2006) or combine gender in the statistical analyses (Bo et al., 2007; Esposito et al., 2004). It is well established that women have higher levels of CRP than their age-matched male counterparts (Ford et al., 2003; Ford et al., 2004b). Research suggests that higher CRP values at baseline can result in larger decreases in CRP with both low-fat diet (Seshadri et al., 2004) and exercise (Goldhammer et al., 2005; Lakka et al., 2005). Thus, analyses of CRP from lifestyle intervention should be kept gender separate. Separate gender analysis revealed similar magnitudes of CRP change in insulin resistant men and women from a diet plus exercise intervention (Haffner et al., 2005), however these results may not be generalizable to individuals with metabolic syndrome who have multiple lipid and metabolic disorders.

The independent effects of low-fat diet and exercise have not been specifically examined for changes in CRP in individuals with metabolic syndrome. However, several lifestyle interventions have explored the results of lifestyle interventions in other high cardiovascular risk adults. Low-fat diet interventions have not shown success in reducing CRP in hyperlipidemic subjects (Jenkins et al., 2005) or hypertensive subjects in comparison to a control group (Erlinger et al., 2003). While exercise training lowered CRP in adults with normal glucose tolerance, insulin resistance and type II diabetes (Oberbach et al., 2006), these changes were not significant in dyslipidemic adults when compared to a control group (Huffman et al., 2006). Dyslipidemia and hypertension are only part of the metabolic syndrome, and the clustering of multiple risk factors may alter changes in CRP in response to low-fat diet or exercise.

The purpose of this study was to explore CRP changes resulting from a low-fat diet and /or exercise intervention, relative to controls, in men and women with and without metabolic syndrome. We hypothesized that the combined intervention of low-fat diet and exercise would be the most effective treatment for reducing CRP in both men and women. We also hypothesized that men and women with metabolic syndrome would benefit most from diet plus exercise, due to their higher overall CRP levels.

Methods

The Diet and Exercise for Elevated Risk Trial (DEER) was a one year single-center randomized controlled clinical trial which began in 1992 at Stanford Medical School. The original DEER objective was to examine the effects of diet and exercise on plasma lipoproteins in men and postmenopausal women (Stefanick et al., 1998). The

present secondary data analysis incorporated a new laboratory test for CRP and a retrospective analysis of metabolic syndrome to compare the effects of low-fat diet and exercise.

Specific eligibility criteria for men were: age 30-64 years, high density lipoprotein cholesterol (HDL) < 45 mg/dL, low density lipoprotein cholesterol (LDL) 126-189 mg/dL and body mass index (BMI) $\leq$ 34 kg/m^2. Eligibility criteria for women were: postmenopausal, age 45-64 years, HDL < 60 mg/dL, LDL 126-209 mg/dL and BMI $\leq$ 32 kg/m^2. In addition, men and women had blood pressure under 160/95 mmHg, triglycerides under 500 mg/dL, fasting glucose under 140 mg/dL and a normal maximal exercise treadmill test. Exclusion criteria included history of heart disease, insulin dependent diabetes mellitus, neuromuscular/orthopaedic disability, use of lipid medication, not euthyroid, low hematocrit, excessive smoking or alcohol consumption, inability to attend sessions or by judgment of a physician.

A variety of mass media was used for recruiting strategies from the Stanford University area in Palo Alto, California. Recruitment methods included worksite contacts, posted flyers, handouts, brochures to area physician offices and clinics, newsletters, mailings and paid advertisements.

Measurement

Potential participants had telephone screening, laboratory blood analysis, dietary screening, exercise habits assessment and a physical examination. Baseline measures were taken before randomization and then repeated at one year follow-up. Clinic staff were blinded to the specific treatment of the participants.

Body composition

Body weight was measured with a standard medical beam balance scale. Height was measured using a Harpenden stadiometer. Waist circumference was taken three times and averaged from the narrowest circumference of the torso when viewed from the front. BMI was calculated by dividing the participant's body weight in kilograms into their height squared (kg/m^2). BMI categories were determined using the guidelines presented by the National Institutes of Health (NIH) (1998). Body density and body composition estimates were made from skinfold measurements. Skinfolds were measured on the right side of the body in triplicate and averaged. For males, the locations of the skinfolds were chest, abdomen and thigh. For women, the skinfold locations were triceps, suprailiac and thigh. Body density was estimated using generalized equations (Jackson et al., 1978; Jackson et al., 1980) and percent body fat was calculated using the Siri equation (Siri, 1961).

Cardiovascular risk factors

Blood pressure was measured from the brachial artery utilizing a mercury sphygmomanometer and stethoscope. Averages for two readings of the first and fifth phase Korotkoff were recorded as systolic and diastolic blood pressure readings in millimeters of mercury (Fortmann et al., 1988).

Prior to venous blood collection, participants abstained from alcohol consumption and vigorous activity for at least 24 hours, food or drink for 12 hours and refrained from smoking for the hour prior. Blood samples were taken in the morning in duplicate at baseline. All blood specimens were mixed with 1.5 mg/mL of EDTA. Serum was

allowed to clot for 30-60 minutes, centrifuged, put on ice, and then plasma was transferred to the freezer for storage at -80° C.

Both fasting blood samples were analyzed for lipoproteins. Total cholesterol and triglycerides were measured using enzymatic procedures (Allain et al., 1974; Sampson et al., 1975). HDL was measured using dextran sulfate–magnesium precipitation (Warnick et al., 1982) as well as enzymatic measurement of non-precipitated cholesterol (Allain et al., 1974). Very low density lipoprotein (VLDL) was calculated as triglycerides divided by five (Friedewald et al., 1972). If triglyceride levels exceeded 400 mg/dL, enzymatic methods were used to measure VLDL according to Friedewald methods (Friedewald et al., 1972). LDL was calculated as total cholesterol minus the sum of HDL + VLDL (Friedewald et al., 1972). Lipoprotein values were averaged between the two baseline fasting values.

C-Reactive Protein

Plasma high sensitivity CRP concentrations were measured utilizing one of the two stored bloods chosen at random. CRP concentration was determined with an immunoturbidimetric assay on the Hitachi 917 analyzer (Roche Diagnostics - Indianapolis, IN) using reagents and calibrators from DiaSorin (Stillwater, MN). In this assay, an antigen-antibody reaction occurs between CRP and an anti-CRP antibody that has been sensitized to latex particles, resulting in agglutination. This antigen-antibody complex causes an increase in light scattering, which is detected spectrophotometrically, with the magnitude of the change being proportional to the concentration of CRP in the sample. Five-point calibration curves were constructed and standardized against CRM470. Values of density and absorption were read at 570 nm with a background

subtract of 800 nm. This assay has a sensitivity of 0.03 mg/L. The day-to-day variability of the assay at concentrations of 0.91, 3.07 and 13.38 mg/L are 2.81, 1.61 and 1.1%, respectively.

Metabolic syndrome definition

The National Cholesterol Education Program Adult Treatment Panel III (NCEP–ATP III) developed a definition to identify metabolic syndrome in which an individual must have three of the following: 1) waist circumference greater than 102 cm in men; 88 cm women, 2) triglycerides greater than or equal to 150 mg/dL, 3) HDL cholesterol less than 40 mg/dL for men; 50 mg/dL for women, 4) blood pressure greater than or equal to 130/85 mmHg and 5) fasting glucose greater than or equal to 100 mg/dL (2001; Grundy et al., 2004a).

Dietary intake

Five unannounced 24 hour dietary recalls captured one weekend day and four weekdays on non-consecutive days. The diet recalls utilized a computer assisted telephone interview by Nutrient Data Systems (NDS), developed by the University of Minnesota Nutrition Coordinating Center. Food database software Version 5a & 6a calculated nutrient intake as the mean from the five recalls.

Physical activity

Physical activity was assessed with the Godin Leisure Time Exercise Questionnaire (Godin) (Godin et al., 1985). The Godin questionnaire is both valid and reliable against maximal oxygen consumption testing in men and women (Godin et al., 1985; Jacobs, Jr. et al., 1993). The specific questions ask, "Considering a seven day period (a week), how many times on average do you do the following kinds of exercises

for more than 15 minutes during your free time?". Intensities assessed include 1) strenuous exercise (heart beats rapidly), 2) moderate exercise (not exhausting), and 3) mild exercise (minimal effort). Total weekly 15 minute bouts for strenuous, moderate and mild exercise are multiplied by 3, 5 and 9, respectively to convert to MET-weighted units. The total MET-weighted units are then summed to identify a physical activity score.

Physical fitness

Physical fitness was measured by oxygen consumption with a maximal graded exercise treadmill test. A semi-automated metabolic analysis system was used whereby oxygen uptake was measured every 30 seconds (Savin et al., 1980). Speed started at two miles per hour at 2.5% grade for three minutes and progressed to three miles an hour with an increase in grade 2.5% every three minutes. Subjects exercised to volitional fatigue and maximal oxygen consumption (VO_2max) was an average of the highest two measures during the final minutes of exercise.

Randomization

Individuals were randomized into four treatment groups: 1) control, 2) diet, 3) exercise and 4) diet plus exercise by the Efron procedure. Participants were assigned to a group by a computer algorithm which balanced groups for sample size, HDL and LDL cholesterol (Efron, 1971).

Figure 6.1 displays the intervention program details for the control, diet, exercise and diet plus exercise groups. The control group was advised to maintain their usual diet and exercise habits for one year. The dietary group followed the dietary goals set by the National Cholesterol Education Program NCEP Step II Guidelines (1993) to reduce total

fat, saturated fat, and dietary cholesterol. The exercise group engaged in a minimum of ten miles of walking/jogging each week at a moderate to vigorous intensity. Diet plus exercise participants received both interventions as individualized treatments. The individuals in the diet plus exercise group had separate nutrition and exercise sessions from the other groups to prevent contamination.

It is important to note that the diet, exercise and diet plus exercise groups did not emphasize weight loss as an intervention goal.

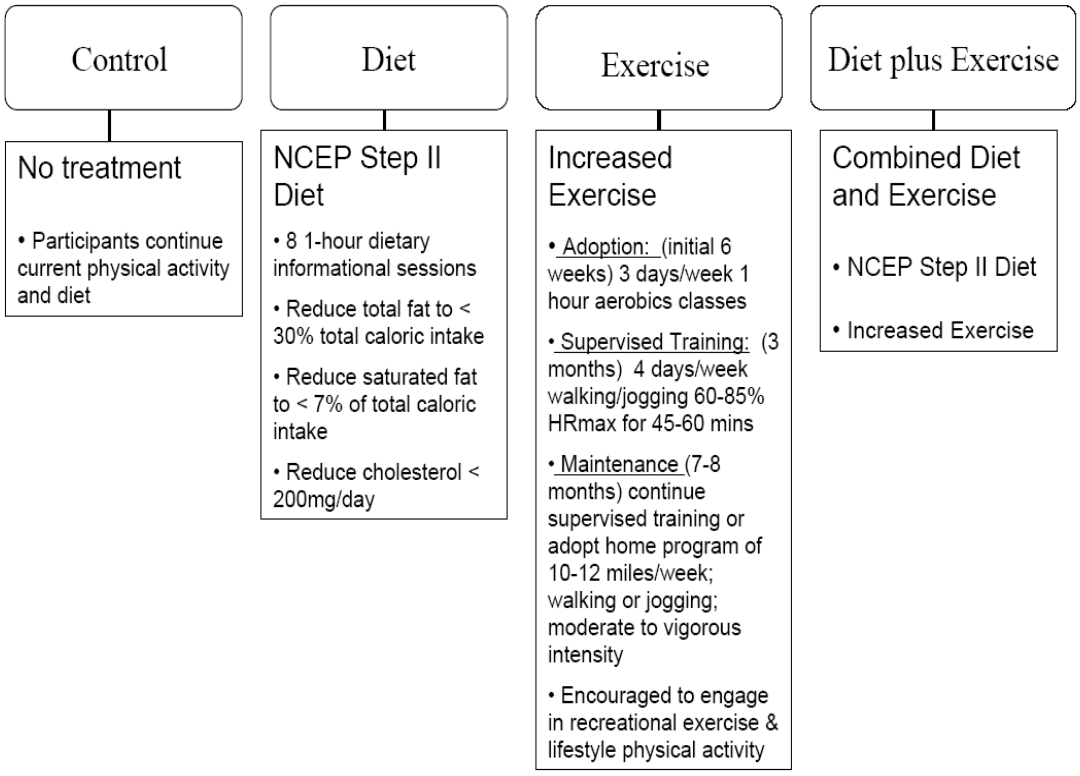


Figure 6.1. Intervention details for Control, Diet, Exercise and Diet plus Exercise Groups.

Statistical Analysis

All statistical analyses were performed by using SAS software version 9.1 (SAS Institute, Cary, NC). Participants with CRP levels over 10 mg/L were removed from the

analysis to eliminate the acute effects of infection (n=9) (Myers et al., 2004). Values were rounded to one decimal point to standardize CRP reporting (Myers et al., 2004). Since the DEER eligibility criteria were different for men and women, all analyses are done separately within gender.

Wilcoxon rank sum tests were used to compare CRP baseline level between metabolic syndrome status groups. χ-square examined differences in the distribution of individuals in baseline high risk CRP categories. Change was calculated as a difference between the follow-up value and the baseline value. Analysis of covariance (ANCOVA) compared the treatment effects on CRP changes separately for individuals with and without metabolic syndrome. Comparisons were made between intervention group differences for the change in CRP from baseline to follow-up: 1) control versus diet, 2) control versus exercise, 3) control versus diet plus exercise, 4) diet versus diet plus exercise and 5) exercise versus diet plus exercise. All models were adjusted for the change in percentage body fat to eliminate its known effect on the change in CRP, as well as baseline CRP, baseline body fat percentage, cohort, change in body fat, cigarettes per day, alcoholic drinks per day and age. Women had an additional covariate of hormonal replacement therapy status (HRT). All models were also analyzed without including the change in body fat as a covariate to note any change in significance. An α level of 0.01 was adopted to control for type I error and account for the multiple statistical comparisons.

Repeated measures generalized linear modeling, with the above mentioned covariates, assessed changes in CRP within each intervention group for those with and without metabolic syndrome. CRP values at baseline and follow-up were positively

skewed to the right, thus to satisfy the normality assumptions, a log transformation was made. Results for within group differences were quantified as Δ log CRP and were considered statistically significant if $p < 0.05$.

Results

Of the total 377 DEER participants who were randomized for the study, 274 participants (73%, 149 men and 125 women) had sufficient data for determining CRP changes and metabolic syndrome status. Participants were excluded from the current analysis due to incomplete data, which was assumed to be missing at random.

Men and women were mostly Caucasian (men: 85%; women: 88%), non-smokers (men: 99% women: 98%) and consumed one or less alcoholic drink per day (men: 92%; women: 95%). Men and women were highly educated: 61% of men and 42% of women had a college degree or greater. Approximately 44% of women were on HRT.

Table 6.1 and Table 6.2 show baseline study characteristics stratified by metabolic syndrome status for men and women, respectively. Classification of obesity according to the National Institutes of Health (NIH) (1998) was 32% in men with metabolic syndrome, while prevalence of obesity in men without metabolic syndrome was 7%. Classification of obesity in women with metabolic syndrome was 33%, whereas obesity prevalence in women without metabolic syndrome was 5%. Baseline CRP was 1.5 ± 1.43 mg/L in men with metabolic syndrome and 1.2 ± 1.24 mg/L in men without metabolic syndrome ($p = 0.24$). Baseline CRP was 2.4 ± 1.66 mg/L in women with metabolic syndrome and 1.7 ± 1.84 mg/L in women without metabolic syndrome ($p = 0.008$). According to the Centers for Disease Control and American Heart Association

classification (Myers et al., 2004), approximately 15% of men with metabolic syndrome and 8% of men without metabolic syndrome were considered to have high CRP at baseline ($p = 0.18$) Approximately 36% of women with metabolic syndrome and 13% of women without metabolic syndrome were considered to have high CRP at baseline ($p = 0.003$).

Changes in body fat, physical activity, fitness (VO_2max), total fat, saturated fat, and cholesterol intake are presented in men with and without metabolic syndrome (Table 6.3), and in women with and without metabolic syndrome (Table 6.4). There was little loss to follow-up for data collection, and retention was 98% and 96% in men and women, respectively (Stefanick et al., 1998).

Table 6.1. Baseline Characteristics in Men With and Without Metabolic Syndrome (mean ± SD).

With Metabolic Syndrome Men (n=47)	**Control**	**Diet**	**Exercise**	**Diet plus Exercise**
N(%)	9(19)	13(28)	11(23)	14(30)
Age	52.7 ± 11.27	52.4 ± 8.81	49.5 ± 10.07	48.7 ± 9.04
Waist Circumference (cm)	104.2 ± 10.50	102.2 ± 9.96	100.9 ± 7.75	100.4 ± 7.83
Body Fat (%)	22.3 ± 4.11	22.8 ± 4.37	22.9 ± 5.58	22.9 ± 5.58
BMI (kg/m^2)	29.0 ± 3.32	28.6 ± 3.05	28.2 ± 2.57	27.8 ± 2.81
CRP (mg/L)	1.8 ± 1.57	0.8 ± 0.29	1.6 ± 1.56	1.9 ± 1.74

Without Metabolic Syndrome Men (n=102)	**Control**	**Diet**	**Exercise**	**Diet plus Exercise**
N(%)	24(24)	26(25)	24(24)	28(27)
Age	47.3 ± 9.00	47.7 ± 9.11	49.5 ± 8.19	48.5 ± 7.80
Waist Circumference (cm)	91.9 ± 6.47	92.8 ± 8.83	92.8 ± 6.60	92.0 ± 7.50
Body Fat (%)	20.6 ± 3.36	20.5 ± 4.42	22.0 ± 4.75	20.9 ± 3.97
BMI (kg/m^2)	25.8 ± 2.72	26.1 ± 2.81	26.4 ± 2.52	26.0 ± 2.33
CRP (mg/L)	1.3 ± 1.44	1.1 ± 1.40	1.1 ± 1.11	1.2 ± 1.05

BMI: body mass index, CRP: C-reactive protein

Table 6.2. Baseline Characteristics in Women With and Without Metabolic Syndrome (mean ± SD).

With Metabolic Syndrome Women (n=39)	**Control**	**Diet**	**Exercise**	**Diet plus Exercise**
N(%)	9(23)	9(23)	9(23)	12(31)
Age	57.4 ± 5.94	59.7 ± 4.18	56.9 ± 5.56	58.1 ± 3.60
Waist Circumference (cm)	95.4 ± 10.15	91.6 ± 2.14	86.2 ± 4.37	93.4 ± 6.86
Body Fat (%)	33.8 ± 5.90	33.3 ± 5.22	31.2 ± 4.13	36.9 ± 2.74
BMI (kg/m^2)	28.8 ± 2.96	28.2 ± 2.87	26.1 ± 1.94	29.7 ± 1.77
CRP (mg/L)	2.6 ± 1.75	2.3 ± 1.83	2.2 ± 2.03	2.4 ± 1.40
Without Metabolic Syndrome Women (n=86)	**Control**	**Diet**	**Exercise**	**Diet plus Exercise**
N(%)	25(29)	23(27)	19(22)	19(22)
Age	58.8 ± 4.47	57.2 ± 5.69	57.7 ± 5.40	55.7 ± 5.12
Waist Circumference (cm)	81.8 ± 10.51	82.6 ± 7.88	82.5 ± 7.79	77.7 ± 5.50
Body Fat (%)	30.9 ± 5.75	31.0 ± 4.60	32.2 ± 5.21	30.1 ± 4.78
BMI (kg/m^2)	25.0 ± 3.86	25.9 ± 2.57	25.7 ± 2.60	24.3 ± 2.58
CRP (mg/L)	2.0 ± 2.41	1.6 ± 1.45	1.6 ± 1.35	1.7 ± 2.08

BMI: body mass index, CRP: C-reactive protein

Table 6.3. Baseline Values and Changes in Body Fat, Physical Activity, Physical Fitness, Dietary Fat, Saturated Fat and Cholesterol Intake in Men With and Without Metabolic Syndrome.[+]

With Metabolic Syndrome	**Baseline**	**Δ Control**	**Δ Diet**	**Δ Exercise**	**Δ Diet plus Exercise**
N	47	9	13	11	14
Body Fat (%)	22.8 ± 0.65	1.9 ± 1.04	-1.7 ± 1.15[*]	-0.9 ± 1.10	-1.92 ± 1.09[*]
Physical Activity Score	21.6 ± 2.94	-10.6 ± 8.71	3.1 ± 1.84	16.9 ± 5.75[*]	21.1 ± 3.83[*]
VO_2max (mL/kg/min)	35.1 ± 0.86	-1.2 ± 0.79	1.0 ± 1.13	1.3 ± 1.40	4.7 ± 1.32[*]
Fat (% of total intake)	32.4 ± 0.93	-4.0 ± 2.00	-7.2 ± 2.46	-4.7 ± 1.60	-9.3 ± 1.69
Saturated Fat (% of total intake)	10.9 ± 0.48	-1.1 ± 0.85	-3.8 ± 1.22	-1.2 ± 0.78	-3.9 ± 0.87[*]
Cholesterol (mg)	291.9 ± 18.86	-60.8 ± 45.01	-107.7 ± 32.74	-36.7 ± 39.14	-129.9 ± 23.81
Without Metabolic Syndrome	**Baseline**	**Δ Control**	**Δ Diet**	**Δ Exercise**	**Δ Diet plus Exercise**
N	102	24	26	24	28
Body Fat (%)	21.0 ± 0.41	-0.02 ± 0.69	-1.4 ± 0.62	-0.3 ± 0.85	-2.0 ± 0.54[*]
Physical Activity Score	30.6 ± 2.01	-2.2 ± 3.58	1.4 ± 4.70	11.6 ± 3.57[*]	21.5 ± 6.57[*]
VO_2max (mL/kg/min)	38.8 ± 0.69	-0.8 ± 0.80	0.9 ± 0.73	2.2 ± 0.94[*]	4.9 ± 0.97[*]
Fat (% of total intake)	29.7 ± 0.73	0.02 ± 1.32	-9.4 ± 1.62	0.9 ± 5.37[*]	-7.8 ± 1.12[*]
Saturated Fat (% of total intake)	9.7 ± 0.31	0.2 ± 0.53	-3.8 ± 0.50[*]	0.2 ± 0.41	-3.9 ± 0.46[*]
Cholesterol (mg)	247.4 ± 12.30	1.7 ± 26.22	-119.4 ± 26.96[*]	9.2 ± 14.5	-100.5 ± 23.02[*]

Adapted from Stefanick et al., 1998

[+] Means ± standard error

[*] $p < 0.05$ for the change in the variable between intervention group and control group

(Change in body fat, physical activity and VO_2max adjusted for age and baseline values; Fat, saturated fat and cholesterol adjusted for baseline values)

Table 6.4. Baseline Values and Changes in Body Fat, Physical Activity, Physical Fitness, Dietary Fat, Saturated Fat and Cholesterol Intake in Women With and Without Metabolic Syndrome.[+]

With Metabolic Syndrome	**Baseline**	**Δ Control**	**Δ Diet**	**Δ Exercise**	**Δ Diet plus Exercise**
N	39	9	9	9	12
Body Fat (%)	34.0 ± 0.77	2.3 ± 1.54	-0.6 ± 1.40	-0.7 ± 1.25	-2.0 ± 1.08
Physical Activity Score	21.2 ± 3.08	31.7 ± 19.87	3.8 ± 8.47*	32.3 ± 17.57	16.6 ± 5.90
VO_2max (mL/kg/min)	24.5 ± 0.61	-1.5 ± 1.35	2.0 ± 2.63	2.0 ± 1.15	3.6 ± 0.67*
Fat (% of total intake)	29.9 ± 1.24	0.6 ± 2.18	-4.2 ± 2.33*	-0.7 ± 1.59	-8.7 ± 2.00*
Saturated Fat (% of total intake)	9.8 ± 0.53	0.3 ± 0.91	-1.2 ± 0.92	-0.4 ± 0.74	-3.3 ± 0.70*
Cholesterol intake (mg)	176.9 ± 11.15	10.5 ± 25.86	-73.2 ± 21.99*	-4.6 ± 27.37	-40.9 ± 20.33*
Without Metabolic Syndrome	**Baseline**	**Δ Control**	**Δ Diet**	**Δ Exercise**	**Δ Diet plus Exercise**
N	86	25	23	19	19
Body Fat (%)	31.1 ± 0.55	1.1 ± 0.64	-1.7 ± 0.94*	0.6 ± 0.92	-2.1 ± 1.20*
Physical Activity Score	24.4 ± 2.86	7.2 ± 6.37	1.7 ± 2.50	10.5 ± 4.95	8.6 ± 10.32
VO_2max (mL/kg/min)	26.7 ± 0.53	-0.9 ± 0.42	0.02 ± 0.53	2.2 ± 0.78*	3.4 ± 1.04*
Fat (% of total intake)	27.3 ± 0.75	-0.1 ± 1.33	-6.6 ± 1.58*	2.3 ± 1.46	-8.5 ± 1.39*
Saturated Fat (% of total intake)	8.6 ± 0.29	0.04 ± 0.54	-2.7 ± 0.61*	0.5 ± 0.77	-3.2 ± 0.58*
Cholesterol intake (mg)	168.9 ± 8.75	6.3 ± 18.96	-64.8 ± 15.51*	20.5 ± 22.08	-68.4 ± 14.17*

Adapted from Stefanick et al., 1998

[+] Means ± standard error

* $p < 0.05$ for the change in the variable between intervention group and control group

(Change in body fat, physical activity and VO_2max adjusted for age and baseline values; Fat, saturated fat and cholesterol adjusted for baseline values)

CRP Changes in Men and Women With Metabolic Syndrome

CRP changes between intervention groups

No significant differences existed for CRP changes between intervention groups in the men with metabolic syndrome ($p = 0.77$) (Figure 6.2). Differences for the change in CRP were found between intervention groups in women with metabolic syndrome ($p < 0.01$) (Figure 6.3). Women with metabolic syndrome had differences for the CRP change between the diet versus control group (-1.2 ± 0.43 mg/L; $p = 0.009$) as well as the diet plus exercise versus control group (-1.3 ± 0.43 mg/L; $p = 0.006$). Women with metabolic syndrome in the diet plus exercise group also had differences in the CRP change when compared to exercise alone (-1.1 ± 0.44 mg/L; $p = 0.02$).

CRP changes within intervention groups

For men with metabolic syndrome, the CRP changes from baseline within the dietary, exercise, or diet plus exercise groups from baseline to follow-up were not significant. In women with metabolic syndrome, only the change in CRP from baseline within the diet plus exercise group was significant (Δ log CRP 0.2 ± 0.039 mg/L; $p=0.0008$).

Removing the change in body fat as a covariate did not alter the significance between or within intervention groups for the response in CRP in either men or women with the metabolic syndrome.

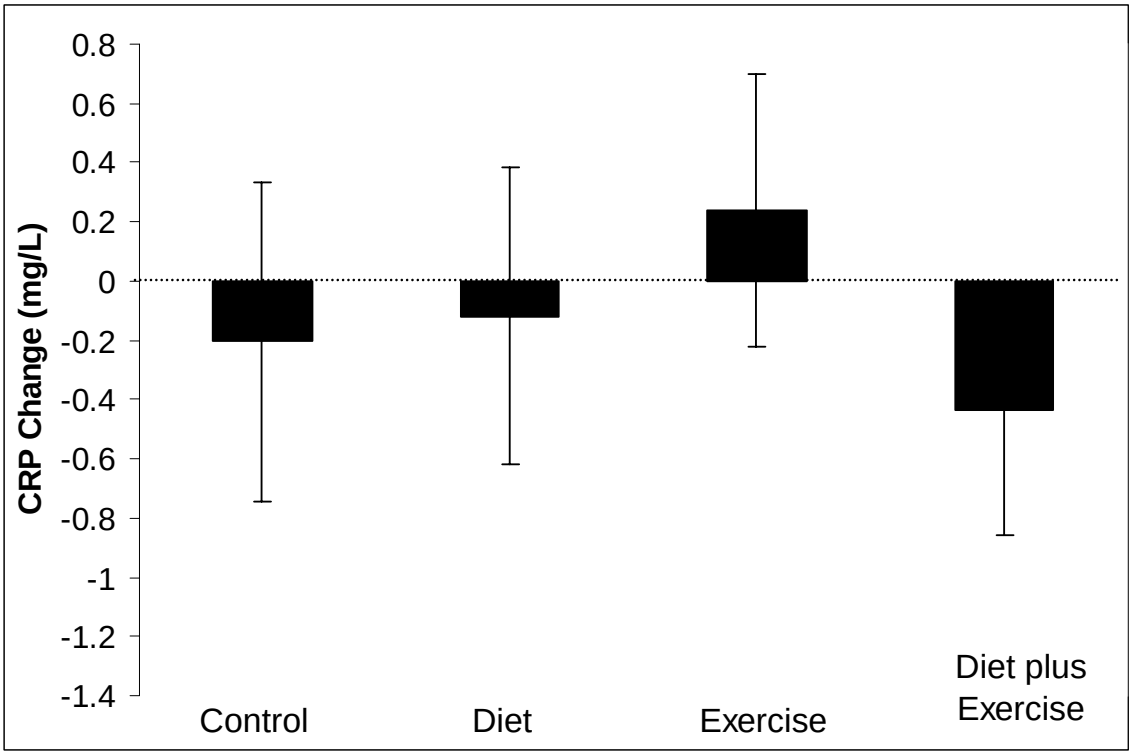
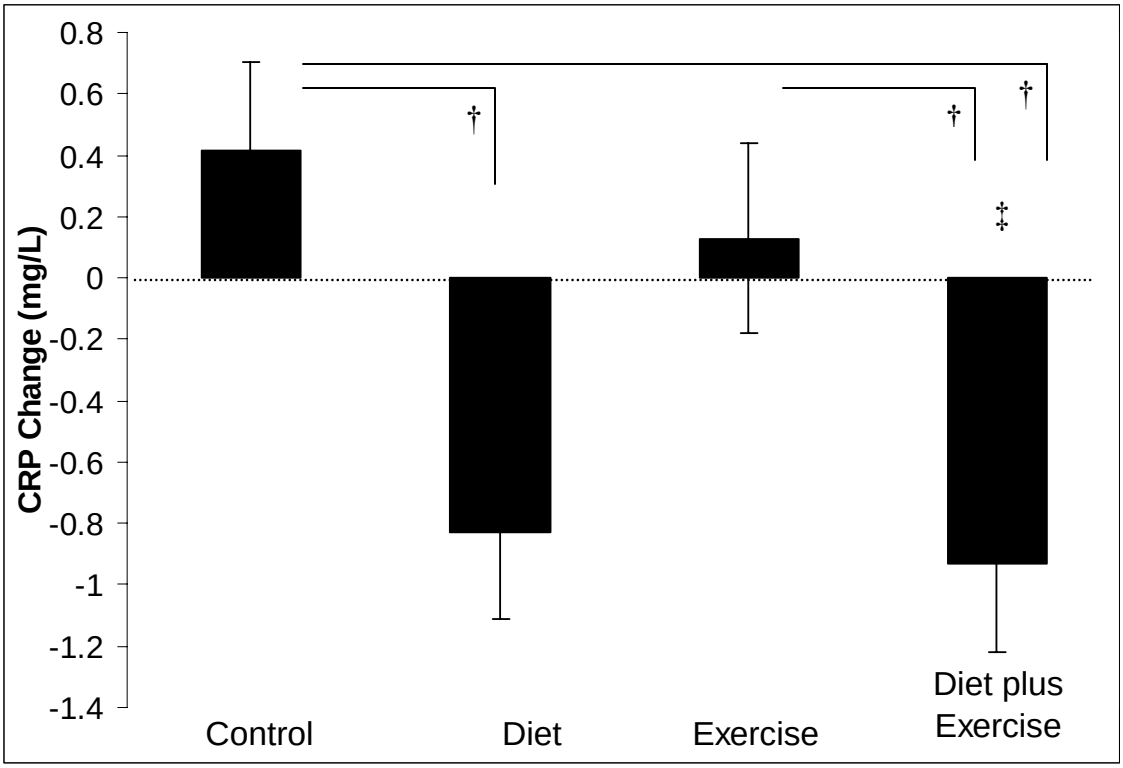


Figure 6.2. Change in CRP for men with metabolic syndrome (n = 47). Adjusted means ± standard error are shown for CRP changes from baseline to follow-up. ANCOVA statistical comparisons between intervention groups adjusted for the following covariates: baseline CRP, baseline body fat percentage, cohort, change in body fat, cigarettes per day, alcoholic drinks per day, and age. No differences existed for the change in CRP between or within control, low-fat diet, exercise or diet plus exercise groups in men with metabolic syndrome.



† significant difference in CRP change between intervention groups
‡ significant reduction in CRP from baseline to follow-up within intervention group

Figure 6.3. Change in CRP for women with metabolic syndrome (n = 39). Adjusted means ± standard error are shown for CRP changes from baseline and follow-up. ANCOVA statistical comparisons between intervention groups adjusted for the following covariates: baseline CRP, baseline body fat percentage, cohort, change in body fat, cigarettes per day, alcoholic drinks per day, age and HRT. CRP changes were different between the control and diet groups ($p = 0.009$), control and diet plus exercise groups ($p = 0.006$) and exercise and diet plus exercise groups ($p = 0.02$). CRP was reduced from baseline within the diet plus exercise group ($p = 0.0008$).

CRP Changes in Men and Women Without Metabolic Syndrome

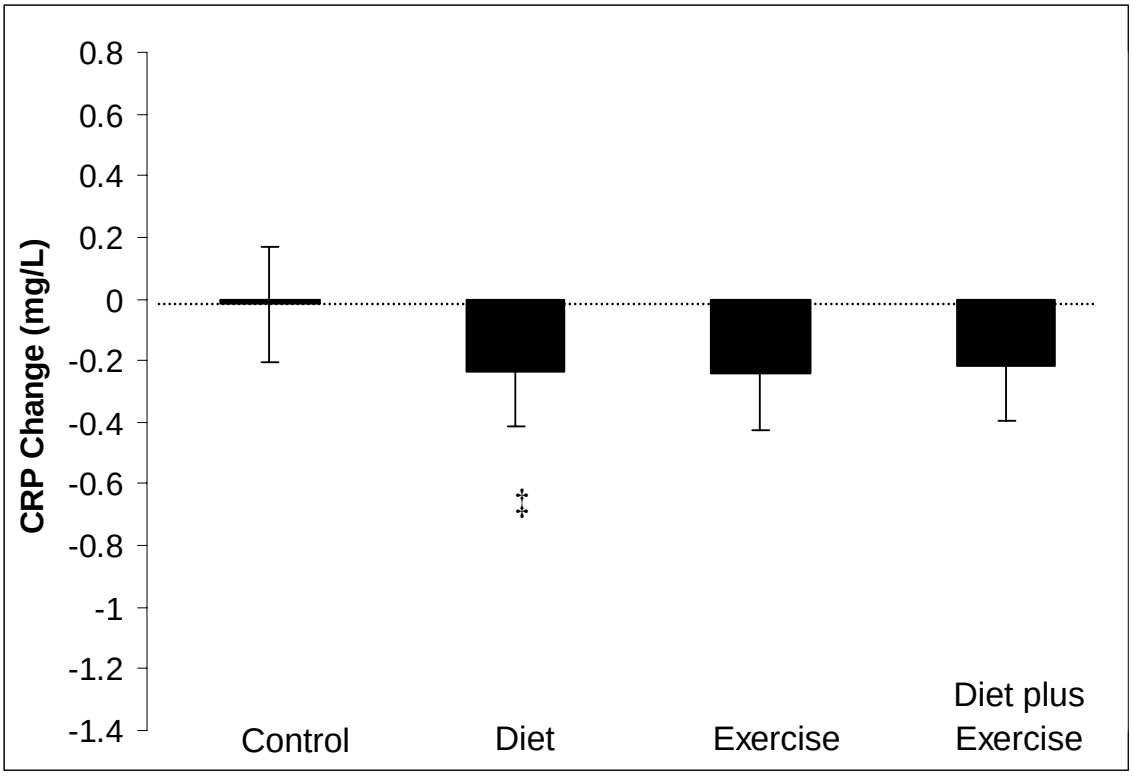
CRP changes between intervention groups

No significant differences were found for the CRP changes between intervention groups in the men without metabolic syndrome ($p = 0.79$) (Figure 6.4) or women without metabolic syndrome ($p = 0.31$) (Figure 6.5).

CRP changes within intervention groups

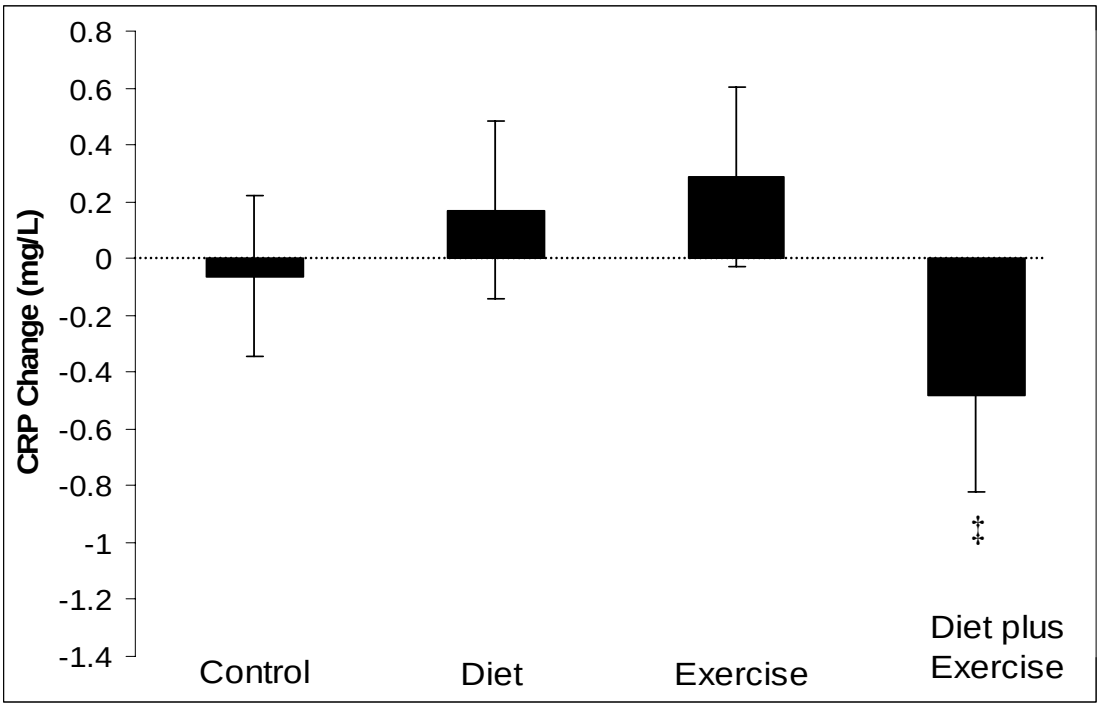
In men without metabolic syndrome, the diet group showed a reduction in CRP from baseline to follow-up (Δ log CRP: 0.1 ± 0.045 mg/L; $p = 0.03$). For women without metabolic syndrome, reductions in CRP from baseline to follow-up were significant within the diet plus exercise group (Δ log CRP: 0.1 ± 0.054 mg/L; $p = 0.02$).

Removing the change in body fat as a covariate did not alter the significance between or within intervention groups for the changes in CRP in men or women without metabolic syndrome.



‡ significant reduction in CRP from baseline to follow-up within intervention group

Figure 6.4. Change in CRP for men without metabolic syndrome (n = 102). Adjusted means ± standard error are shown for CRP changes from baseline to follow-up. ANCOVA statistical comparisons between intervention groups adjusted for the following covariates: baseline CRP, baseline body fat percentage, cohort, change in body fat, cigarettes per day, alcoholic drinks per day, and age. No differences existed for the change in CRP between intervention groups for the men without metabolic syndrome. CRP change from baseline in men without metabolic syndrome was significant within the diet only group ($p = 0.03$).



‡ significant reduction in CRP from baseline to follow-up within intervention group

Figure 6.5. Change in CRP in women without metabolic syndrome (n = 86). Adjusted means ± standard error are shown for CRP changes from baseline to follow-up. ANCOVA statistical comparisons between intervention groups adjusted for the following covariates: baseline CRP, baseline body fat percentage, cohort, change in body fat, cigarettes per day, alcoholic drinks per day, age and HRT. No differences existed between intervention groups for the changes in CRP in women without metabolic syndrome. CRP was reduced from baseline within the diet plus exercise group ($p = 0.02$) in women without metabolic syndrome.

Discussion

To our knowledge, this is the first paper to explore CRP changes in a low-fat diet and/or exercise intervention in men and postmenopausal women with and without the metabolic syndrome. When compared to the control group, women with metabolic syndrome had significant reductions in CRP for the low-fat diet and diet plus exercise group. In addition, women with metabolic syndrome had significant reductions for CRP in diet plus exercise when compared to the exercise group. In men with metabolic syndrome, no differences were found between CRP changes for control, low-fat diet, exercise and diet plus exercise. For both men and women without metabolic syndrome, no significant changes in CRP were found between the intervention and control conditions.

Explanations for why we only found significant results in women with metabolic syndrome may be due to their elevated baseline CRP values. In the present study, women with metabolic syndrome had significantly higher CRP levels than women without metabolic syndrome at baseline. Some low-fat diet and exercise interventions have found decreased CRP levels only in individuals who initially had elevated levels of CRP at baseline (Goldhammer et al., 2005; Lakka et al., 2005; Seshadri et al., 2004). CRP levels in men with metabolic syndrome were not significantly elevated above their healthy counterparts, which may have weakened the CRP response to low-fat diet and/or exercise. Future studies are needed to clarify whether the significant CRP reductions were an influence of gender or a result of higher baseline values.

Women with metabolic syndrome reduced their CRP levels with low-fat diet and diet plus exercise, however, no differences were found between these two groups. In

addition, no independent effects for exercise on CRP were found. These results suggest that low-fat diet may be the most important component for reducing CRP levels in women with metabolic syndrome. Our findings are supported by work from Nicklas et al., who also found significant reductions for CRP from low-fat diet and diet plus exercise, but not from exercise (2004). However, Nicklas et al., involved a group of elderly, arthritic, obese adults with purposeful weight loss (2004). Thus our study is the first to confirm the benefits of low-fat diet alone and in combination with exercise, while controlling for the change in body fat, on CRP levels in individuals with the metabolic syndrome.

The independent success of low-fat diet was also confirmed in a study of dyslipidemic adults (Jenkins et al., 2005), though a portfolio diet was utilized (combination of cholesterol lowering foods: viscous fibers, plant sterols, soy foods and almonds), making it difficult to distinguish the successful factor or factors. Our null findings for the independent changes in CRP from exercise training in comparison to control are confirmed by Huffman et al., who also studied the CRP response in dyslipidemic adults (Huffman et al., 2006). Our study supports previous low-fat diet and exercise work in dyslipidemic adults, while translating these findings to individuals with multiple metabolic and lipid abnormalities.

Combined diet and exercise interventions show decreases in CRP in insulin resistant adults (Haffner et al., 2005), women with multiple cardiovascular risk factors (Wegge et al., 2004) and in adults with metabolic syndrome (Esposito et al., 2004). However, several diet plus exercise interventions also incorporated weight loss (Haffner et al., 2005; Esposito et al., 2004) which has been suggested as the most important

contributor for changes in CRP for a combined intervention (Haffner et al., 2005). Even after controlling for changes in body fat (Esposito et al., 2004) or allowing ad-libitum dietary consumption (Wegge et al., 2004; Roberts et al., 2006), CRP reductions were still significant within the diet plus exercise group which is consistent with our research.

In contrast to our findings in individuals without metabolic syndrome, evidence for CRP changes in healthy men and women have shown positive effects from a low-fat diet (Clifton et al., 2005; Heilbronn et al., 2001; O'Brien et al., 2005; Tchernof et al., 2002), exercise (Mattusch et al., 2000; Obisesan et al., 2004; Okita et al., 2004) and diet plus exercise (Dvorakova-Lorenzova et al., 2006; Esposito et al., 2003; Marfella et al., 2004; Nicklas et al., 2004; You et al., 2004). However, Lakka et al., found that only healthy adults with initially high CRP values were able to lower their CRP with exercise and weight loss (Lakka et al., 2005). Since our individuals without metabolic syndrome did not have an overall elevated mean, and the intervention did not emphasize weight loss, there may not have been the appropriate stimulus for CRP change. In order to clarify the ideal treatment for lowering CRP in individuals with and without metabolic syndrome, future studies should clarify the added effect of weight loss, and more specifically fat loss, with low-fat diet and exercise.

The underlying physiological mechanisms to explain the reduction of CRP through low-fat diet and exercise remain unknown. Low-fat meals may increase intake of naturally anti-inflammatory foods (Middleton E Jr, 1998) and limit the postprandial glucose response (McCarty, 2005). These alterations may inhibit the cytokine release into the bloodstream (McCarty, 2005) and CRP release from the endothelium (De, Liao, & Libby, 2000). Increased physical activity increases the release of IL-6, which initiates

a negative feedback loop for secretion of anti-inflammatory cytokines (Pedersen, 2006; Petersen et al., 2005). Future studies are needed to uncover the biological mechanisms to explain the additive effects of low-fat diet and exercise for the changes in CRP for individuals with metabolic syndrome.

Limitations of the present study include a highly educated, highly motivated, and mostly Caucasian group of men and postmenopausal women which limits overall generalizability. The statistical analysis to determine differences in CRP between intervention groups for individuals with metabolic syndrome had a reduced sample size, which may have reduced statistical power. Therefore, although significant findings were found, these analyses were exploratory, and further study is warranted.

In conclusion, men with metabolic syndrome showed no changes in CRP levels within and between control, diet, exercise and diet plus exercise. In contrast, women with metabolic syndrome successfully reduced CRP levels with low-fat diet and diet plus exercise programs in comparison to control. Men and women without metabolic syndrome were not successful at decreasing CRP levels between any intervention groups.

Chapter 7

Summary, Conclusions and Future Directions

Few randomized controlled clinical trials have compared the response of a low-fat diet and increased physical activity, on CRP and metabolic syndrome. In this dissertation, we compared: 1) the effects of a low-fat diet and exercise, alone and in combination, on CRP levels in a high risk adult population, 2) the effects of a low-fat diet and exercise, alone and in combination, on metabolic syndrome, and 3) the changes in CRP in men and women with and without metabolic syndrome in response to a low-fat diet and/or exercise. This chapter serves as an executive summary of the findings from all three studies with recommendations for future research.

Comparisons Between Low-Fat Diet, Exercise and Diet Plus Exercise on CRP

In postmenopausal women, CRP was significantly reduced within the low-fat diet plus exercise from baseline (Δ log CRP 0.2 ± 0.035 mg/L; $p = 0.0002$). The change in CRP for the diet plus exercise group was significantly different in comparison to the control group (-0.7 ± 0.33 mg/L, $p = 0.04$) and the exercise group (-0.9 ± 0.32 mg/L, $p = 0.004$). The independent effects of low-fat diet alone and exercise alone, while controlling for the change in body fat, appear to be ineffective for lowering CRP levels in women. Men did not show differences for CRP between control, diet, exercise or diet plus exercise.

This dissertation provides evidence for the combination of diet plus exercise changes, in order to reduce CRP in postmenopausal women. Future studies are also

needed to elucidate the response of CRP to lifestyle intervention in men and explore possible biological mechanisms to explain the possible gender discrepancy.

Comparisons Between Low-Fat Diet, Exercise and Diet Plus Exercise on Metabolic Syndrome

No differences were found in metabolic syndrome prevalence between control, low-fat diet, exercise and diet plus exercise in either men or postmenopausal women. There were also no differences between intervention groups for the change in number of abnormal cardiometabolic risk factors in either men or women. Despite the null findings between groups, there were: 1) significant net reductions for metabolic syndrome prevalence from baseline within the diet plus exercise group in men (-57%), 2) significant net reductions for metabolic syndrome prevalence within the diet group in women (-76%), 3) decreases in the number of abnormal risk factors within the diet, exercise and diet plus exercise groups in both men and women.

This dissertation clarifies recommendations from the NCEP for treating metabolic syndrome. Our research implies that after controlling for the changes in body fat, there are no differences between control, diet, exercise and diet plus exercise for reversing metabolic syndrome in men and women. Low-fat dietary patterns such as the DASH and Mediterranean diet need to replicate findings for reversal of metabolic syndrome without concurrent weight loss. Future studies should continue to explore the effect of varying dose of physical activity on metabolic syndrome (Johnson et al., 2007). Further research is needed to identify the ideal combination of lifestyle treatment for reversing metabolic syndrome.

Comparisons Between Low-Fat Diet, Exercise and Diet Plus Exercise on CRP in Adults with and without Metabolic Syndrome

To our knowledge, this is the first paper to explore CRP changes in a low-fat diet and/or exercise intervention in men and postmenopausal women with and without metabolic syndrome. In women with the metabolic syndrome, the change from baseline to follow-up CRP within the diet plus exercise group was significant (Δ log CRP 0.2 ± 0.039 mg/L; p=0.0008). The difference between the change in CRP between the diet plus exercise group and control group was significant (-1.3 ± 0.43 mg/L; p = 0.006), as well as between the diet plus exercise group and exercise only group (-1.1 ± 0.44 mg/L; p = 0.02). Finally, women with metabolic syndrome had a significant difference for the change in CRP between the low-fat diet and control groups (-1.2 ± 0.43 mg/L; p = 0.009). Our study confirms the benefits of low-fat diet alone and in combination with exercise, in women with metabolic syndrome.

For men with metabolic syndrome, CRP changes were not different between control, low-fat diet, exercise and diet plus exercise. Furthermore, in both men and women without metabolic syndrome, no significant changes in CRP were found between any of the intervention and control conditions.

Replication of these findings is needed to explain whether the significant CRP reductions were an influence of metabolic syndrome, gender or a result of higher baseline CRP values.

In summary, this dissertation describes the change of CRP and metabolic syndrome prevalence utilizing a randomized controlled trial of low-fat diet, exercise and diet plus exercise. Weight loss was not a major intervention focus, and all changes in

body fat were controlled for in the statistical analysis, eliminating a known influence on both CRP and metabolic syndrome. All analyses were gender stratified in order to characterize the results from low-fat diet and exercise independently in men and women. Finally, changes in CRP from lifestyle interventions were characterized separately in adults with and without the presence of metabolic syndrome. This dissertation provides evidence for the effectiveness of diet and diet plus exercise for reducing CRP in women with metabolic syndrome.

Appendices

Appendix I: Definitions of Common Terms

*Body Composition***:** Body composition refers to a component of fitness that relates to the proportion of metabolically active and inactive tissues of the body which may include muscle, adipose tissue, bone and fluids.

*Exercise***:** Exercise is a term used to describe a planned and structured approach to repetitive bodily movement done to improve or maintain components of physical fitness.

*Intervention***:** Intervention studies are research in which subjects are enrolled into a specific exercise program, measured at baseline and then at follow-up to ascertain the changes in various variables.

*MET***:** A MET indicates a unit of resting metabolic rate and is an indication of exercise intensity (1-3 – light; 4-6 – moderate; 6+ - heavy) (Pate et al., 1995).

*Physical Activity***:** Physical activity will be operationally defined as "any bodily movement produced by skeletal muscle that results in energy expenditure" (pg. 126) (Caspersen, Powell, & Christenson, 1985). Physical activity is a complex behavior that can be categorized into many subgroups including formal, informal, planned, unplanned, voluntary or involuntary. Physical activity is usually characterized in an "amount" of frequency, intensity and duration of activity. Frequency refers to the number of times the activity is performed while duration is the total amount of time in a specified time period.

Examples of intensity for physical activity can be described as light, moderate and/or vigorous. Physical activity can also be stratified into types including but not limited to leisure time, housework/yardwork, work-related.

Physical Fitness: Physical fitness is a set of attributes that people have or achieve that relate to the ability to perform physical activity (Caspersen et al., 1985). Being physically fit is defined as "the ability to carry out daily tasks with vigor and alertness without undue fatigue and with ample energy to enjoy leisure time pursuits and to meet unforeseen emergencies" (1971). Physical fitness can be thought of an objective and current assessment of physical activity levels. Physical fitness health-related components include cardiorespiratory aerobic endurance, muscular strength, muscular endurance, flexibility and body composition.

VO2max: Cardiorespiratory endurance is a specific component of physical fitness that relates to the ability of the circulatory and respiratory systems to supply fuel during sustained physical activity. The volume of oxygen utilized by various body tissues per unit of time will be referred to as VO_2max. Ideally, VO_2max is measured by a treadmill graded exercise test, in which intensity increases as expired air is analyzed until volitional exhaustion.

Appendix II: Limitations of the Study

1. This dissertation was a secondary and retrospective data analysis. The original DEER study was not designed for the outcomes for CRP change or metabolic syndrome prevalence.
2. The diet protocol from DEER has been updated by the National Cholesterol Education Program in 2001 to:
    - 25-35% total fat per day of total caloric intake
    - < 7% saturated fat per day of total caloric intake
    - < 200 mg/day cholesterol
    - 50-60% carbohydrate intake of total caloric intake
    - 20-30g/day fiber
    - ~ 15% protein intake of total caloric intake
3. Baseline values for CRP and metabolic syndrome prevalence were lower than expected which reduced statistical power to examine differences between groups.
4. Participants were limited to the Stanford University area of Palo Alto, California which included a high socioeconomic status, mostly Caucasian and highly educated study sample.
5. All study participants had to meet the study inclusion criteria: be between the ages of 30 and 64 years for men; and 45 and 64 years for postmenopausal women. All participants had high LDL between 126-189 mg/dL for men, and between 126-209 mg/dL for women. All participants also had low HDL below 45 mg/dL for men and below 60 mg/dL for women. All participants were non-

diabetic and without any history of cardiovascular disease, cancer or any other medical conditions. Finally, none of the participants were on any lipid lowering medications. Therefore, the study results may not be generalizable to a wider population.

6. Due to the different eligibility criteria, statistical analyses were stratified by gender. It is possible that statistical power was reduced from a lower sample size.
7. Participants in the control group were specifically asked not to change their exercise or dietary habits for a year. While baseline and follow-up measures were taken to assess their compliance, it is important to note that these individuals could have already possessed active and healthy eating lifestyles.
8. In order to assess change in body composition, body fat and body fat change was assessed using skinfold measures. It is widely recognized that DEXA is the current gold standard for body composition measurement. Percent body fat was included as a covariate for all analyses.
9. Women were all postmenopausal and 45% were on HRT. HRT is no longer a recommended course of treatment, however, to control for any differences for CRP or metabolic syndrome, HRT was included as a covariate in all analyses.

Appendix III: Body Fat Changes Between Intervention Groups (mean ± SE)

Men	**Control**	**Diet**	**Exercise**	**Diet plus Exercise**
Baseline Body Fat (%)	21.1 ± 0.63	21.3 ± 0.72	22.3 ± 0.84	21.6 ± 0.64
Follow-up Body Fat (%)	21.6 ± 0.69	19.8 ± 0.71	21.8 ± 0.91	19.7 ± 0.72
Body Fat Change (%)	0.5 ± 3.38	-1.5 ± 3.45[*]	-0.5 ± 3.96	-1.96 ± 3.33[*+]

Women	**Control**	**Diet**	**Exercise**	**Diet plus Exercise**
Baseline Body Fat (%)	31.7 ± 0.96	31.8 ± 0.83	31.6 ± 0.94	32.8 ± 0.87
Follow-up Body Fat (%)	33.2 ± 0.99	30.5 ± 0.92	32.0 ± 1.04	30.6 ± 0.99
Body Fat Change (%)	1.5 ± 3.60	-1.3 ± 4.31[*]	0.4 ± 3.92	-2.2 ± 4.59[+ #]

[*] significantly different from control
[+] significantly different from exercise
[#] significantly different from diet

Appendix IV: Unadjusted CRP levels (mg/L) at Baseline and Follow-up in Men and Women*

Men (n=149)				
	Control	**Diet**	**Exercise**	**Diet plus Exercise**
n	33	39	35	42
CRP baseline	1.4 ± 1.47	1.0 ± 1.16	1.3 ± 1.26	1.4 ± 1.34
CRP follow-up	1.3 ± 1.09	1.0 ± 1.01	1.2 ± 1.60	1.1 ± 1.02
CRP change	-0.1 ± 1.71	-0.04 ± 0.66	-0.1 ± 1.21	-0.3 ± 1.32

Women (n=129)				
	Control	**Diet**	**Exercise**	**Diet plus Exercise**
n	34	32	30	33
CRP baseline	2.2 ± 2.24	1.8 ± 1.56	1.9 ± 1.56	2.0 ± 1.80
CRP follow-up	2.1 ± 1.53	1.6 ± 1.32	2.2 ± 2.17	1.3 ± 1.00
CRP change	-0.1 ± 1.88	-0.2 ± 1.04	0.3 ± 1.47	-0.7 ± 1.33

* means ± standard deviation

Appendix V: Frequencies of CRP Risk Categories[*] Between Intervention Groups in Men and Women

Men (n=149)	**Control**		**Diet**		**Exercise**		**Diet plus Exericse**	
	Baseline	Follow-up	Baseline	Follow-up	Baseline	Follow-up	Baseline	Follow-up
High Risk n(%)	3(9)	3(9)	2(5)	1(3)	5(14)	2(6)	5(12)	3(7)
Low Risk n(%)	30(91)	30(91)	37(95)	38(97)	30(85)	33(94)	37(88)	39(93)

Women (n=129)	**Control**		**Diet**		**Exercise**		**Diet plus Exericse**	
	Baseline	Follow-up	Baseline	Follow-up	Baseline	Follow-up	Baseline	Follow-up
High Risk n(%)	9(26)	9(26)	6(19)	3(9)	5(17)	7(23)	5(14)	3(8)
Low Risk n(%)	25(74)	25(74)	26(81)	29(91)	25(83)	23(77)	28(85)	30(91)

[*]High Risk CRP > 3 mg/L; Low Risk CRP ≤ 3 mg/L (Myers et al., 2004)

Appendix VI: Metabolic Syndrome Prevalence Changes in Men and Women

Men	**With Metabolic Syndrome**			
	Baseline n(%)	**Follow-up n(%)**	**Absolute Change n(%)**	**Net Reduction from Baseline (%)**
Control	9(20)	9(20)	0(0)	0
Diet	15(32)	8(17)	-7(-15)	-47
Exercise	13(31)	6(14)	-7(-17)	-55
Diet plus Exercise	16(35)	7(15)	-9(-20)	-57

Women	**With Metabolic Syndrome**			
	Baseline n(%)	**Follow-up n(%)**	**Absolute Change n(%)**	**Net Reduction from Baseline (%)**
Control	10(27)	10(27)	0(0)	0
Diet	12(30)	3(8)	-9(-22)	-73
Exercise	11(33)	7(21)	-4(-12)	-36
Diet plus Exercise	14(39)	8(22)	-6(-17)	-44

Appendix VII: Unadjusted Mean CRP levels (mg/L) in Men and Women With and Without Metabolic Syndrome*

Men: Metabolic Syndrome					
	Total	**Control**	**Diet**	**Exercise**	**Diet + Exercise**
n	47	9	13	11	14
CRP baseline	1.5 ± 1.43	1.8 ± 1.57	0.8 ± 0.29	1.6 ± 1.46	1.9 ± 1.74
CRP follow-up	1.4 ± 1.62	1.4 ± 1.24	1.0 ± 0.57	1.7 ± 2.53	1.5 ± 1.43
CRP change	-0.1 ± 1.46	-0.3 ± 1.67	0.2 ± 0.58	0.1 ± 1.80	-0.3 ± 1.66
Without Metabolic Syndrome					
	Total	**Control**	**Diet**	**Exercise**	**Diet + Exercise**
n	102	24	26	24	28
CRP baseline	1.2 ± 1.24	1.3 ± 1.44	1.1 ± 1.40	1.1 ± 1.11	1.2 ± 1.05
CRP follow-up	1.0 ± 0.92	1.2 ± 1.05	1.0 ± 1.24	1.0 ± 0.58	1.0 ± 0.71
CRP change	-0.1 ± 1.15	-0.03 ± 1.75	-0.2 ± 0.67	-0.2 ± 0.85	-0.2 ± 1.14
Women: Metabolic Syndrome					
	Total	**Control**	**Diet**	**Exercise**	**Diet + Exercise**
n	39	9	9	9	12
CRP baseline	2.4 ± 1.67	2.6 ± 1.75	2.3 ± 1.82	2.2 ± 2.02	2.4 ± 1.40
CRP follow-up	2.0 ± 1.39	3.0 ± 1.78	1.5 ± 0.72	2.2 ± 1.61	1.6 ± 0.90
CRP change	-0.4 ± 1.10	0.4 ± 0.86	-0.8 ± 1.32	-0.04 ± 0.92	-0.8 ± 0.88
Without Metabolic Syndrome					
	Total	**Control**	**Diet**	**Exercise**	**Diet + Exercise**
n	86	25	23	19	19
CRP baseline	1.7 ± 1.87	2.0 ± 2.41	1.6 ± 1.45	1.6 ± 1.35	1.7 ± 2.08
CRP follow-up	1.6 ± 1.54	1.7 ± 1.29	1.6 ± 1.50	2.0 ± 2.15	1.1 ± 1.09
CRP change	-0.1 ± 1.60	-0.3 ± 2.12	0.01 ± 0.82	0.3 ± 1.46	-0.6 ± 1.62

* means ± standard deviation

Appendix VIII: Human Subjects Institutional Review Board Application

UNIVERSITY OF MARYLAND
INSTITUTIONAL REVIEW BOARD

2100 Blair Lee Building
College Park, Maryland 20742-5121
301.405.4212 TEL 301.314.1475 FAX
irb@deans.umd.edu
www.umresearch.umd.edu/IRB

October 29, 2007

To: **Investigator:** Deborah Sue Rohm Young
Co-Investigator(s): Not Applicable
Student Investigator(s): Not Applicable
Department: KNES - Kinesiology

From: Roslyn Edson
IRB Manager
University of Maryland, College Park

Re: IRB Application Number 07-0495 (PAS# 1875)
THE EFFECTS OF PHYSICAL ACTIVITY AND DIET ON C-REACTIVE PROTEIN AND METABOLIC SYNDROME IN HIGH RISK ADULTS

Approval Date: 10-29-2007

Expiration Date: 10-29-2008

Type of Application: New Application

Type of Research: Non-Exempt

Type of Review: Expedited

The University of Maryland, College Park Institutional Review Board (IRB) approved your IRB application. The research was approved in accordance with the University's IRB policies and procedures and 45 CFR 46, the Federal Policy for the Protection of Human Subjects. Please include the above-cited IRB application number in any future communications with our office regarding this research.

Recruitment/Consent: For research requiring written informed consent, the IRB-approved and stamped informed consent document is enclosed. The IRB approval expiration date has been stamped on the informed consent document. Please keep copies of the consent forms used for this research for three years after the completion of the research.

Continuing Review: If you want to continue to collect data from human subjects or analyze data from human subjects after the expiration date for this approval, you must submit a renewal application to the IRB Office at least 30 days before the approval expiration date.

Modifications: Any changes to the approved protocol must be approved by the IRB before the change is implemented except when a change is necessary to eliminate apparent immediate hazards to the subjects. If you want to modify the approved protocol, please submit an IRB addendum application to the IRB Office.

Unanticipated Problems Involving Risks: You must promptly report any unanticipated problems involving risks to subjects or others to the IRB Manager at 301-405-0678 or redson@umresearch.umd.edu.

Student Researchers: Unless otherwise requested, this IRB approval document was sent to the Principal Investigator (PI). The PI should pass on the approval document or a copy to the student researchers. This IRB approval document may be a requirement for student researchers applying for graduation. The IRB may not be able to provide copies of the approval documents if several years have passed since the date of the original approval.

Additional Information: Please contact the IRB Office at 301-405-4212 if you have any IRB-related questions or concerns.

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