Impact of Low- and High-Carbohydrate Diets On Fasting Plasma Fatty Acid and Inflammatory Marker Concentrations

by

Danielle Joy Podesta

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CERTIFICATE OF APPROVAL

This is to certify that the Master's Thesis of

Danielle Joy Podesta

has been approved

Diane Stadler, PhD, RD, LD

Melanie Gillingham, PhD, RD, LD

Sonja Connor, MS, RD, LD

Bart Duell, MD

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LIST OF ABBREVIATIONS

AA	Arachidonic Acid
ALA	α-Linolenic Acid
BHT	Butylated hydroxytoluene
BMI	Body Mass Index
CVD	Cardiovascular Disease
CDC	Center for Disease Control and Prevention
CI	Confidence Interval
DEXA	Duel-energy x-ray absorptiometry
DHA	Docosahexaenoic acid
EER	Estimated Energy Requirement
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme-linked immunosorbent assay
EPA	Eicosapentaenoic acid
GC/MS	Gas Chromatography-Mass Spectrometry
HC	High-Carbohydrate Diet
HCL	Hydrochloric acid
HDL	High-density lipoprotein
CRP	C-reactive protein
IL-6	Interleukin-6
LA	Linoleic Acid
LC	Low-Carbohydrate Diet
LDL	Low-density lipoprotein
MeCN	Acetonitrile

MeOH	Methanol
n-3	Omega-3
n-6	Omega-6
NaOH	Sodium hydroxide
NDSR	Nutrition Data System for Research
NF-κβ	Nuclear factor kappa-light-chain-enhancer of β cells
OCTRI	Oregon Clinical & Translational Research Institute
OHSU	Oregon Health & Science University
PFB	Pentaflorabenzene
VLCD	Very Low-Carbohydrate Diet

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ABSTRACT

Background: Low-carbohydrate diets are used successfully for weight loss but may induce a state of heightened inflammation due to their high fat content. However, little research has explored the impact of low- and high-carbohydrate diets on circulating fatty acid and inflammatory marker concentrations.

Objective: A secondary analysis of data obtained from the Energy Balance Study was used to determine whether changes in circulating concentrations of fatty acids and inflammatory markers were different after six weeks of low- or high-carbohydrate dietary intervention in obese adults. Relationships between changes in fatty acid and inflammatory marker concentrations were also determined within groups.

Methodology: Twenty-three obese, but otherwise healthy adults participated in a randomized controlled feeding study. Participants consumed a standard diet for three weeks and then either an *ad libitum* low- (n=10) or high- (n=13) carbohydrate diet for six weeks. Fasting blood samples collected at the end of the second and ninth weeks of dietary intervention were analyzed for concentrations of saturated, omega-6 (n-6) and omega-3 (n-3) fatty acids, C-reactive protein (CRP) and interleukin-6 (IL-6). Wilcoxon signed-rank tests and rank sum tests were used to determine whether differences in the change from baseline of each variable were significant, within and between groups, respectively. Changes from baseline, adjusted for baseline values and weight loss, were compared between groups using robust regression analyses. Spearman correlation analyses were used to determine relationships between

the change in concentrations of fatty acids and the change in concentrations of inflammatory markers within groups after adjusting for weight loss.

Results: Subjects in the low-carbohydrate diet group lost -5 ± 3 kg and subjects in the high-carbohydrate diet group lost -3 ± 2 kg with the low-carbohydrate diet group losing more weight than the high-carbohydrate diet group ($p=0.05$). Plasma saturated fatty acid concentrations were significantly lower after the low- and high-carbohydrate dietary interventions than before but the changes from baseline were not different between groups ($p=0.6$). Plasma n-6 fatty acid concentrations were not significantly different from baseline after the low- and high-carbohydrate diet interventions and change from baseline was similar between the two diet groups ($p=0.4$). Plasma n-3 fatty acid concentration was higher after the high-carbohydrate diet intervention ($p=0.02$) but not after the low-carbohydrate diet intervention; however changes from baseline between groups were not different ($p=0.2$). As a result, the n-6/n-3 fatty acid concentration ratio was lower after the high-carbohydrate diet intervention but not the low-carbohydrate diet intervention ($p<0.01$). There were no differences in the change in inflammatory marker concentrations between groups. When mean plasma fatty acid and inflammatory marker concentrations were adjusted for baseline values and weight loss the only comparison that was significant between groups was the plasma n-6/n-3 fatty acid ratio ($p=0.02$); the plasma n-6/n-3 fatty acid ratio was lower in the high-carbohydrate diet group than the low-carbohydrate diet group. The change in plasma concentrations of saturated fatty acids was positively correlated with the change in concentrations of CRP in the high-carbohydrate

diet group (p=0.01), but not the low-carbohydrate diet group (p=0.4). The change in plasma concentrations of n-3 fatty acids was negatively correlated with the change in concentrations of CRP (p=0.05) in the low-carbohydrate diet group, but not the high-carbohydrate diet group (p=0.2).

Conclusions: During active weight loss, changes in circulating concentrations of saturated, n-6 and n-3 fatty acids, and IL-6 and CRP were similar between groups despite extreme differences in the macronutrient content of the low- and high-carbohydrate diets. These results are different from what we anticipated, as we hypothesized that the low-carbohydrate diet would heighten inflammation. Although a randomized, well controlled feeding study was used to test these hypotheses, the study was not powered, a priori, to detect differences in our outcome variables. Therefore, our results should be interpreted with caution due to the high risk of a type II error (β=0.37) that may have produced results showing no differences, when differences actually existed. To confirm the results described here, additional studies with larger sample sizes that are powered to detect differences in changes in plasma fatty acid and inflammatory marker concentrations after low- and high-carbohydrate dietary interventions should be performed.

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CHAPTER 1. INTRODUCTION

In 2000 the United States Department of Health and Human Services started the public health promotion and disease prevention program *Healthy People 2010*. One objective of this ambitious program was to reduce nation-wide obesity rates from 23% in 2000 to 15% by 2010 (1). However, in 2007, according to the Center for Disease and Control (CDC) ~26% of adults in the United States were still considered obese with over 30% of state populations in Alabama, Tennessee and Mississippi categorized as obese (1). These high rates of obesity are not only a domestic problem but also a global problem. In 2007, the International Day for Evaluation of Abdominal Obesity study evaluated the current prevalence of obesity in ~170,000 participants living in 65 different countries. Populations in France, Switzerland, Italy, Greece, Poland, Russia, Hungary, Israel, Morocco, Egypt, Saudi Arabia, China, India, Australia, and Canada were included in this cross-sectional study to highlight obesity rates on an international level (2). Waist circumference and body mass index (BMI) were measured in adult men (~70,000) and women (~99,000). The prevalence of obesity based on a BMI of $\geq$30 kg/m^2 was 24% among men and 27% among women. Obesity rates are of great public health importance, as obesity can be a major contributor to the rise in co-morbid conditions, as well as markers of inflammation.

In addition to classification by BMI, obesity can also be measured by waist circumference or by percent of ideal body weight for height. In the International Day for Evaluation of Abdominal Obesity study, participants in the highest quintile for waist circumference (>102 cm for men and >88 cm for women) were 2.2 times more likely to have cardiovascular disease, an inflammatory related disease, than participants with lower waist circumferences (2). The same link between obesity and cardiovascular disease was illustrated at the 26 year follow-up of Framingham Heart Study participants. Current weight as a percentage of desirable weight based on 1959 Metropolitan Life Insurance Company Desirable Weight Tables was evaluated in ~5000 participants. On average, ~19% of male participants and 21% of female participants were above their desirable weight. Men above their desirable weight had twice the risk of developing coronary heart disease than men at or below their desirable weight. Women above their desirable weight had 2.4 times the risk of developing coronary heart disease than women at or below their desirable weight (3).

Two commonly measured circulating markers of inflammation are C-reactive protein (CRP) and interleukin-6 (IL-6). CRP is an acute phase protein that is a strong predictor of cardiovascular disease (4). IL-6 is a pro-inflammatory cytokine that stimulates the liver to produce C-reactive protein, possibly contributing to tissue inflammation (5). In 2007, Nishida et al. examined 326 healthy Japanese men and reported that with increased abdominal obesity measured by waist circumference there was a direct correlation with increased circulating concentrations of CRP and IL-6. IL-6 concentrations were significantly higher among those with abdominal obesity (1.5 ± 1.25 pg/ml) compared to those without abdominal obesity (1.25 ± 0.25 pg/ml, $p<0.05$). CRP concentrations were also significantly higher among those with abdominal obesity (1.0 ± 0.75 mg/L) compared to those without abdominal obesity (0.65 ± 1.0 mg/L, $p<0.0001$) (6). One strategy to reduce inflammation associated with obesity is through dietary interventions that result in weight loss.

Weight loss among overweight and obese individuals has been achieved with two popular diets, very low-carbohydrate diets (VLCD) and high complex carbohydrate diets (HCD). Tay et al. conducted a parallel cohort study of 88 adults randomly assigned to either a VLCD or HCD diet for 24 weeks. Weight was measured every four weeks and CRP concentrations were measured at baseline and at the end of the study. Both diets resulted in significant weight loss with the VLCD group losing an average of -11.9 ± 6.3 kg and the HCD group losing an average of -10.1 ± 5.7 kg ($p < 0.0001$). Both groups demonstrated significant reductions in CRP concentrations from baseline to 24 weeks. CRP was reduced by −1.11 ± 1.46 mg/L in the VLCD group and by −1.27 ± 1.95 mg/L in the HCD group ($p < 0.05$). However differences in change between groups were not significant (7). Weight loss through dietary intervention may not be the only factor to reduce markers of inflammation. The composition of diet may also contribute to changes in markers of inflammation.

Plasma saturated and polyunsaturated fatty acids have been shown to affect circulating concentrations of markers of inflammation (8-10). Clarke et al. reported that the mortality rate due to coronary heart disease was associated with plasma concentrations of saturated and polyunsaturated fatty acids (11). Participants with higher plasma saturated fatty acid concentrations had a two-fold higher risk of coronary heart disease mortality than participants with lower plasma concentrations of saturated fatty acids, a finding that is sometimes attributed to higher plasma LDL cholesterol levels. Higher plasma saturated fatty acids were also significantly and positively correlated with increased CRP concentrations (95% Confidence Interval (CI): 1.1- 4.0). Participants with higher dietary concentrations of polyunsaturated fatty acids had one-half the coronary heart disease mortality risk as those with lower polyunsaturated fatty acid intakes. Plasma polyunsaturated fatty acid concentrations were also inversely associated with plasma CRP concentrations (95% CI: 0.26-0.94) (11). Thus differing plasma concentrations of saturated and polyunsaturated fatty acids may affect circulating concentrations of markers of inflammation.

Polyunsaturated fatty acids, specifically the essential omega-6 (n-6) and omega-3 (n-3) fatty acids, are known to influence circulating concentrations of markers of inflammation. In 2007, Block et al. reported that 786 participants with acute coronary heart disease had on average 20% lower concentrations of n-3 fatty acids in fasting serum compared to 768 healthy matched participants ($p<0.001$) (12). Other studies reported that imbalances between plasma n-6 and n-3 fatty acid concentrations affect circulating concentrations of markers of inflammation. Kiecolt-Glacer et al. reported a significant, positive correlation between high ratios of n-6 to n-3 plasma fatty acid concentrations and high IL-6 concentrations in fasting blood samples collected from 43 elderly adults ($p=0.02$) (13). The results of these studies suggest that imbalances in circulating blood n-6 and n-3 fatty acid concentrations, along with obesity and high dietary saturated fatty acid intake, may have an effect on inflammatory status.

The primary purpose of this study was to determine the effects of six weeks of low- and high-carbohydrate dietary intervention on circulating concentrations of fatty acids and inflammatory markers in obese adults.

SPECIFIC AIMS AND HYPOTHESES

Aim 1: Measure fasting plasma concentrations of fatty acids by gas chromatography-mass spectrometry before and six weeks after consumption of an *ad libitum* low-carbohydrate diet or a high-carbohydrate diet.

Hypothesis 1a: Participants in the *ad libitum* high-carbohydrate diet group will demonstrate a greater reduction from baseline in total circulating omega-6 (n-6) and saturated fatty acid concentrations and in the n-6: n-3 fatty acid concentration ratio than those in the *ad libitum* low-carbohydrate diet group.

Hypothesis 1b: Participants in the *ad libitum* low-carbohydrate diet group will demonstrate a greater reduction from baseline in total circulating omega-3 (n-3) fatty acid concentrations than those in the *ad libitum* high-carbohydrate diet group.

Aim 2: Measure fasting plasma concentrations of C-reactive protein (CRP) using a high sensitivity latex-enhanced turbidimetric *in vitro* immunoassay and Interleukin-6 (IL-6) using a high sensitivity ELISA assay before and six weeks after consumption of an *ad libitum* low-carbohydrate diet or high-carbohydrate diet.

Hypothesis 2: Participants in the *ad libitum* high-carbohydrate diet group will demonstrate a greater reduction from baseline in fasting plasma CRP and IL-6 concentrations than those in the *ad libitum* low-carbohydrate diet group.

Aim 3: Correlate the change in plasma total saturated fatty acid, total n-6 fatty acid, and total n-3 fatty acid concentrations along with the change in the plasma n-6: n-3 fatty acid concentration ratio with changes in circulating CRP and IL-6 concentrations after consumption of an *ad libitum* low-carbohydrate diet or high-carbohydrate diet for six weeks.

Hypothesis 3a: In the *ad libitum* high-carbohydrate diet group a reduction in circulating total n-6 fatty acid concentrations, saturated fatty acid concentrations and in the n-6: n-3 fatty acid concentration ratio will be directly correlated with reductions in circulating CRP and IL-6 concentrations. Conversely, in the *ad libitum* low-carbohydrate diet group an increase in circulating total saturated fatty acid, n-6 fatty acid concentrations and in the n-6: n-3 fatty acid concentration ratio will be directly correlated with increases in circulating CRP and IL-6 concentrations.

Hypothesis 3b: In the *ad libitum* low-carbohydrate diet group a decrease in circulating n-3 fatty acid concentrations will be indirectly correlated with an increase in circulating CRP and IL-6 concentrations. Conversely in the *ad libitum* high-carbohydrate diet an increase in circulating n-3 fatty acid concentrations will be indirectly correlated with a decrease in circulating CRP and IL-6 concentrations.

CHAPTER 2. BACKGROUND

National Obesity Rates in the United States

According to the National Health and Nutrition Examination Study the current prevalence of overweight and obese adults (>20 years of age) in the United States is 66.3% (14). The prevalence of obesity is projected to increase 0.77% annually. If this projected rate is realized, all adults in the United States will either be overweight or obese by 2050 (14). Individuals can be categorized as underweight, normal weight, overweight, or obese on the basis of body mass index (BMI). The BMI is the ratio of body weight in kilograms divided by height in meters-squared. Underweight individuals have BMIs <18.9 kg/m^2, normal weight individuals have BMIs between 18.9-24.9 kg/m^2; overweight individuals have BMIs between 25 and 29.9 kg/m^2, and obese individuals have BMIs ≥ 30 kg/m^2. Obesity rates in the United States have been continuously monitored to determine obesity trends and to predict of future health-care issues, such as the rise in morbidity and mortality associated with co-morbid conditions.

In 2006, Ogden et al. analyzed obesity prevalence trends from height and weight measurements of 4431 adults (>20 years old) collected between 1999-2004 for the National Health and Nutrition Examination Survey (NHANES) (15). Individuals were grouped into three categories based on BMI: overweight, 25.0 - 29.9 kg/m^2, obese, > 30 – 39.9 kg/m^2, and extremely obese, > 40 kg/m^2. As of 2003- 2004, the prevalence of obesity was ~32% in all adults; and 2.8% of men and 6.9% of women were considered extremely obese. The prevalence of obesity in men had increased significantly from 27.5% between 1999-2000 to 31.1% between 2003-2004. However, the prevalence of obesity in women did not change significantly in this same time interval ranging from 33.4% to 33.2% (15). These current and projected obesity rates in the United States indicate that obesity is a nationwide epidemic and that effective interventions need to be designed and implemented to curb these increasing rates.

Health promotion measures such as losing weight and implementing healthy dietary interventions may have multiple health benefits, which might reduce death related to obesity in the United States. In 2005 the United States had a death rate of ~2.5 million people /year. Danaei et al. estimated the proportion of the United States deaths in 2005 that were associated with modifiable dietary and lifestyle risk factors (16). Although smoking and high blood pressure were the leading risk factors associated with death in the United States, overweight and obesity may have contributed to 216,000 deaths (95% CI: 188,000–237,000). This work indicates that the combination of overweight and obesity may be a contributory factor in 1 out of 10 deaths in the United States. Dietary risk factors were also evaluated in their relationship to United States deaths. One of the most potent dietary risk factors associated with increased death was low omega-3 (n-3) fatty acid consumption, which was linked to 84,000 deaths in 2005 (95% CI: 72,000–96,000) (16). It is important to improve our understanding of the potential health benefits of lowering rates of overweight and obesity, and decreasing dietary risk factors, such as low n-3 fatty acid intake, in the United States.

Rising Rates of Overweight and Obesity Globally

This rising prevalence of obesity is not only occurring in the United States, but also is occurring in almost all global populations. In 2008, Lilja et al. reported on BMI and obesity trends from a longitudinal cross-sectional survey of 250 men and 250 women living in northern Sweden in 1986, 1990, 1994, and 2004 (17). Study participants were grouped into five age groups: 25 - 34, 35 - 44, 45 - 54, 55 - 64, and 65 - 75 years of age. The average BMI increased in males from 1986-2004, but, men in the 25 - 34 year age group showed the greatest absolute change in BMI of +1.8 kg/m^2. The mean BMI increased in this age group from 24.2 ± 3 kg/m^2 to 26.0 ± 3.8 kg/m^2 ($p < 0.0005$). In women, the changes in average BMI from 1986 to 2004 were only significant in the 25 - 34 and the 35 - 44 year age groups. The mean BMI of women in the 25 - 34 year age group increased from 22.9 ± 3.3 kg/m^2 in 1986 to 24.9 ± 4.9 kg/m^2 in 2004 ($p < 0.0005$); the mean BMI of women in the 35 - 44 year age group increased from 24.0 ± 4.1 kg/m^2 in 1986 to 25.8 ± 5.1 kg/m^2 in 2004 ($p < 0.005$).

The prevalence of obesity also increased. Men aged 35 - 44 years had the largest increase of 11.6% in obesity prevalence, increasing from 6.6% in 1986 to 18.2% in 2004 ($p < 0.0005$). Among women, the prevalence of obesity increased from 4.8% to 14.6% in the 25 - 34 year age group and from 8.3% to 14.6% in the 35 - 44 years of age group ($p < 0.005$) (17). Thus, rates of obesity have increased two to three-fold in Sweden during an 18 year period.

The rise in prevalence of obesity is not unique to the Western Hemisphere, but is also occurring in Asian populations. In Japan, a large-scale population-based cohort study, the Japan Public Health Center Study, recruited ~65,000 men and women from the main island Honshu and Okinawa in Japan. The participants were grouped into four year age intervals from 40 to 65 years of age. Participants were sub-grouped based on BMI. Overweight individuals had BMIs between 25 and 30 kg/m^2 and obese individuals had BMIs > 30 kg/m^2. After ten years of follow-up, the prevalence of obesity was significantly higher in Japanese women in the youngest age group (40 - 44 years of age) from both Honshu and Okinawa ($p < 0.0001$ and $p < 0.0002$, respectively). The prevalence of obesity in Japanese men was also higher in the youngest age group (40 - 44 years of age) in Honshu and Okinawa ($p < 0.02$ and $p < 0.008$, respectively) (18). The obesity trends world-wide raise concerns about possible correlations with increases in detrimental health problems related to obesity.

Markers of Inflammation and Obesity

Obesity is associated with a chronic inflammation (19). The relationship between obesity and co-morbid conditions such as atherosclerosis and diabetes can be correlated with concentrations of inflammatory-related markers circulating in the body (19). Two commonly measured inflammatory markers are the cytokine, interleukin-6 (IL-6), and the acute phase protein, C-reactive protein (CRP).

IL-6 is an important circulating pro-inflammatory cytokine. One role of IL-6 is to stimulate production of the acute phase protein, CRP (20). IL-6 is synthesized and released at critical sites of inflammation by monocytes and macrophages. Monocytes are white blood cells that react quickly to initial responses of acute tissue injury. Monocytes are attracted to sites of injury through chemotaxis, through the release of chemical signals produced by bacteria and other foreign substances. Phagocytosis of foreign substances is the primary role of monocytes at sites of tissue damage. Once inside the affected tissue, monocytes can differentiate into macrophages which continue phagocytosis of foreign substances. Another important action of macrophages is the release of cytokines such as IL-6, which not only promote production of acute phase proteins but further attract monocytes to the site of infection or inflammatory and injury. During chronic inflammation, IL-6 has stimulatory effects on T lymphocytes (T cells) and induces the differentiation of B lymphocytes (B cells) (21). IL-6 synthesis is regulated by the pro-inflammatory transcription factor, nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), which is found in most cells including adipocytes (22). Two common IL-6 receptors are the non-signaling IL-6 receptor alpha (IL-6Rα) and the non-ligand-binding signal transducer receptor glycoprotein 130 (gp130). IL-6Rα has a half life of 2-3 hours and the estimated half-life of gp130 is 2.5 hours (23). IL-6 receptors are found on T-cells, B cells and macrophages as well as epithelial, fibroblastic, and hemtopoietic cells (24). However, IL-6 receptors act on T cells and B cells at different stages. IL-6 receptors are down regulated during IL-6 activation of T-cells and are required only at the final stages of B lymphocyte maturation. Sheu et al. determined that deoxyribonucleic acid binding of the transcriptionally active NF-κB form in circulating mononuclear cells was 38.6% higher ($p < 0.05$) in obese subjects compared to normal weight subjects (25). Because IL-6 is an indicator of inflammation, elevated circulating concentrations of IL-6 may suggest that those who are obese are actually in a chronic state of inflammation.

In 2000, Roytblat et al. demonstrated the relationship between IL-6 and obesity. Serum IL-6 concentrations were measured in and compared between 25 healthy obese women with BMI > 35 kg/m² and 12 healthy normal weight women (26). The mean IL-6 concentration was significantly lower in normal weight women (1.28 ± 0.85 pg/ml) compared to obese women (7.69 ± 5.06 pg/mL; $p < 0.05$) (26). The higher IL-6 concentrations in obese women compared to the normal weight women suggests that obesity is a pro-inflammatory condition.

Although IL-6 is an important marker of inflammation within the body, it also induces production of the acute phase protein, CRP, primarily by hepatocytes (27). Because of its long half-life and relative stability in plasma, CRP has been used as a marker of inflammation in the body. CRP has characteristics similar to Immunoglobulin G, which activates macrophages and endothelial cells and generates a secondary antibody response to inflammation within the body (28, 29). In 1981 Shine et al. determined normal serum levels of CRP in healthy adults using two rapid, sensitive radioimmunassay methods (30). The first method was a single antibody method, using solid phase anti-CRP, providing a sensitivity of 50 μg/L with a 1-hour incubation time and intra- and inter-assay coefficients of variation of 10%. The second method was a double antibody method, using fluid phase rabbit anti-CRP serum and solid phase sheep anti-rabbit Immunoglobulin G serum, providing a sensitivity of 3 mg/L with an overnight incubation and intra- and inter-assay coefficients of variation of 10%. Four hundred and sixty blood samples were collected from healthy adults and analyzed. The median CRP concentration was 0.8 mg/L with an interquartile range of 0.3 - 1.7 mg/L. Ninety percent of the samples had CRP concentrations less than 3.0 mg/L and 99% of the samples had CRP values < 10 mg/L (30). A more general guideline suggests that normal circulating concentrations of CRP are < 2 mg/L (4). Healthy men and healthy non-pregnant women have similar CRP concentrations which remain constant with regard to time of collection; thus serum samples collected from healthy subjects at any time during the day provide “true” circulating CRP concentrations (4).

Elevated concentrations of CRP and IL-6 have been associated with cardiovascular risk factors such as hypertension. In 2002, Bermudez et al. performed a cross-sectional study recruiting 340 healthy women who were participants in the prospective Women's Healthy Study (31). Participants reported their history of hypertension, an inflammatory related risk factor for the development of cardiovascular disease. The American Heart Association defines hypertension as systolic pressure ≥ 140 mm Hg and diastolic pressure ≥ 90 mm Hg. Blood samples were analyzed for IL-6 and CRP concentrations. Participants had an average age of 60 ± 9 years with an average BMI of 26 ± 5 kg/m^2. The percentage of women with a systolic blood pressure ≥ 140 mm Hg was 20.6% and the percentage of women with a diastolic blood pressure ≥ 90 mm Hg was 10.3%. Women with hypertension had an average IL-6 concentration of 1.94 ± 2.01 pg/ml which was significantly higher than the concentration in women without hypertension 1.35 ± 1.74 pg/ml ($p < 0.0001$). Women with hypertension had a mean CRP concentration of 3.94 ± 2.81 mg/L which was significantly higher than the mean CRP concentration of 2.35 ± 3.03 mg/L in women without hypertension ($p < 0.0001$) (31). This study illustrated that elevated circulating CRP and IL-6 concentrations are associated with hypertension, a cardiovascular risk factor.

Elevated CRP and IL-6 concentrations are not only associated with inflammatory-related diseases but also with obesity, particularly visceral adiposity. In 2006, Bahceci et al. evaluated the relationship between adipocyte volume and the inflammatory markers, CRP and IL-6 (19). Thirty-two men and 68 women with a mean age of 47.3 ± 11.2 years were divided into 4 different groups. Group 1 included healthy, lean adults with an average BMI of 24.2 ± 1.4 kg/m^2 (n = 30); group 2 included non-diabetic, obese adults with an average BMI 30.2 ± 2.9 kg/m^2 (n = 30); group 3 included type 2 diabetic, obese adults with an average BMI of 30 ± 3.2 kg/m^2 (n = 20); and group 4 included type 2 diabetic, non-obese adults with an average BMI of 22.2 ± 1.5 kg/m^2 (n = 20). The BMI of each obese and diabetic group was significantly higher than the control group ($p < 0.0001$). All patients had an elective abdominal surgery which included hernia repair, cholecystectomy and gastroplasty (n = 76) or hysterectomy for myoma uteri (n = 24). Blood samples collected to measure CRP and IL-6 concentrations were obtained before the operation and adipose tissue (1 x 2 cm in size) samples were collected during the operation. The Goldrick's formula (πd [3(SD)2+d2)] / 6; d=diameter of 100 adipocyte cells) was used to determine volume of adipocytes from collected tissue. Obese and type 2 diabetic, obese patients had significantly higher adipocyte volumes, 0.29 ± 0.04 and 0.56 ± 0.11 µg lipid/cell, respectively, than the type 2 diabetic, non-obese patients and the controls, 0.21 ± 0.02 and 0.22 ± 0.04 µg lipid/cell, respectively ($p<0.0001$) (19).

The concentrations of inflammatory markers from the Bahceci et al. study were also significantly different between four groups (19). Both type 2 diabetic groups (obese and non obese) and the obese group had significantly higher IL-6 concentrations than the controls. The type 2 diabetic, obese group also had a significantly higher mean CRP concentration of 13.8 ± 14.6 mg/L compared to the control group with a mean CRP concentration of 3.6 ± 2.2 mg/L ($p < 0.0001$).

The non-diabetic, obese group with an average CRP concentration of 7.2 ± 4.2 mg/L and the type 2 diabetic non-obese group with an average CRP concentration of 8.3 ± 4.0 mg/L, were significantly higher than the control group ($p < 0.05$). Thus, obesity correlated with increased circulating IL-6 and CRP concentrations. The rise in obesity rates and obesity-related co-morbid conditions, such as diabetes, presses the issue of exploring strategies to promote weight loss to reverse the obesity epidemic and the associated rise in inflammatory-related diseases.

Impact of Weight Loss on Markers of Inflammation

Possible health-related risks associated with obesity include increased concentrations of circulating inflammatory markers. Weight loss is one possible strategy that can be used to decrease the inflammatory risk associated with obesity. In 2004, Nicklas et al. conducted an 18 month randomized controlled study of 252 overweight and obese adults to determine if weight loss reduced circulating concentrations of markers of inflammation (32). The participants were assigned to one of four groups: exercise-induced weight loss, diet-induced weight loss, exercise and diet-induced weight loss, or control. Participants randomized to the diet-induced weight loss treatment attended monthly nutrition education sessions that encouraged reduced energy intake and consumption of a well-balanced diet. The goal of the diet-induced weight loss treatment was for participants to lose and maintain a 5% weight loss by the end of the study. Fasting serum concentrations of CRP and IL-6 were measured. The average BMI at baseline was 34.4 ± 4.9 kg/m^2 for the diet-induced weight loss group, 34.6 ± 5.8 kg/m^2 for the exercise-induced weight loss group, 33.9 ± 5.6 kg/m^2 for the diet- and exercise- induced weight loss group, and 34.5 ± 5.3 kg/m^2 for the control group. Participants in the diet-induced weight loss group lost 5.7% of baseline body weight or an average of 12.8 ± 19.2 kg, which was the largest weight loss of all groups. This weight loss was significantly different compared to the control group which lost 1.3% or 2.3 ± 11.6 kg from baseline ($p<0.0001$). Participants in the diet- and exercise- induced weight loss group lost 4.4% of their body weight from baseline, or 8.2 ± 13.3 kg, which was also significantly different compared to the control group ($p<0.0001$). The exercise- induced weight loss group lost 2.6% or 4.1 ±11.1 kg which was not significantly different from the control group. The change in BMI from baseline in the diet-induced weight loss group was - 2.0 ± 3.1 kg/m² and in the diet- and exercise- induced weight loss group was -1.48 ± 2.2 kg/m² which was significantly different from the change in BMI of the control group, -0.96 ± 3.7 kg/m² ($p<0.0001$). The change in BMI of the control group was not significantly different from the change in BMI of the exercise-induced weight loss group, -1.2 ± 3.1 kg/m².

The diet- induced weight loss group and the diet- and exercise- induced weight loss group lost a significant amount of weight at follow up and also had significantly lower circulating CRP and IL-6 concentrations. The log change in CRP concentrations was significantly greater in the diet-induced weight loss group than the exercise-induced weight loss group (-0.26 ± 0.07 μg/mL; $p=0.01$) and the control group ((0.04 ± 0.07 μg/mL; $p=0.01$). The log change in CRP concentrations for the control group was the only group to have increased from baseline (+ 0.15 ±0.05 μg/mL). The reduction in mean IL-6 concentration in the diet-induced weight loss group was significantly greater (-0.13 ± 0.04 pg/ml) compared with the exercise-induced weight loss group (-0.01 ± 0.04 pg/mL, $p=0.009$) (32). Weight loss and changes from baseline in circulating IL-6 and CRP concentrations were not significantly different in the control or exercise group alone. The results of this study suggest that circulating concentrations of inflammatory markers are significantly lowered after weight loss of as little as 2.6% to 4.4% of baseline body weight.

In 2006, in another study performed by Villareal et al. circulating concentrations IL-6 and CRP also were lowered after weight loss than before (33). Twenty-four obese (BMI ≥ 30 kg / m^2), older (≥ 65 years old) subjects were randomized into two groups: the first group was placed on a low-calorie diet in combination with an exercise training schedule (treatment group; *n* = 15) and the second group was a control group without any intervention (control group; *n* = 9) for 24 weeks. Supervised exercise training sessions lasted for 90 minutes, 3 times/week. The diet was designed to yield an energy deficit of ~750 kcals/day and was comprised of ~30% fat, 50% carbohydrate, and 20% protein. Fasting blood samples were obtained for CRP and IL-6 analysis before and after the intervention. At the end of 24 weeks the treatment group lost on average of 8.2 ± 5.7 kg of body weight, compared to a weight loss of 0.7 ± 2.7 kg in the control group (p< 0.001). Baseline concentrations of for CRP in the control group were 5.8 ± 4.5 mg/L and in the treatment group 6.1 ± 5.4 mg/L. End of study concentrations were 6.6 ± 4.5 mg/L in the control group were and 3.5 ± 3.6 mg/L in the treatment group. The absolute change in CRP was significantly different between the two groups; the treatment group demonstrated a reduction in CRP of -2.5 ± 4.3 mg/L whereas the control group demonstrated an increase in CRP of 0.8 ± 2.8 mg/L; p < 0.01. Baseline concentrations of IL-6 were 3.6 ± 1.6 pg/mL in the control group and 4.6 ± 4.5 pg/mL in the treatment group. End of study concentrations were 4.8 ± 4.1 pg/mL in the control group and 2.3 ± 1.2 pg/mL in the treatment group. The reduction in IL-6 concentration was also significantly greater in the intervention group (-2.4 ± 4.7 pg/ml) than in the control group (-1.6

± 4.3 pg/ml, $p < 0.001$) (33). The results from these studies indicate that weight loss is associated with significantly lower circulating concentrations of markers of inflammation.

High- and Low-Carbohydrate Diets and Markers of Inflammation

Research conducted on two popular weight-loss diets, a high-complex carbohydrate diet and a very low-carbohydrate diet has shown conflicting results with respect to changes in markers of inflammation. In 2008, Peairs et al. studied 19 overweight men and women who consumed a very low-carbohydrate, high-fat, energy restricted diet with antioxidant or placebo supplementation for one week (34). Fasting blood samples were collected to measure concentrations of CRP and IL-6 at baseline and after eight days of the intervention. Both groups lost weight, however, the differences in weight loss were not significant. Serum CRP concentrations in the placebo group tended to increase from baseline from 2.66 ± 0.5 mg/L to 3.98 ± 1.1 mg / L ($p>0.05$) and tended to decrease from baseline in the antioxidant supplemented diet group from 2.63 ± 1.4 mg/L to 1.8 ± 0.6 mg/L ($p>0.05$); although both of these trends were not significant. Also, the analysis of covariance for CRP concentration by group and time was not significantly different ($p= 0.076$) nor was the difference in the change in CRP concentrations from baseline between the antioxidant supplemented versus the placebo groups ($p=0.119$). In this study, waist circumference correlated significantly with circulating IL-6 concentrations (correlation: 0.677, $p < 0.004$). Baseline values for IL-6 were 0.87 ± 0.15 pg/mL in the antioxidant group and 0.7

± 0.12 pg/mL in the placebo group. At the end of study, the mean IL-6 concentration in the antioxidant group was 0.7 ± 0.12 pg/ml and for the placebo group was 1.15 ± 0.24 pg/mL. However, the changes from baseline in mean IL-6 concentrations were not significantly different between groups. Although the results from this study were not significant, and the length of this study is a limitation. The CRP concentration trends suggest that additional studies are needed to determine if a very low-carbohydrate diet fed for short periods of time may lower circulating CRP and IL-6 concentrations if antioxidants are taken concurrently (34).

Noakes et al. demonstrated that an energy-restricted, high-protein, low-fat diet did not have a significant effect on circulating CRP concentrations compared to a high-carbohydrate, low-fat diet (35). Participants consumed one of two diets for 12 weeks: a high-protein, low-saturated-fat diet or a high-carbohydrate, low-saturated-fat diet. Participants attended individual education consultations with dietitians and food preparation sessions every four weeks. CRP was measured in fasting serum samples at baseline and four, eight and 12 weeks after the start of intervention. Weight loss was not different between the two diet groups; those in the high-protein diet group lost 7.6 ± 0.4 kg and those in high-carbohydrate diet group lost 6.9 ± 0.5 kg (p=0.29). The CRP concentration at baseline in the high-protein diet group was 6.6 ± 0.7 mg/L and was 4.8 ± 0.5 mg/L in the high-carbohydrate diet group. The end of study CRP concentration in the high-protein diet was 4.9 ± 0.6 mg/L and in the high-carbohydrate diet group was 4.0 ± 0.4 mg/L. CRP concentrations were significantly lower in both groups at the end of intervention ($p<0.001$), but the change in CRP concentrations from baseline to week 12 was not different between groups: high-protein diet, -1.7 ± 0.4 mg / L; high-carbohydrate diet, -0.8 ± 0.3 mg / L ($p=0.447$) (35).

In contrast, Rankin et al. showed an increase in circulating CRP concentrations after weight loss in individuals consuming a low-carbohydrate, high-fat diet (36). In 2007, 29 overweight women were randomized to either a low-carbohydrate or high-carbohydrate diet for four weeks. Fasting serum samples were collected every week for CRP and IL-6 analyses. Both groups lost significant amounts of weight; the low-carbohydrate diet group went from 87.3 ± 15.2 kg at baseline to 83.5 ± 14.8 kg at the end of the study ($p<0.01$) and the high-carbohydrate diet group went from 79.2 ± 16 kg at baseline sto 77.1 ± 15.9 kg at the end of the study ($p<0.01$). CRP concentrations decreased significantly after weight loss (2.7 ± 2.9 mg/L) compared to baseline (4.8 ± 4.2 mg/L) with the high-carbohydrate diet ($p < 0.001$). However, the CRP concentrations increased significantly after weight loss (7.1 ± 6.1 mg/L) compared to baseline (5.7 ± 5.5 mg/L) with the low-carbohydrate diet ($p < 0.05$). IL-6 concentrations were not significantly different after the dietary intervention in either group. IL-6 baseline values in the low-carbohydrate diet group were 1.6 ± 0.78 pg/mL and in the high-carbohydrate diet group were 1.2 ± 0.76 pg/mL. The end of study IL-6 concentrations were 1.25 ± 0.44 pg/mL in the low-carbohydrate diet group and were 1.18 ± 0.75 pg/mL in the high-carbohydrate diet group (36).

Saturated Fat and Markers of Inflammation

Saturated fatty acids are distinguished from monounsaturated and polyunsaturated fatty acids in that they have no double bonds in their chemical structure. Food sources high in saturated fatty acids include red meat and whole-fat dairy products. Two predominant saturated fatty acids that are linked to inflammation are palmitic acid and stearic acid. In 2003, Weigert et al. performed cell culture studies to assess the correlations between palmitic acid, stearic acid, unsaturated fatty acids and the production of IL-6 (37). Primary skeletal muscle cells from the quadriceps femoris of healthy, normal weight subjects were grown from satellite cells. Cells were plated in a 1:1 mixture of alpha-minimum Eagle's media and Ham's F-12 media supplemented with 20 % fetal bovine serum, 1 % chicken embryo extract and 0.2 % antibiotic antimycotic solution. The control cells were treated with essentially fatty acid-free bovine serum albumin. IL-6 mRNA was measured using real time quantitative polymerase chain reaction analysis. IL-6 mRNA expression was significantly higher in the presence of 0.5 mM stearic acid and 0.5 mM and 0.25 mM palmitic acid after 48 hours of incubation ($p < 0.05$). The unsaturated fatty acid, linoleate acid, at 0.25 mM lowered, IL-6 mRNA expression compared to palmitic acid after 48 hours ($p < 0.05$). Addition of palmitic acid also increased NF-κβ expression compared to cells treated with essential fatty acid-free bovine serum albumin ($p < 0.01$). This study suggests that saturated fatty acids induce pro-inflammatory responses in muscle tissue cultured in vitro.

Dietary saturated fat content has also been assessed in low- and high-carbohydrate diets in relation to inflammation. In 2008, Keogh et al. conducted an eight week randomized controlled trial with 100 participants to determine the inflammatory effect of a very low-carbohydrate, high-saturated fat, weight loss diet compared to a high-carbohydrate, low-saturated fat, weight loss diet (38). The high-saturated fat diet provided 35% of energy as protein, 61% of energy as fat (of which 20% of energy was saturated fat), and 4% of energy as carbohydrate. The low-saturated fat diet consisted of 24% of energy as protein, 30% of energy as fat (of which < 8% of energy was saturated fat), and 46% of energy as carbohydrate. The diets restricted energy intake by ~ 30%. The diets were designed to include specific amounts of food to meet established energy and macronutrient requirements. The mean energy content provided in the diet was ≈ 6000 kJ (≈ 1400 kcal) for women and ≈ 7000 kJ (≈ 1700 kcal) for men. Foods for each diet were supplied every 2 weeks for the duration of the 8 week study. These foods were listed in a food record that the participants completed daily. Body weight was measured to the nearest 0.05 kg with a calibrated electronic digital scale and body composition was assessed by dual-energy X-ray absorptiometry (DEXA). The average energy consumed on the low-carbohydrate diet for the eight weeks was 6608 ± 664 kJ (≈ 1580 ± 160 kcal) and for the high-carbohydrate diet was 6590 ± 717 kJ (≈ 1575 ± 171 kcal). At the end of the intervention, both groups lost weight, however subjects on the very low-carbohydrate, high-saturated-fat diet lost more weight (7.9 ± 2.0% of baseline weight) than those on the high-carbohydrate, low-saturated-fat diet (6.5 ± 2.8% of baseline weight) ($P < 0.01$). The very-low-carbohydrate, high-saturated-fat diet group also lost more abdominal fat (–0.6 ± 0.4 kg) than those in the high-carbohydrate, low-saturated-fat diet group (–0.4 ± 0.3 kg) ($p < 0.001$). Circulating CRP concentrations were significantly lower in both groups after weight loss. Those consuming the very low-carbohydrate, high-saturated-fat diet demonstrated a reduction in circulating CRP of -0.27 ± 1.5 mg/L ($p < 0.05$) but those consuming the high-carbohydrate, low-saturated-fat diet group demonstrated a greater reduction in circulating CRP of -1.1 ± 0.63 mg/L ($p < 0.05$). This difference in change in circulating CRP concentrations between groups was statistically significant ($p<0.05$) (38). These findings suggest that weight loss diets lower in saturated-fat may reduce markers of inflammation to a greater extent than weight loss diets with higher saturated fat content. More research is necessary to better understand this relationship.

Omega-6 Fatty Acids and Markers of Inflammation

Essential fatty acids cannot be synthesized by the body and must be acquired from the diet. One important class of essential fatty acids is omega-6 (n-6) fatty acids. The n-6 fatty acid family has two primary constituents: linoleic acid and arachidonic acid. Linoleic acid is obtained from plant sources and oils derived from corn, safflower and sunflower seeds. Linoleic acid is the most basic n-6 fatty acid and is metabolized through an enzymatic cascade to generate the most metabolically active form of an n-6 fatty acid, arachidonic acid. In addition to being produced endogenously, arachidonic acid is also found in foods such as egg yolks and other animal products. Arachidonic acid is a component of cell membranes and contributes to the cell membrane structure. At high concentrations, arachidonic acid is converted to pro-inflammatory leukotrienes, prostaglandins and thromboxanes (39, 40).

In contrast, low dietary intakes of n-6 fatty acids have been associated with decreased risk of inflammation. In 2004, Sundrarjun et al. conducted a 24-week randomized, double-blind, placebo-controlled study of 60 patients with active rheumatoid arthritis, a chronic autoimmune condition (41). Three intervention diets were used in the study: a low n-6 fatty acid diet containing less than 12.5 g/day of n-6 fatty acid from cooking oil, a high n-3 fatty acid diet containing ~3.36 g/d of fish oil, and a placebo diet (control individuals were not given dietary education and were asked not to change their current dietary patterns for the duration of the study). Participants in each group completed weekly food logs and recorded their food intake during one weekend day and two weekdays for the duration of the study. CRP and IL-6 concentrations were measured in serum at baseline and at three time points throughout the study. After the 24-week study, IL-6 concentrations were significantly lower than baseline in the high n-3 fatty acid diet group (94 ± 16 pg/ml vs.59 ± 9 pg/ml, $p < 0.05$). The concentration of IL-6 was also significantly lower at the end of the study in the low dietary n-6 fatty acid group (61 ± 13 pg/ ml vs. 40 ± 9 pg / ml, $p < 0.05$). There was also a significant reduction in serum CRP concentration in the high n-3 fatty acid group which was 11% lower than baseline ($p < 0.05$). The percentage change in CRP concentrations in the high n-3 fatty acid group was significantly greater (67%) than in the intervention group but not the control group ($p < 0.05$, $p< 0.16$; respectively) (41). These results suggest that diets high in n-6 and n-3 fatty acids may have an anti-inflammatory effect in individuals with chronic inflammation.

Omega-3 Fatty Acids and Markers of Inflammation

Omega-3 (n-3) fatty acids are polyunsaturated fatty acids that have an anti-inflammatory effect in the body (42). The n-3 fatty acid family has three important constituents: alpha-linolenic acid (ALA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). ALA is found in plant sources such flaxseeds and walnuts. ALA is the most basic form of an n-3 fatty acid and is metabolized to the most active forms of n-3 fatty acids, EPA and DHA. EPA and DHA can be derived from the diet with the consumption of fatty fish, such as salmon or cod. EPA induces the production of leukotrienes (LTA5), prostaglandins (PGG3 and PGH3) and tromboxanes (TXA3) (39).

In 2004, Moriguchi et al. reported that Japanese individuals living in Brazil had higher prevalence rates of obesity and cardiovascular disease (CVD) and lower intakes of n-3 fatty acids than those living in Japan (43). These researchers conducted a cross-sectional observational study and recruited 234 Japanese men and women living in Okinawa and 160 Japanese men and women living in Brazil who were within 45-69 years of age. Individual medical and dietary histories, blood pressure, electrocardiograph, blood tests and 24 hour food records were collected. The dietary history included questions on frequency of consumption of meat and fish per week. Plasma was collected to measure concentrations of EPA and DHA. Mean BMI was significantly higher among those living in Brazil than those living in Okinawa ($p < 0.001$). The prevalence of hypertension was also significantly higher in those living in Brazil than those living in Okinawa ($p < 0.01$). Significant dietary differences were also recognized between the two populations. Fish intakes were significantly lower among those living in Brazil than those living in Okinawa ($p < 0.0001$) and, as such, EPA and DHA intakes were also significantly lower among those living in Brazil than those living in Okinawa ($p < 0.001$) (43). These findings suggest that lower dietary intakes of n-3 fatty acids may be associated with pro-inflammatory-related diseases.

High consumption of n-3 fatty acids has been associated with lower risk of inflammatory-related diseases due to an inverse association with circulating CRP concentrations. In a cross-sectional study, a food frequency survey was administered to ~1000 elderly (>70 years of age) Japanese men and women. One time serum samples were collected to measure circulating CRP concentrations. Subjects with circulating CRP concentrations ≥ 10 mg/L were excluded. The mean serum CRP was 1.14 ± 1.52 mg/L. After analysis, subjects were divided into quartiles based on dietary intake of EPA and DHA. The mean dietary intake of EPA and DHA was 1.38 ± 0.82 g / d in men and 1.17 ± 0.67 g / d in women. In both men and women, those with the highest dietary intakes of EPA and DHA (4-7 g / 2000 kcal) had a log mean serum CRP concentration of 0.28 ± 0.03 mg/L. Those with the lowest dietary intakes of EPA and DHA (0.5 - 3 g /

2000 kcal) had a log mean serum CRP concentration of 0.35 ± 0.03 mg / L. Though not significantly different (p = 0.051), there was an inverse association between higher intakes of n-3 fatty acids and lower circulating concentrations of CRP (44).

Ratio of Omega-6 to Omega-3 Fatty Acids and Markers of Inflammation

Both n-6 and n-3 fatty acids are essential components of our diet. However, different balances of these two essential fatty acids may have an effect on inflammation in the body. In the 1970s, a high intake of fish was correlated with a low incidence of inflammatory-related diseases (45). This relationship was first described in Greenland Eskimos who consumed more fish and who had a lower incidence of inflammatory-related disorders than Danish Eskimos. A higher intake of fish in the diet increases the concentration of circulating n-3 fatty acids. The Greenland Eskimo diet was high in n-3 fatty acids (~14 g/3000 kcal/day) and low in n-6 fatty acids (~5 g/3000 kcal/day) with an average n-6 to n-3 fatty acid ratio of ~5:14 (~0.4:1). The Danish Eskimo diet provided a n-3 fatty acid intake of ~3 g/3000 kcal / day, an n-6 fatty acid intake of ~10 g/ 3000 kcal/day, and a n-6/n-3 fatty acid ratio of ~10/3 (~4/1) (46).

The typical Western diet is estimated to provide a n-6/n-3 fatty acid ratio of ~10/1 (47). The current Japanese diet provides n-6/n-3 fatty acid ratio of ~4:1. This difference in n-6/n-3 fatty acid ratio may contribute to the lower rate of inflammatory-related diseases in Japanese populations compared to Western populations (48). In 2008, Kazumasa et al. conducted a prospective study with ~

58,000 Japanese men and women who participated in the Japan Collaborative Cohort Study for Evaluation of Cancer Risk. Participants completed a food frequency questionnaire. The n-3 fatty acid content in their diet was correlated with the risk of developing cardiovascular disease within a twelve and half year follow-up period. Participants were separated into quartiles based on their fish consumption with the first quartile having the lowest consumption of fish and the fourth quartile having the highest consumption of fish. In the lowest quartile, men had a n-6/n-3 fatty acid ratio of 6/1 and women had a n-6/n-3 fatty acid ratio of 5/1. Men in the highest quartile had an n-6/n-3 fatty acid ratio of ~3/1 and women had a n-6/n-3 fatty acid ratio of ~3/1. When the results for men and women were combined those in the higher quartiles of fish consumption had significantly lower rates of CVD than those in the lower quartiles ($p < 0.001$) (49).

Recent studies have correlated a lower ratio of n-6/n-3 fatty acids with lower concentrations of the inflammatory markers, CRP and IL-6. In 2004, Esposito et al. performed a randomized, double-blind study to assess the impact of diets with higher and lower n-6/n-3 fatty acid ratios, on circulating IL-6 and CRP concentrations (50). One hundred and eight Italian participants with metabolic syndrome were randomly assigned to either a control diet group or Mediterranean diet group for 24 months. The Mediterranean diet included whole grains, olive oil, fruit and nuts. The control group followed a prudent diet that consisted of < 30% fat, 15-20% protein and 50-60% carbohydrate. Participants completed weekly three day diet records throughout the duration of the study. Compliance to the study was based on attendance at the monthly meetings for the first year, bimonthly meetings for the second year and completion of the weekly three day diet records. The average dietary n-6/n-3 fatty acid ratio at follow-up for the Mediterranean diet group (~7/1) was significantly lower than the control diet group (~11/1, $p < 0.001$). At the two year follow-up, IL-6 and CRP concentrations were significantly lower in participants who consumed the Mediterranean diet compared to those who consumed the control diet ($p<0.04$ and $p<0.01$, respectively). Baseline values for CRP concentrations in the control diet group were 3.2 ± 1.8 mg/L and in the Mediterranean diet group were 3.9 ± 2.8 mg/L. After 2 years, the mean CRP concentrations in the control group were 2.9 ± 2.0 mg/L and in the Mediterranean group were 2.8 ± 2.1 mg/L. The results from this study suggest that lower dietary n-6/n-3 fatty acid ratios are associated with lower concentrations of the inflammatory markers, CRP and IL-6 (50).

Conclusion

The typical Western diet contains low amounts of fatty fish, tree nuts, and flaxseed, all rich sources of n-3 fatty acids, and high amounts of red meat and processed foods, which are high in saturated fat and n-6 fatty acids. As the Westernized diet becomes more global, obesity levels and the risk for inflammatory-related diseases, such as the CVD, may increase (51).

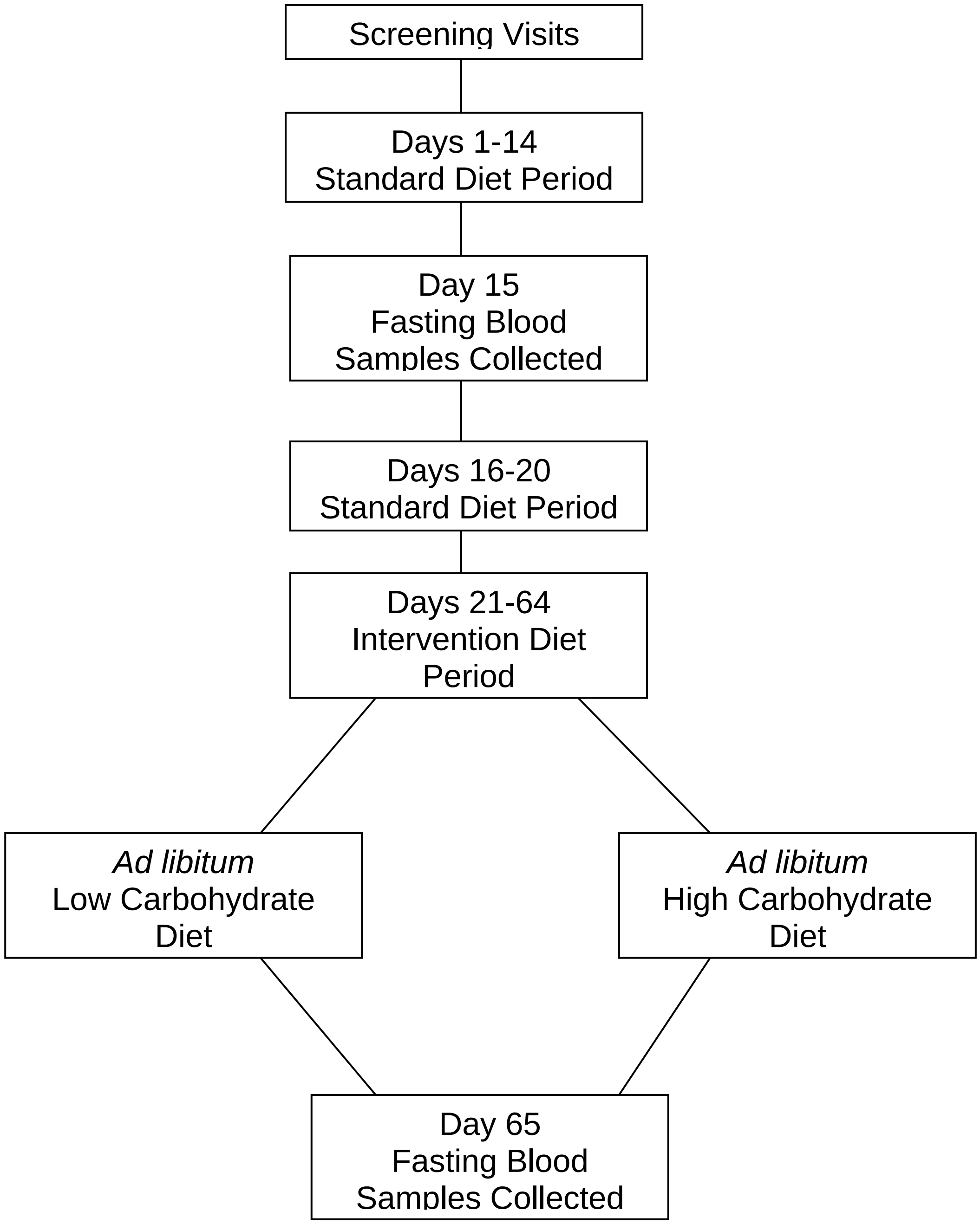
The research reviewed here suggests a relationship between dietary saturated fat intake and the n-6/n-3 fatty acid ratio with the inflammatory markers, CRP and IL-6. However, there is limited research comparing this relationship among individuals following various diets, such as low-carbohydrate diets or high carbohydrate diets for weight loss. The purpose of this study was to examine the impact of these two very different diets, with very different amounts of saturated, n-6, and n-3 fatty acids, on changes in circulating concentrations of the inflammatory markers, CRP and IL-6.

CHAPTER 3. RESEARCH AND DESIGN METHODS

General Design of the Energy Balance Sub-Study

A sub-analysis of samples collected as a part of a prospective, randomized, clinical trial were used to determine the impact of low- and high-carbohydrate diets on circulating fatty acid and inflammatory marker concentrations. Figure 1 illustrates the study design. Participants ate a standardized weight maintenance diet for two weeks after which fasting blood samples were obtained. Participants were then randomized to one of two intervention diets: a low-carbohydrate *ad libitum* diet or a high-carbohydrate *ad libitum* diet. Participants consumed either the low- or high-carbohydrate intervention diet for six weeks, after which a second set of fasting blood samples were obtained. Plasma fatty acid and inflammatory marker concentrations were measured at each time point. Changes from baseline to the end of the study were calculated to assess the impact of diet and weight loss on these outcomes.

Figure 1. Design of Energy Balance Study



Recruitment and Screening

Twenty-three obese (BMI: 30-50 kg/m^2) but otherwise healthy participants were recruited from the Portland, Oregon area into this randomized, controlled feeding study. Recruitment was through advertisements posted on the Oregon Health & Science University (OHSU) research participation website, in local newspapers, and by flyers posted on the OHSU and Portland State University campuses. Individuals who participated in this study completed and passed a series of initial medical history and health screenings. First, a telephone interview was conducted to determine eligibility for full screening assessment based on weight, medication use, and inclusion and exclusion criteria as described in Table 1. If eligible, a follow-up visit was conducted to obtain medical history and demographic information, to explain risks and benefits of the study, and to obtain written informed consent to participate in the screening process. If potential participants remained eligible and interested, a second clinic visit was scheduled to obtain a fasting blood sample and to assess liver, kidney and cardiovascular function. Those who remained eligible and interested were invited to meet with the study's medical director who performed a physical examination and reviewed the participant's medical history. If the participant met all inclusion criteria and remained interested in participating in the study a meal trial week was scheduled which involved consuming a standard breakfast meal prepared by the OCTRI metabolic kitchen staff for three days and performing other standard study-related measurements (weight, blood pressure, etc). The purpose of the meal trial week was to expose potential participants to the study protocol and the time that would be required of them regularly during the controlled nine week dietary intervention. Those who remained interested were invited to participate and asked to review and sign the consent form for the main study intervention.

Table 1. Inclusion and Exclusion Criteria Established for the Energy Balance Study

Inclusion Criteria	Exclusion Criteria
• BMI: 30-50 kg/m² • Age: 21-65 years • Permission from primary care providers • Willingness to eat either a low-carbohydrate or high-carbohydrate diet • Willingness to stop taking vitamin, minerals, or other dietary supplements for the duration of the study	• Major debilitating mental or physical illness that would interfere with participation (as determined by the participant's primary care provider) • At risk for anemia • Renal or hepatic disease, diabetes • History of stroke • Heart disease of any kind • Pregnancy or lactation within past 12 months • History of gallbladder disease • Current/history of swallowing disorders, esophageal or bowel strictures, fistulas, or gastrointestinal obstructions • Hyperthyroidism or untreated hypothyroidism • Chronic use of prescription pain medications • Poorly controlled hypertension • Weight instability (gain or loss of more than 10 pounds or 3-5% of body weight in 6 months) • Current participation in a commercial or self-directed weight loss program • Vegetarian, vegan, or kosher food restrictions • Food allergies to eggs, nuts or wheat or lactose intolerance to cheese • Current moderate or excessive use of alcohol • Current/recent chronic use of tobacco or recreational drugs • Plans to leave area in next year

Weight and Height Measurements and BMI Calculation

During the screening visits, at baseline (Week 2), and the end of the study (Week 9) body weight was measured after an overnight fast with participants wearing only hospital gowns and underwear. Weight was measured to the nearest 0.001 kg using a stand-on-scale with shielded remote digital display (Scale-Tronix, Model 5002; White Plains, NY). Height was measured (without shoes) using a wall-mounted stadiometer to the nearest 0.1 centimeter (Holtain Ltd, UK). Body mass index (BMI) was calculated by dividing weight (in kg) by height (in meters-squared).

Calculation of Estimated Energy Requirements

The estimated energy requirement to maintain weight for each participant was calculated using the Boothby and Berkson Food Nomogram that takes into account a participant's sex, age, weight, height, and physical activity level (52). Weight and height measured at the third screening visit were used for this calculation. Calculations were performed by a bionutritionist of the Oregon Clinical & Translational Research Institute (OCTRI).

Diet Allocation

Participants were assigned to the low- or high-carbohydrate dietary intervention by a covariate-adaptive randomization scheme known as the minimization method (53). This method assigned participants to intervention groups to achieve balance in gender, age, initial BMI, and fasting cholesterol and triglyceride concentrations. In implementing the minimization method, the first four participants were assigned randomly, without regard to balancing factors, to one of the two diet groups. After these first subjects were assigned, new assignments were made that took into consideration the distribution of variables of interest for the previous subjects and the current subject. For each new eligible subject, this method assigned that subject with high probability to the diet group that would yield the greatest balance between the two treatment groups for these covariates.

Description of the Standardization and Intervention Diet Protocols

This study was a nine week controlled dietary intervention. Participants consumed a standard diet for three weeks and either a low- or high-carbohydrate diet for six weeks. Participants were asked to eat foods provided to them by the OCTRI kitchen staff and nothing else for the duration of the study. Each of the three diets followed a six day repeating menu that provided a variety of foods and food combinations. Participants ate breakfast in the OCTRI dining room three times a week (typically Monday, Wednesday, and Friday) and collected food for the remainder of the day and the following day to eat off-site. Weekend meals and snacks were packaged and provided to participants on Fridays. As food preferences necessitated, individual food substitutions were made that maintained the macronutrient composition of the day's menu. Participants were asked to return all uneaten food and food containers to be weighed, recorded and used to calculate energy and nutrient consumption by each participant. Those taking vitamin, mineral or other dietary supplements were asked to discontinue this practice for the duration of the study. A standard adult-strength multivitamin/mineral supplement was provided each day to each participant for the duration of the nine week intervention. Participants were also asked to maintain a consistent exercise profile throughout the study.

Standardized Diet

The purpose of the standard diet was to allow participants to habituate to a common weight maintenance diet so that differences in outcome variables that are influenced by weight and diet were minimized. This diet consisted of common foods distributed in three meals and one snack each day that provided approximately 100% of each participant's estimated energy requirements for weight maintenance. The macronutrient composition of the standard diet was 52 ± 0.6% carbohydrate, 15 ± 0.3% protein and 36 ± 0.5% fat. This diet consisted of a variety of typical foods and beverages common to the U.S. diet and was designed to be widely acceptable. The weight of each food and beverage item was increased or decreased to create similarly composed menu plans at 250 kcal increments. Unit foods of the same macronutrient composition that provided 250 kcal each were added to or subtracted from a participant's diet as necessary to maintain body weight within one kilogram.

Low-Carbohydrate Diet

The low-carbohydrate dietary intervention was modeled after the induction phase of the Atkins Diet (54). The food provided contained no more than 28 grams of carbohydrate per day regardless of energy intake and was comprised of 4 ± 0.5% carbohydrate, 29 ± 3% protein, and 66 ± 2% fat. All moderate-to-high-carbohydrate containing foods were excluded including: fruits, fruit juices, starchy vegetables, grains and dairy products (except for cheese and cream). Food choices included meat, fish, poultry, eggs, cheese, whipping cream, oils and butter and small amounts of non-starchy vegetables. Participants assigned to this intervention were offered pre-weighed meals that provided 120 ± 2% of their estimated energy requirements for weight maintenance and allowed to eat as much or as little of any of the foods available to satisfy their hunger.

High-Carbohydrate Diet

The high-carbohydrate intervention was modeled after the Dietary Approaches to Stop Hypertension (DASH) diet (55) and the diet was comprised of 57 ± 2% carbohydrate, 18 ± 1% protein, and 29 ± 2% fat. The high carbohydrate diet emphasized consumption of fruits, vegetables, low-fat dairy products and lean meats. It included whole grains, poultry, fish and nuts, and was reduced in fats, red meat, sweets and sugar-containing beverages. Like the DASH diet, the high-carbohydrate diet, was also high in calcium, magnesium, potassium and fiber and low in saturated fat and cholesterol. Participants assigned to this group were provided 120% of their estimated energy requirement for weight maintenance and allowed to eat as much or as little of the food provided each day to satisfy their hunger.

Nutrient Analyses

All food items and recipe ingredients provided to participants were matched to foods in the USDA Standard 17 database and accessed using the nutrient analysis software ProNutra (Viocare, Princeton, NJ). ProNutra was the diet planning software used by the OCTRI bionutritionists to develop and analyze the energy and nutrient content of the standardized and the low- and high-carbohydrate diets. Food items and nutrients not included in the USDA database were added to Pronutra using similar foods identified in another nutrient analysis database, Nutrition Data System for Research (NDSR 2008, Nutrition Coordinating Center, University of Minnesota, Minneapolis, MN) (56). Values obtained from NDSR were used to complete the master ProNutra database. For the purpose of this study the nutrient composition of a food item was determined to be complete when the energy content and amounts of carbohydrate, protein, and fat (including saturated, polyunsaturated and monosaturated fat and the fatty acids 18:2, 18:3, 20:4, 20:5, 22:6) were entered into the master ProNutra database. If a food item or recipe ingredient was not in either database, the food company was contacted to obtain nutrient information to complete the nutrient content of the food or ingredient item in the master ProNutra database. If the nutrient information was not available from the company then the best estimate was determined using foods with similar macronutrient contents from either database. ProNutra was used to calculate each participant's energy and nutrient intake for each day of the study. These values were then imported to an Excel database and used for analysis.

Blood Sample Collections

Fasting blood samples were collected into phlebotomy tubes at the end of week 2 and the end of week 9. All tubes were centrifuged for 12 minutes at 2600 rpm under 4˚C refrigerated conditions; tubes for plasma samples were processed immediately; after collection tubes for serum samples were allowed to clot for 20 minutes before processing. Serum and ethylene-diamine-tetra-acetic acid (EDTA) plasma samples were collected, divided into polypropylene aliquote tubes, and stored for batched analysis at the end of the study. All storage tubes were frozen at -20°C and then transferred within 24 hours to -80°C freezers until the time of analysis. Samples for fatty acid and IL-6 analysis underwent one prior freeze/thaw cycle before analysis; samples for CRP analysis underwent one freeze/thaw cycle as part of the analysis.

Analytical Methods

Inflammatory Marker Analyses

Interleukin-6 (IL-6) and C-reactive protein (CRP) concentrations were measured at the OCTRI Core Laboratory at OHSU. IL-6 was measured in fasting serum using a commercial enzyme-linked immunosorbent assay **(**ELISA) (Catalog # HS600B, R & D Systems, Minneapolis, MN). R& D Systems reports a sensitivity range for this IL-6 assay between 0.016-0.110 pg/mL (57). The interassay coefficient of variation reported by the OCTRI Core Laboratory was between 7.49 – 12.27% depending on the concentration of the control sample. CRP concentration was measured in fasting EDTA plasma using a high sensitivity latex-enhanced turbidimetric *in vitro* immunoassay (Catalog # LKCR1, Immulite/Immulite 1000 (Siemens), Deerfield, IL). Siemens reported the sensitivity for the CRP assay at 0.1 mg/L (58). The interassay coefficient of variation reported by the OCTRI Core Lab was between 6.95-7.56%.

Plasma Fatty Acids

Plasma samples for fatty acid analysis were prepared in Dr. Melanie Gillingham's laboratory and analyzed in the Bioanalytical Shared Resource Pharmacokinetics Core Laboratory at OHSU using the method of Lagerstedt et al (59). The method was as follows: All glass tubes were rinsed with 0.1 N hydrochloric acid (HCL) followed by hexane. A stock solution of one mg/ml butylated hydroxytoluene (BHT) in one ml of 2 $CHCl_3$:1 MeOH was prepared. Individual internal standard stock solutions were sonicated for 15 minutes. A mixture of internal standards was prepared and contained 1 µg of d_3C10, 2 µg of d_3C14, 20 µg of d_3C16, 20 µg of d_3C18, 10 µg of d_3C20, and 2 µg of d_3C22 (Cambridge Isotope Laboratories, Andora, MA). A mixture of internal standards was diluted with 2 $CHCl_3$: 1 MeOH to yield a total of 5 ml. 100 µl of BHT and 200 µl of the internal standard mixture were added to each sample tube and the tubes were dried with nitrogen.

Standard Curve

To prepare solutions to generate a standard curve, free fatty acid stock solutions were sonicated for 15 minutes. The standard curve mixture included 5 µg/ml of C8:0, 5 µg/ml of C10:0, 50 µg/ml of C14:0, 5 µg/ml of C14:1, 500 µg/ml of C16:0, 50 µg/ml of C16:1, 150 µg/ml of C18:0, 500 µg/ml of C18:1, 500 µg/ml of C18:2, 5 µg/ml of C18:3, 150 µg/ml of C20:4, 50 µg/ml of C20:5, and 50 µg/ml of C22:6 (Nu-Chek Prep, INC; Elysian, MN). Fatty acid standards were brought to a final volume of 1 ml in a volumetric flask with $CHCl_3$: 1 MeOH. A blank tube was prepared with 100 µl of 2 $CHCl_3$: 1 MeOH. Known volumes of the standard mixture were added to prepared tubes as follows: tube 1, 300 µl; tube 2, 200 µl; tube 3, 100 µl; tube 4, 50 µl; tube 5, 25 µl; and tube 6, 10 µl. Then, 25 µl of plasma was added to subsequent tubes. A mixture of 90:10 acetonitrile (MeCN) : 6 N HCL was prepared and 2 ml was added to each tube. This mixture was mixed twice for 30 seconds and placed into a 100 ˚ C oven for 45 minutes. The tubes were then cooled to room temperature. During this time, a 90:10 mixture of methanol (MeOH): sodium hydroxide (NaOH) was prepared and 2 ml was placed into each tube. Each tube was mixed twice for 30 seconds and placed in a 100˚ C oven. After 45 minutes the tubes were cooled to room temperature and 350 µl 6 N HCL and 2 ml of hexane were added to each tube, mixed twice for 30 seconds and spun at 2100 rpm for 10 minutes. The hexane layer in each tube was transferred to clean conical 13 x100 mm tubes and stored overnight at 4 ˚C.

All tubes were dried under nitrogen. A 90:10 mixture of MeCN: pentaflorabenzene (PFB) was prepared. Ten µl of triethylamine and 50 µl of the 90:10 MeCN: PFB solution were added to each tube, mixed twice for 30 seconds, and allowed to sit at room temperature for at least 30 minutes. 150 µl of 0.1 N HCL and 1 ml of hexane were added to each tube and mixed twice at 30 seconds. Tubes were then spun at 600 rpm for 10 minutes. The top layer of hexane in each tube was then transferred to a clean 13 X 100 round bottom tube and allowed to dry under nitrogen. Once the tube was dried, 1 ml of hexane was added to each tube, and mixed. Gas chromatography vials were then filled with 250 µl of the mixture from each tube and were loaded into a DSQ II Single

Quadrupole gas chromatographer/mass spectrometer (GCMS) (Hewlett-Packard 6890 with 5973 mass selective detector, Palo Alto, CA). The GCMS was programmed to operate in the negative chemical ionization mode with methane as the reagent gas. Fatty acid PFB-esters were separated on a DB-5mx capillary column (30m x 0.25mm x 0.25μm film thickness; ThermoFisher Scientific, Inc., Waltham, MA) with helium as the carrier gas.

The fatty acids analyzed included: C14:0, C14:1, C16:0, C16:1, C18:0, C18:1, C18:2, C18:3, C20:4, C20:5 and C22:6, and were detected using selected ion monitoring for the corresponding free fatty acid resulting from the loss of PFB. Each fatty acid was matched to the deuterated internal standard closest in length and retention time. Peak area ratios of known amounts of standard fatty acids and the internal standards were used to generate calibration curves to quantify unknowns using Xcalibur software (ThermoFisher Scientific, Inc., Waltham, MA). Individual fatty acid peaks were compared to internal standards of known concentration and identified by molecular mass and retention time. The sums of saturated (C8:0, C10:0, C14:0, C16:0, C18:0), monounsaturated (C14:1, C16:1, C18:1), polyunsaturated (C18:2, C18:3, C20:4, C20:5, C22:6), n-3 fatty acids (C18:3, C20:5, C22:6) and n-6 fatty acids (C18:2, C20:4) were calculated for each subject at each time point by adding together absolute concentrations of respective individual fatty acids within each summed group. Fatty acid concentrations were reported in μmol/L.

Sample Size Consideration

In the parent Energy Balance Study a sample size of 10 participants per group provided 90% power to detect differences in weight loss between groups. Thus, this dictated the sample size for this subanalysis. This study was a secondary analysis of samples collected as part of the Energy Balance Study. As such, it was not designed, a priori, to meet specific power requirements to detect differences in changes in concentrations of the analytes measured herein, but rather to provide preliminary data for future studies that will examine the effect of low- and high-carbohydrate diets on circulating fatty acid and inflammatory marker concentrations.

Statistical Analysis

Circulating fatty acid, IL-6 and CRP concentrations at baseline (Week 2) and the end of the study (Week 9) were the primary outcome variables of the study. Baseline characteristics of participants in the low- and high-carbohydrate diet groups were analyzed using tests of proportions and t-tests. Values at baseline, at the end of the study, and the change from baseline to the end of the study (Week 9 minus Week 2) were summarized using the mean, median, standard deviation and interquartile range (IQR, 75th percentile minus 25th percentile) for each outcome variable. Skewness and existence of outliers for each dietary group were visually assessed using box-whisker plots; measurements exceeding a 1.5 interquartile range from the 25th or 75th percentile were considered outliers. To maintain maximal sample size, these outliers were retained and robust statistical methods based on ranks were employed to reduce influence of any extreme values. Specifically, the Wilcoxon signed-rank test was used to assess changes over time within groups, and the rank sum test was used to assess differences in changes over time between groups. All statistical analyses were performed using STATA 11.1 (Stata / SE 10.1 StataCorp, College Station, TX). Significance was set to 0.05 for all tests.

Regression Analysis

Robust regression models were constructed to determine whether the change in fatty acid or inflammatory marker variables (the outcomes) differed between groups, after controlling for weight loss and baseline (Week 2) concentrations of the outcome of interest. Robust analysis was used as fatty acid concentrations were not normally distributed. For each outcome, the overall model was initially tested for significance of any explanatory variable (group or weight loss or baseline value) at the 0.15 level. If this initial screening test was significant ($p<0.15$), then individual explanatory variables were examined separately and judged for significance at the 0.05 level. If the initial screening test was not significant ($p>0.15$), the analysis proceeded to the next marker.

Correlation Analysis

Spearman correlation tests were used to measure the relationships between changes in fatty acid and inflammatory marker concentrations within the low- and high-carbohydrate diet groups. The same correlations were also re-analyzed after controlling for weight loss.

CHAPTER 4. RESULTS

This sub-analysis of samples, obtained as part of a randomized, controlled feeding study, was performed to determine the impact of low- and high-carbohydrate diets on changes in circulating concentrations of fatty acids and inflammatory markers. Correlations within groups, between changes in fatty acid and inflammatory marker concentrations, were also assessed.

Subject Characteristics at Baseline

Demographic and anthropometric characteristics of the study participants at baseline are presented in Table 2. Most of the participants in the study were female; 90% in the low-carbohydrate diet group and 77% in the high-carbohydrate diet group. Forty percent of participants in the low-carbohydrate diet group and 23% in the high-carbohydrate diet group were of racial and ethnic groups other than Caucasian. The average age of all participants was 47 ± 9 years with an average weight of 99 ± 13 kg and an average BMI of 35 ± 5 kg/m^2. The majority of participants, 80% in the low-carbohydrate diet group and 85% in the high- carbohydrate diet group, were classified as Class I obese with BMIs between 30-34.9 kg/m^2. The use of the minimization method to balance groups in this study was successful as the frequencies and mean values for each prognostic variable were similar between groups ($p>0.05$).

Table 2. Subject Characteristics at Baseline

Characteristic	Low-Carbohydrate Diet (n=10)	High-Carbohydrate Diet (n=13)
Male	1	3
Female	9	10
Race, Caucasian	6	10
Race, Other	4	3
Age (y)	46 ± 8	48 ± 11
Weight (kg)	103 ± 13	99 ± 14
BMI (kg/m^2)	37 ± 5	35 ± 5
Class I Obesity: 30-34.9 (kg/m^2)	8	11
Class II Obesity: 35-39.9 (kg/m^2)	2	2

Frequency or Mean ± SD; Proportion tests were used to assess differences between groups for sex, race and obesity classification; t-tests were used to assess differences between groups in age, weight and BMI. There were no differences between groups at baseline ($p>0.05$).

Dietary Composition

All subjects tolerated the meals served and compliance was high as assessed by watching participants eat breakfast at the OCTRI, by visually inspecting food containers upon return to the OCTRI kitchen, and by self-reporting by the participants.

Composition of Standard Diet

The average energy and nutrient content of the standard diet provided to and consumed by participants in the low- and high-carbohydrate diet groups is presented in Table 3. The participants randomized to the low-carbohydrate diet group consumed 2861 ± 323 kcal/day and the participants randomized to the high-carbohydrate diet group consumed 2770 ± 390 kcal/day during the standard diet phase of the study. Energy intake by the two groups was similar (p=0.9) and reflected the average energy requirement for weight maintenance. The average macronutrient composition of the standard diet consumed by all participants was 52 ± 0.6% carbohydrate, 15 ± 0.3% protein, and 36 ± 0.5% fat. The fat composition of the standard diet was 11 ± 2% saturated fat, 13 ± 2% monounsaturated fat, and 9 ± 2% polyunsaturated fat.

Table 3. Energy and Nutrient Content of the Standard Diet Offered to and Consumed by Subjects in the Low- and High-Carbohydrate Diet Groups*

Dietary Variables	Standard Diet			
	Low-Carbohydrate Diet (n=10)		High-Carbohydrate Diet (n=13)	
	Offered	Consumed	Offered	Consumed
Energy (kcal)	2921 ± 161	2861 ± 323	2922 ± 374	2770 ± 390
Energy (% EER)	~100%	96 ± 4	~100%	96 ± 2
Protein (g)	106 ± 6	104 ± 11	106 ± 13	100 ± 13
Carbohydrate (g)	379 ± 21	373 ± 42	380 ± 49	362 ± 51
Total Fat (g)	116 ± 7	113 ± 13	116 ± 13	109 ± 16
Saturated Fat (g)	37± 6	36 ± 3	37 ± 7	36 ± 5
PA, 16:0 (g)	19 ± 2	19 ± 2	19 ± 3	18 ± 3
SA, 18:0 (g)	9 ± 1	9 ± 1	9 ± 2	9 ± 1
MUFA (g)	41 ± 6	39 ± 5	42 ± 8	38 ±6
PUFA (g)	28 ± 6	26 ± 3	29 ± 7	25 ± 4
n-6 (g)	25 ± 6	23 ± 3	25 ± 6	23 ± 4
LA, 18:2 (g)	25 ± 6	23 ± 3	25 ± 6	23 ± 3
AA , 20:4 (g)	0.1 ± 0.06	0.01 ± 0.01	0.1 ± 0.07	0.1 ± 0.02
n-3 (g)	3 ± 1	2.7 ± 0.4	3 ± 1	3 ± 0.4
ALA, 18:3 (g)	3 ± 1	2.6 ± 0.4	3 ± 1	2 ± 0.4
EPA, 20:5 (g)	0.02 ± 0.04	0.02 ± 0.04	0.02 ± 0.04	0.02 ± 0.01
DHA, 22:6 (g)	0.1 ± 0.2	0.1 ± 0.02	0.1 ± 0.2	0.1 ± 0.02
n-6/n-3	9 ± 3	9 ± 0.6	9 ± 3	9 ± 0.6
AA/(EPA + DHA)	3 ± 1	1 ± 0.2	3 ±1	1 ± 0.2

*Mean ± SD
PA=Palmitic Acid; SA=Stearic Acid; MUFA=Monounsaturated Fatty Acid; PUFA=Polyunsaturated Fatty Acid; N-6=omega-6 Fatty Acid; LA=Linoleic Acid Fatty Acid; AA=Arachidonic Acid; N-3=omega-3 Fatty Acid; ALA= α-Linolenic Acid; EPA=Eicosapentaenoic Acid; DHA=Docosahexanoic Acid; Estimated Energy Requirement (EER) to maintain weight as calculated by the Boothby and Berkson Food Nomogram (52).
% EER=(actual energy intake/EER)*100

Composition of Low- and High-Carbohydrate Diets

The energy and macronutrient composition offered to and consumed by the low- and high-carbohydrate diet groups during the six-week intervention are displayed in Table 4. The average amount of energy consumed by participants on the low- and high-carbohydrate diets was similar at 2222 ± 432 kcal/day and 2227 ± 512 kcal/day, respectively ($p=0.9$) and represented 76 ± 14 and 77 ± 11% of the estimated energy intake for weight maintenance. The macronutrient distribution of the low-carbohydrate diet was 4 ± 0.6% carbohydrate, 30 ± 2% protein, and 66 ± 2% fat. Furthermore, 27 ± 1% of energy was derived from saturated fat, 23 ± 1% was derived from monounsaturated fat and 7 ± 1% was derived from polyunsaturated fat. The macronutrient distribution of those consuming the high-carbohydrate diet was 52 ± 3% carbohydrate, 19 ± 1% protein 29 ± 2 % fat. In addition, 7 ± 0.5% of energy was derived from saturated fat, 13 ± 1% was derived from monounsaturated fat, and 8 ± 0.5% was derived from polyunsaturated fat. Those consuming the low-carbohydrate diet consumed ~4 times more saturated fat ($p<0.001$), ~2 times more monounsaturated fat and about the same amount of polyunsaturated fat as those consuming the high-carbohydrate diet. Consumption of n-3 fatty acids was significantly different between the low- and high-carbohydrate diet groups ($p=0.03$) as was the n-6/n-3 fatty acid ratio ($p<0.001$) and the AA/ (EPA+DHA) fatty acid ratio ($p<0.001$). However, n-6 fatty acid consumption was not significantly different between those in the two diet groups ($p=0.1$).

Table 4. Energy and Nutrient Content of Diets Offered to and Consumed by Subjects In the Low- and High-Carbohydrate Diet Groups*

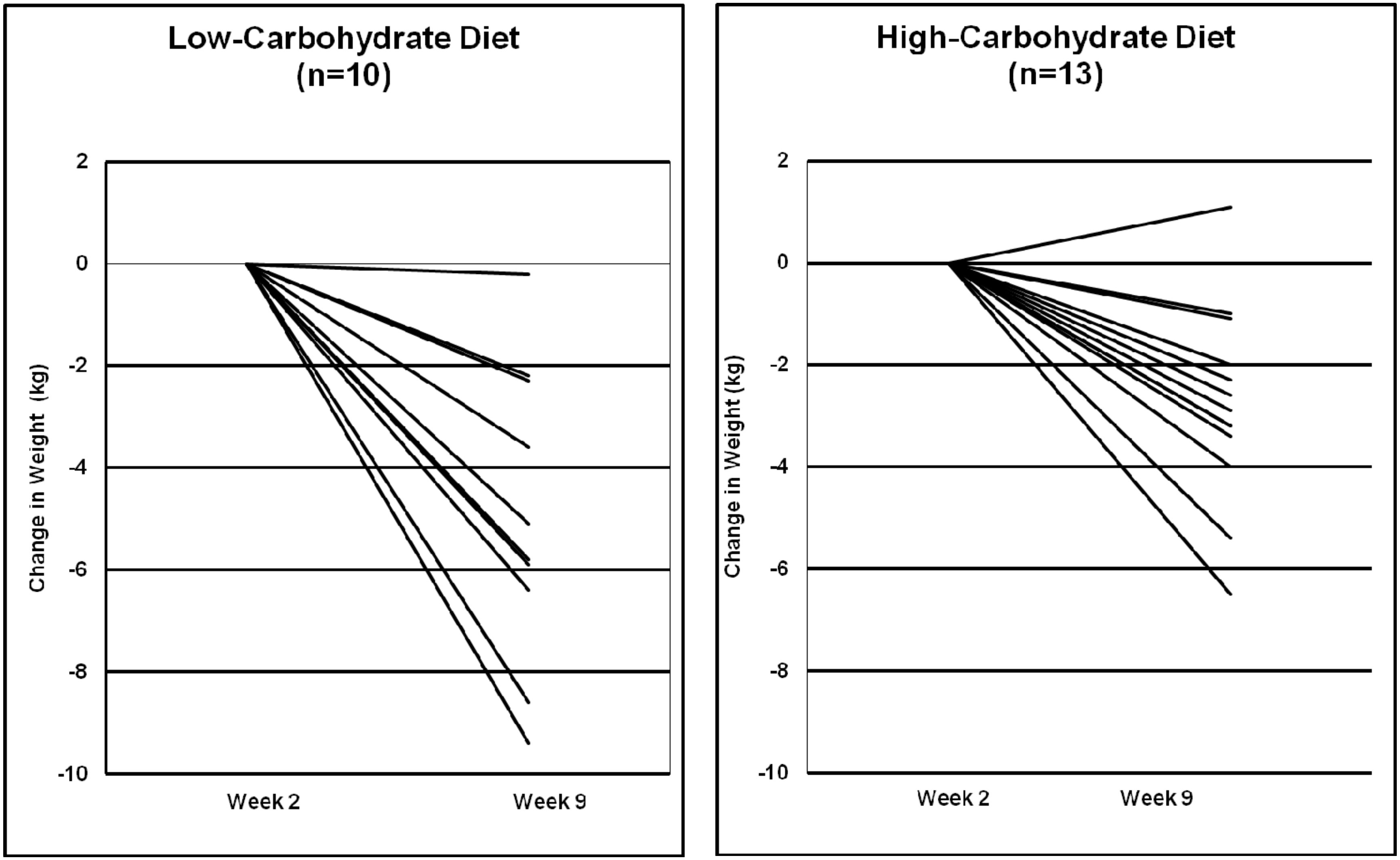
Dietary Variables	Low-Carbohydrate Diet (n=10)		High-Carbohydrate Diet (n=13)	
	Offered	Consumed	Offered	Consumed
Energy (kcal)	3526 ± 331	2223 ± 432	3346 ± 523	2227 ± 512
Energy (% EER)	~120%	76 ± 14	~120%	77 ± 11
Protein (g)	258 ± 33	165 ± 33	150 ± 26	104 ± 23
Carbohydrate (g)	35 ± 7	23 ± 4	475 ± 79	298 ± 75
Total Fat (g)	258 ± 26	161 ± 32	106 ± 15	76 ± 17
SAT (g)	106 ± 17	66 ± 14	24 ± 6	17 ± 5
PA, 16:0 (g)	56 ± 7	35 ± 7	14 ± 3	10 ± 3
SA, 18:0 (g)	26 ± 4	16 ± 3	5 ± 2	4 ± 1
MUFA (g)	91 ± 13	57 ± 12	45 ± 9	31 ± 6
PUFA (g)	31 ± 9	19 ± 3	28 ± 5	21 ± 5
n-6 (g)	27 ± 8	17 ± 3	26 ± 5	19 ± 5
LA, 18:2 (g)	27 ± 8	16 ± 3	26 ± 5	19 ± 5
AA, 20:4 (g)	0.7 ± 0.2	0.4 ± 0.1	0.1 ± 0.1	0.1 ± 0.02
n-3 (g)	4 ± 1	3 ± 0.4	3 ± 1	2 ± 0.5
ALA, 18:3 (g)	4 ± 1	2 ± 0.4	3 ± 0.8	2 ± 0.4
EPA, 20:5 (g)	0.06 ± 0.08	0.04 ± 0.01	0.2 ± 0.3	0.1 ± 0.04
DHA, 22:6 (g)	0.3 ± 0.3	0.2 ± 0.04	0.3 ± 0.5	0.2 ± 0.06
n-6/ n-3	7 ± 2	6 ± 0.3	9 ± 3	9 ± 0.8
AA / (EPA +DHA)	10 ± 12	2 ± 0.3	2 ± 1	0.8 ± 0.4

*Mean ± SD
PA=Palmitic Acid; SA=Stearic Acid; MUFA=Monounsaturated Fatty Acid; PUFA=Polyunsaturated Fatty Acid; N-6=omega-6 Fatty Acid; LA=Linoleic Acid; AA=Arachidonic Acid; N-3=omega-3 Fatty Acid; ALA= α-Linolenic Acid; EPA=Eicosapentaenoic Acid; DHA=Docosahexanoic Acid
Estimated Energy Requirement (EER) to maintain weight as calculated by the Boothby and Berkson Food Nomogram (52).% EER=(actual energy intake/EER)*100

Changes in Body Weight

Individual changes in weight of participants in the low- and high-carbohydrate diet groups are displayed in Figure 2. All participants in the low-carbohydrate diet lost weight ranging from -0.2 to -9.4 kg (mean -5 ± 3 kg; compared to baseline p=0.005). Of those assigned to the high-carbohydrate diet one participant gained 1.1 kg while all the others lost weight. The average change in weight from baseline in the high-carbohydrate diet group was -3 ± 2 kg (p=0.003) with a range from +1.1 to -6.5 kg. The mean change in weight was significantly different between groups with the low-carbohydrate diet group losing more weight than the high-carbohydrate diet group (p=0.05).

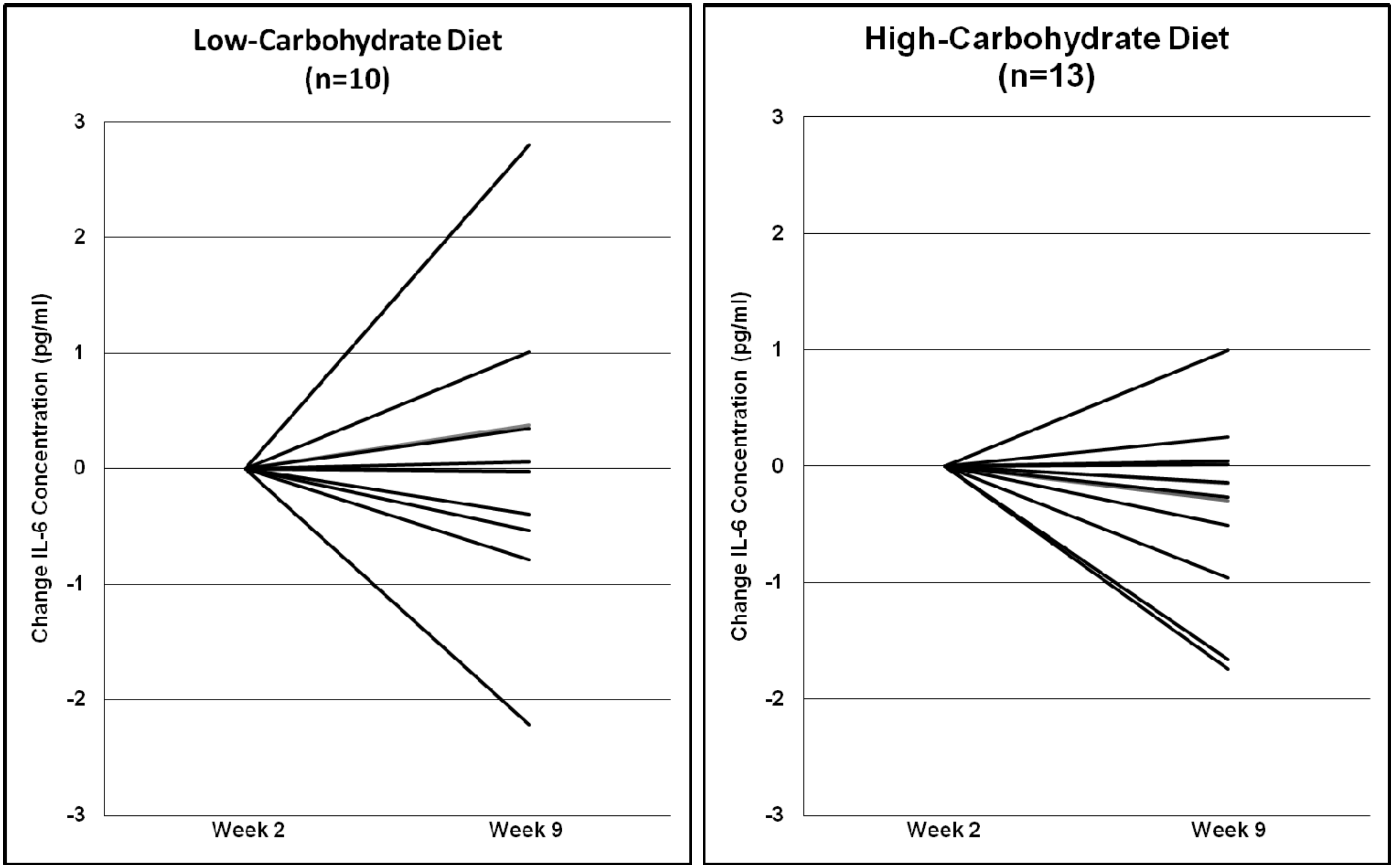
Figure 2 Effect of Low- and High-Carbohydrate Diets on Weight Loss



Serum Interleukin-6 (IL-6) Concentrations

Serum IL-6 was analyzed as a marker of systemic inflammation. Figure 3 illustrates the individual changes from baseline in serum IL-6 concentrations among participants in the low- and high-carbohydrate groups. The mean serum IL-6 concentrations at baseline was 3 ± 4 pg/mL in the low-carbohydrate group and 2 ± 1 pg/mL in the high-carbohydrate diet group ($p=0.6$). The mean serum IL-6 concentrations at the end of the study was 3 ± 4 pg/mL in the low-carbohydrate group and 2 ± 1 pg/mL in the high-carbohydrate diet group ($p=0.5$). The mean change in serum IL-6 concentration from baseline to end of study was at 0.06 ± 1 pg/ml for the low-carbohydrate group and 0.4 ± 0.7 pg/ml for the high-carbohydrate group and was not different between groups ($p=0.4$). Fifty percent of participants in the low-carbohydrate group and 70% of participants in the high-carbohydrate group had lower serum IL-6 concentrations after the six week dietary intervention than before. The range of the change in serum IL-6 concentration was larger in the low-carbohydrate group (-2.2 to 2.8 pg/mL) than the high-carbohydrate group (-1.7 to 0.99 pg/mL). Although there was a trend for lower serum concentrations of IL-6 in both groups, the results were not significant.

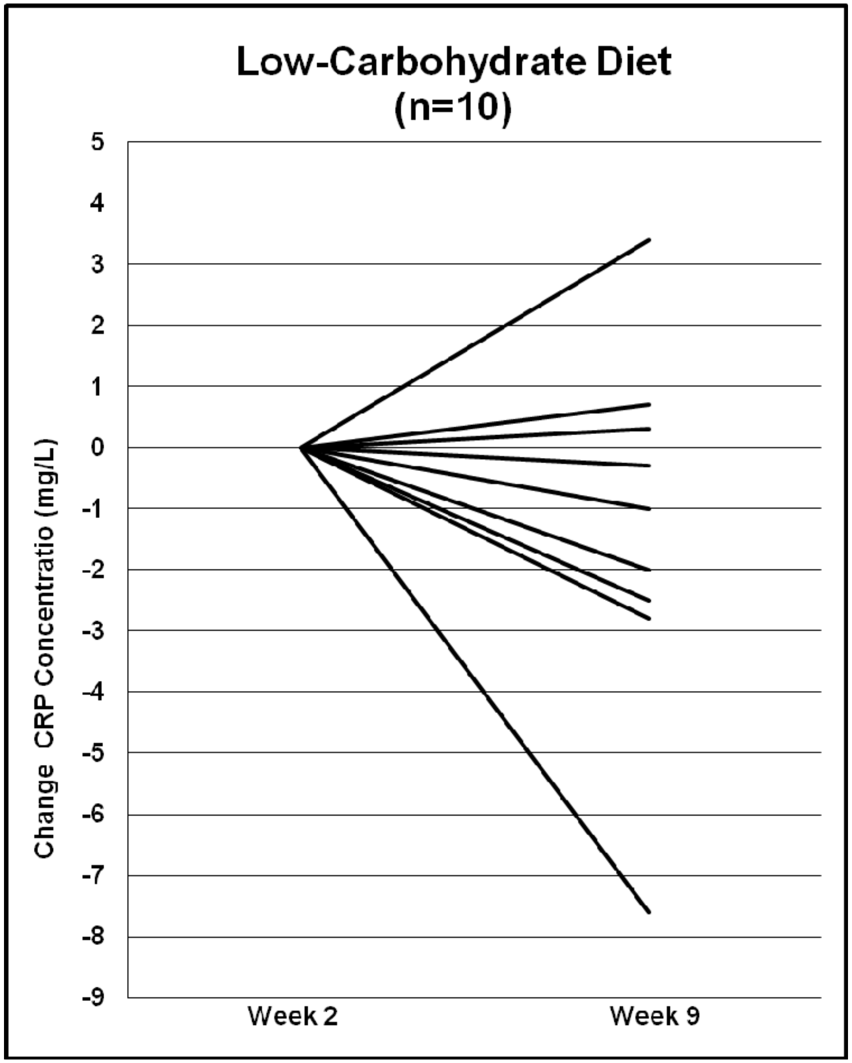
Figure 3. Effect of Low- and High-Carbohydrate Diets on Change in Serum IL-6 Concentrations

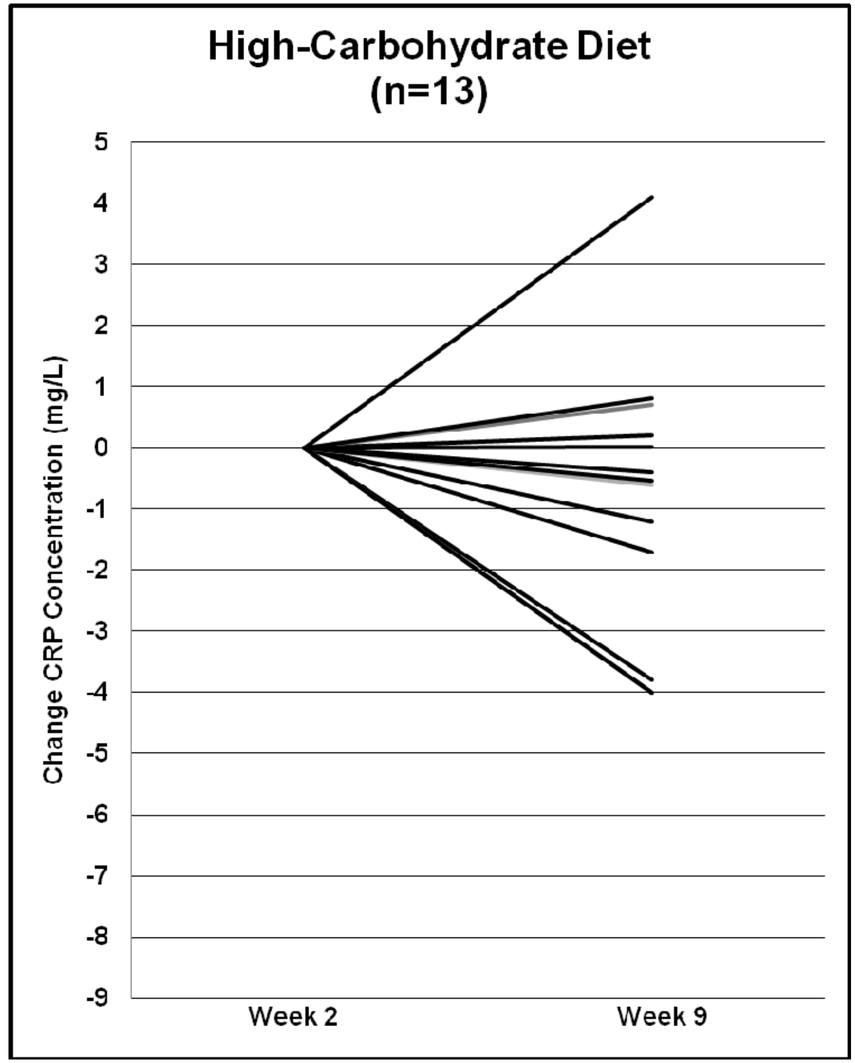


Plasma C-Reactive Protein (CRP) Concentrations

Plasma CRP concentration was also assessed as a marker of systemic inflammation. Figure 4 illustrates the individual changes from baseline in CRP concentrations among participants in the low- and high-carbohydrate diet groups. One participant in the low-carbohydrate diet group who had an end of study CRP concentration of 51.6 mg/L and a change in CRP concentration of 50.9 mg/L from baseline to end of study, was not included in the figure as the end of study value was considered to be an extreme outlier. However, this participant's CRP concentrations were included in the nonparametric and robust statistical analyses. The mean baseline concentration of CRP was 5 ± 4 mg/L for both the low- and high-carbohydrate diet groups (p=0.9). The mean CRP concentration at the end of the study was 9 ± 15 mg/L (including the outlier) and 4 ± 2 mg/L (excluding the outlier) for the low-carbohydrate diet group and 4 ± 3 mg/L for the high-carbohydrate diet group (p=0.4). The mean change from baseline in CRP concentrations in the low-carbohydrate diet group was 4 ± 17 mg/L (including the outlier; median was -0.7 mg/L and the interquartile range was 3 mg/L) and -1.3 ± 3 mg/L (excluding the outlier). The mean change in CRP concentration in the high-carbohydrate diet group was -0.8 ± 2 mg/L (median -0.5 mg/L and the interquartile range was at 2 mg/L) suggesting that changes in CRP in the two diet groups were associated with weight loss despite differences in diet composition. Sixty percent of participants in both the low- and high-carbohydrate diet groups demonstrated reductions in plasma CRP concentration from baseline. The range of the change in plasma CRP concentrations in the low-carbohydrate diet group with the outlier included was -8 to +51 mg/L, but was -8 to +3 mg/L after excluding the outlier. The range of the change in plasma CRP concentrations in the high-carbohydrate diet group was -4 to 4 mg/L. There were no differences in mean CRP concentrations at baseline or at the end of study within groups (low-carbohydrate p=0.6, high-carbohydrate p=0.2) or between groups (low-carbohydrate vs. high-carbohydrate p=0.9).

Figure 4. Effect of the Low- and High-Carbohydrate Diets on Change in Plasma CRP Concentrations





*One participant was excluded from this illustration as the value at the end of the study was considered to be an extreme outlier 51.6 mg/L generating a change from baseline of 50.9 mg/L

Plasma Fatty Acid Concentrations

Means, standard deviations, medians and interquartile ranges for plasma fatty acid concentrations at baseline, the end of the study, and the change from baseline for the low- and high-carbohydrate diet groups are presented in Table 5. Surprisingly, circulating concentrations of saturated fatty acids, specifically palmitic and stearic acid concentrations, were significantly lower in both groups after dietary intervention ($p=0.6$) despite large differences in dietary saturated fatty acid content between the low- and high-carbohydrate diets. Changes in plasma saturated fatty acid concentrations were not significantly different between groups. Plasma monounsaturated fatty acid concentrations were lower in both groups after dietary intervention, however, the change was not significantly different within or between groups. Plasma polyunsaturated fatty acid concentrations were not significantly different from baseline after the dietary intervention in either group. The difference from baseline in circulating n-6 fatty acid concentrations between groups was not significant. Circulating n-3 fatty acid concentrations rose significantly from baseline by 177 ± 283 µmol/L in the high-carbohydrate diet ($p=0.02$) but not the low-carbohydrate diet group. The change in n-3 fatty acid concentrations in the high-carbohydrate diet group was driven by a significant increase from baseline in circulating docosahexaenoic acid concentration ($p=0.03$). Despite this difference, the difference in the change from baseline in n-3 fatty acid concentrations was not significant between the high-carbohydrate diet group and the low-carbohydrate diet group ($p=0.08$).

The changes from baseline in plasma n-6/n-3 and AA/(EPA+DHA) fatty acid concentration ratios were significantly different between the low- and high-carbohydrate diet groups. The mean circulating n-6/n-3 fatty acid concentration ratio was significantly lower than baseline in the high-carbohydrate diet group but not the low-carbohydrate diet group. As a result the difference in change in the n-6/n-3 fatty acid ratio from baseline between groups was significantly different (p=0.02). The reduction in the plasma n-6/n-3 fatty acid ratio seen in the high-carbohydrate diet group was likely due to the increase in circulating n-3 fatty acid concentrations; specifically, the increase in docosahexaenoic fatty acid concentration. Likewise, the significant reduction in the AA/(EPA+DHA) fatty acid ratio in the high-carbohydrate diet group was likely due to the significant increase in circulating docosahexaenoic acid concentrations.

Table 5. Change in Plasma Fatty Acid Concentrations in Subjects Consuming a Low- or a High-Carbohydrate Diet for Six Weeks

Plasma Fatty Acids		Baseline (Week 2)		End of Study (Week 9)		Change from Baseline	
		Mean ± SD	Median/IQR	Mean ± SD	Median/IQR	Mean ± SD	Median/IQR
Saturated Fat, µmol/L	LC	2320 ± 2456	1536/643	1721 ± 1792	1235/588	**-599 ± 906**	**-294/662**
	HC	1432 ± 435	1309/656	1172 ± 322	1093/313	**-260 ± 281**	**-261/259**
Palmitic Acid, 16:0, µmol/L	LC	1422 ± 2427	718/648	1095 ± 1695	567/538	-326 ± 859	-16/497
	HC	615 ± 343	501/36	529 ± 283	419/374	-86 ± 186	-75/152
Stearic Acid,18:0, µmol/L	LC	874 ± 88	871/136	619 ± 165	562/221	**-255 ± 201**	**-254/286**
	HC	799 ± 164	806/210	633 ± 75	639/60	**-166 ± 133**	**-168/167**
Monounsaturated Fat, µmol/L	LC	37 ± 8	38/11	32 ± 12	29/6	-6 ± 11	-6/13
	HC	45 ± 23	40/22	42 ± 15	41/26	-3 ± 22	-3/14
Polyunsaturated Fat, µmol/L	LC	2403 ± 381	2407/394	2530 ± 812	2755/625	127 ± 744	48/914
	HC	2676 ± 1118	2484/861	2948 ± 1165	2641/1182	271 ± 1107	253/887
N-6 Fatty Acids, µmol/L	LC	2043 ± 283	2032/288	2233 ± 500	2333/666	190 ± 512	35/786
	HC	2014 ± 778	2080/588	2349 ± 984	2188/1023	336 ± 706	215/804
Linoleic Acid, 18:2, µmol/L	LC	1205 ± 114	1184/104	1051 ± 320	1150/257	-155 ± 358	-84/285
	HC	1570 ± 959	1397/536	1345 ± 477	1256/335	-225 ± 957	-45/396
Arachidonic Acid, 20:4, µmol/L	LC	837 ± 204	849/356	1094 ± 362	1200/409	**256 ± 321**	**125/492**
	HC	761 ± 340	835/514	1058 ± 489	988/509	**297 ± 342**	**154/541**
N-3 Fatty Acids, µmol/L	LC	360 ± 113	393/119	395 ± 133	433/156	35 ± 99	35/128
	HC	339 ± 151	386/162	516 ± 319	450/159	**177 ± 283**	**44/248**
α-Linolenic Acid, 18:3, µmol/L	LC	33 ± 3	32/4	29 ± 7	31/4	-4 ± 9	-2/2
	HC	41 ± 24	36/13	36 ± 12	34/9	-5 ± 24	-1/9
EPA, 20:5, µmol/L	LC	154 ± 76	196/128	155 ± 81	205/131	1.6 ± 27	4/35
	HC	120 ± 77	128/148	158 ± 84	191/113	38 ± 73	8/76
DHA, 22:6, µmol/L	LC	174 ± 48	190/57	202 ± 69	220/60	28 ± 70	17/104
	HC	185 ± 86	182/84	352 ± 228	279/235	**167 ± 201***	**110/211**
n-6/n-3 fatty acid ratio	LC	6.2 ± 2.1	5/1	6.0 ± 1.4	5/2	-0.20 ± 1.4	-0.06/0.4
	HC	6.2 ± 1.1	6/2	5.0 ± 1.0	5/0.5	**-1.2 ± 1.4***	**-0.7/1**
AA/ (EPA+DHA) fatty acid ratio	LC	2.8 ± 0.8	2/0.4	3.3 ± 0.8	3/2	**0.5 ± 0.8**	**0.2/0.5**
	HC	2.6 ± 0.50	3/0.4	2.1 ± 0.74	2/0.6	**-0.5 ± 0.6****	**-0.2/0.6**

IQR= Interquantile Range; Bold Font Indicates Significantly Different Change from Baseline Using Wilcoxon Signed Rank Tests; *p<0.01 between group comparison using Rank Sum Tests; **p<0.001 between group comparison using Rank Sum Tests; EPA=Eicosapentaenoic Acid; DHA= Docosahexaenoic Acid

Regression Analysis

Regression models were designed to analyze the impact of the low- and high-carbohydrate diets on changes in circulating fatty acid and inflammatory marker concentrations, adjusted for weight loss, baseline values, and dietary assignment. Weight loss and baseline values were centered to a median that fit both the low- and high-carbohydrate diet groups. As illustrated in Table 6, when weight loss and baseline values were centered for each variable, end of study means were significantly different between the low- and high-carbohydrate diet groups for the n-6/n-3 ($p=0.02$) and the AA/(EPA+DHA) ($p=0.005$) fatty acid concentration ratios, only. In each case, the means were higher in the high-carbohydrate than the low-carbohydrate group. Whether these differences are clinically relevant is not known.

Table 6. End of Study Plasma Fatty Acid and Inflammatory Marker Concentrations Adjusted for Change in Weight and Baseline Values*

Variables	Low-Carbohydrate Diet (n=10)	High-Carbohydrate Diet (n=13)	Difference in Means between Diets	p-value
Saturated Fat (µmol/L)	1347 (1094,1601)	1184 (1007, 1360)	-164 (-480,151)	0.3
n-6 fatty acid (µmol/L)	2227 (1690,2764)	2321 (1909, 2733)	94 (-593, 781)	0.8
n-3 fatty acid (µmol/L)	421 (323, 519)	435 (358, 512)	14 (-110, 138)	0.8
n-6/n-3 fatty acid ratio	5.4 (5.3, 5.6)	5.2 (5.0, 5.3)	-0.24 (-0.4, -0.04)	**0.02**
AA / (EPA + DHA) fatty acid ratio	2.7 (2.5, 2.9)	2.3 (2.0, 2.5)	-0.4 (-0.7, -0.1)	**0.005**
IL-6 (pg/mL)	1.7 (1.1, 2.3)	1.5 (1.0, 2.0)	-0.2 (-1, 0.6)	0.6
CRP (mg/L)	3.6 (2, 5)	3.2 (2.0, 4.5)	-0.4 (-2.4,1.6)	0.7

*Mean (95% CI); Bolded Cells Indicate Significant Differences In End of Study Means Between the LC and HC Diet Groups
SFA= saturate fatty acid; n-6=omega 6 fatty acid; n-3=omega 3 fatty acid;
AA=arachidonic acid, EPA= eicosapentaenoic acid, DHA=docosahexaenoic acid
IL-6=Interleukin-6; CRP= C-reactive protein

Correlational Analysis

Correlation coefficients relating changes from baseline in fatty acid and inflammatory marker concentrations are presented in Table 7 for the low- and high-carbohydrate diet groups. Significant correlations were only observed between the changes in concentrations of saturated fatty acids and CRP in the high-carbohydrate diet group ($p=0.01$) and the changes in concentrations of n-3 fatty acids and CRP in the low-carbohydrate diet group ($p=0.05$). Although both groups demonstrated significant reductions in plasma saturated fatty acid concentrations as seen in Table 5, changes in saturated fatty acids and CRP concentrations were only correlated in the high-carbohydrate diet group. This relationship suggests that as plasma saturated fatty acid concentrations decreased, plasma CRP concentrations also decreased among those in the high-carbohydrate diet group. On the other hand, the negative correlation observed between the change in plasma n-3 fatty acid concentrations and the change in CRP concentrations in the low-carbohydrate diet group suggests that as n-3 fatty acid concentrations increased, CRP concentrations decreased. No other correlations were significant between changes in fatty acid and changes in inflammatory marker concentrations in either the low- or high-carbohydrate diet groups.

Table 7. Correlations between Change in Fatty Acid and Inflammatory Marker Concentrations within the Low- and High-Carbohydrate Diet Groups, Adjusted for Change in Weight

Change in Plasma Fatty Acid Concentration	Dietary Group	Change in CRP Concentration (mg/L)		Change in IL-6 Concentration (pg/mL)	
		Corr. Coeff.	p-value	Corr. Coeff.	p-value
Saturated Fat (µmol/L)	LC	-0.07	0.9	0.3	0.4
	HC	**0.7**	**0.01**	0.4	0.2
n-6 fatty acid (µmol/L)	LC	-0.3	0.3	-0.08	0.8
	HC	0.4	0.1	0.2	0.5
n-3 fatty acid (µmol/L)	LC	**-0.6**	**0.05**	-0.07	0.9
	HC	0.3	0.2	0.3	0.3
n-6/n-3 fatty acid ratio	LC	0.4	0.3	0.1	0.7
	HC	0.2	0.6	0.1	0.7

Corr. Coeff.=Correlation Coefficient; Bolded Values Indicate a Significant Correlation
LC=Low-Carbohydrate Diet Group; HC= High-Carbohydrate Diet Group;
SFA= saturated fatty acid; n-3= omega 3 fatty acid; n-6= omega 6 fatty acid;
IL-6=Interleukin 6; CRP= C-Reactive Protein

CHAPTER 5. DISCUSSION

Our study provides insight into how dietary composition and weight loss affect inflammatory marker and fasting plasma fatty acid concentrations. We observed that during active weight loss, low- and high-carbohydrate diets impact inflammatory marker and fasting plasma fatty acid concentrations similarly. Our results are consistent with those reported by others in that both the low- and high-carbohydrate dietary interventions induced significant weight loss. However, our results are different from those reported by others in that, despite significant weight loss, we did not observe significant changes in inflammatory marker concentrations, regardless of dietary composition. Table 8 summarizes the results of these studies, including the results reported here, that have tested the effect of diet and weight loss on inflammatory marker concentrations.

We chose to evaluate low- and high-carbohydrate diets because they are among the most popular diets used to achieve weight loss. After six weeks of dietary intervention, both the low- (-5 ± 3 kg) and high- (-3 ± 2 kg) carbohydrate diet groups lost weight, with the low-carbohydrate diet group losing more weight than the high-carbohydrate diet group ($p<0.05$). Other studies also report significant weight loss among participants consuming these two diets. Gardner et al. compared weight loss in participants assigned to low- or high-carbohydrate diets for 12 months (60). Women consuming the low-carbohydrate diet lost an average of -4.7 kg (95%CI: -6.3 to -3.1 kg) which was significantly more than women consuming the high-carbohydrate diet who lost an average of -1.6 kg (95%CI: -2.8 to -0.4 kg; $p<0.05$). Dansinger et al. and Noakes et al. also reported significant weight loss by participants randomized to either a low- or high-carbohydrate diet. In the Dansinger et al. study (35), after 8 weeks of dietary intervention, participants in the low-carbohydrate diet group lost -4.7 ± 2.9 kg and participants in the high-carbohydrate diet group lost -4.6 ± 3.8 kg (p = NS). After 12 weeks of dietary intervention, participants in the Noakes et al. study (61) lost -7 ± 4 kg consuming the low-carbohydrate diet while those consuming the high-carbohydrate diet lost -5 ± 4 kg (p=0.04). Keogh et al. also studied the effect of a low- and high-carbohydrate diets on weight loss (38). After eight weeks of dietary intervention the low-carbohydrate diet group lost more weight (-7.5 ± 2.6 kg) than the high-carbohydrate diet group (-6.2 ± 2.9 kg; p<0.01). In combination, the results of these studies suggest that weight loss can be achieved using either a low- or high-carbohydrate diet; however, a low-carbohydrate diet may induce greater weight loss, at least in the short term.

Weight loss by those who are overweight or obese is associated with reduced disease risk which may be explained, at least in part, by a reduced state of inflammation. Two commonly measured markers of inflammation are Interleukin-6 (IL-6) and C-reactive protein (CRP). We reported changes in IL-6 concentrations of 0.06 ± 1 pg/ml and 0.4 ± 0.7 pg/ml in the low- and high-carbohydrate diet groups, respectively. We also reported changes in CRP concentrations of -1.3 ± 3 mg/L and of -0.8 ± 2 mg/L, in the low- and high-carbohydrate diets, respectively. Unlike our hypothesis, these changes in inflammatory marker concentrations were not significantly different within or between groups. Dansigner et al. (61), however, reported significant differences in the reduction in CRP concentrations among those consuming a low- (-0.42 ± 1.8 mg/L) or high- (-0.27 ± 2.1 mg/L) carbohydrate diet ($p<0.001$). Interestingly, despite similar weight loss, the reduction in CRP concentration was greater in the low-carbohydrate diet group than the high-carbohydrate diet group. Noakes et al.(35) also reported a significantly greater mean reduction in CRP concentration after a high-protein (-1.7 ± 0.4 mg/L) than a high-carbohydrate (-0.8 ± 0.3 mg/L) dietary intervention ($p<0.001$).

Other studies, however, report significant increases in mean CRP concentrations after consumption of a low-carbohydrate diet. Keogh et al. reported that the mean CRP concentration increased significantly and to a greater extent in participants consuming a high-carbohydrate diet (1.1 ± 0.7 mg/L) than participants consuming a low-carbohydrate diet (0.3 ± 0.2 mg/L; $p<0.05$) (38). Rankin et al. (36) reported a significant increase in mean CRP concentrations among those consuming a low-carbohydrate diet (1.4 ± 0.6 mg/L) and a significant decrease in mean CRP concentrations among those consuming a high-carbohydrate diet (-2.1 ± 1.3 mg/L). The difference in change between groups was also significant ($P<0.001$) (36). Rankin et al. also described how high- and low-carbohydrate diets affect IL-6 concentrations. Like our results, they did not observe a significant difference in mean change from baseline between diet groups (low-carbohydrate diet: 0.18 ± 0.05 pg/ml; high-carbohydrate diet: 0.27 ± 0.27 pg/ml).

Contrary to our predictions, mean changes in fasting plasma fatty acid concentrations were not different between groups after six weeks of dietary intervention. However, we did detect a significant difference in the change in the fasting plasma n-6/n-3 fatty acid concentration ratio between groups. In our study, the high-carbohydrate diet group demonstrated a greater reduction (-1 ± 1) in the fasting plasma n-6/n-3 fatty acid concentration ratio than the low-carbohydrate diet group (-0.2 ±2; $p<0.01$).This mean reduction in plasma fatty acid ratio occurred despite a significantly higher mean dietary n-6/n-3 fatty acid ratio in the high-carbohydrate diet (9 ± 0.8) than the low-carbohydrate group (6 ± 0.3; $p<0.001$).

In a study comparing a Mediterranean-style dietary intervention to a prudent-style dietary intervention, Esposito et al. (50), reported significant differences in changes in mean fasting plasma n-6/n-3 fatty acid concentration ratios and changes in mean inflammatory marker concentrations after a two-year dietary intervention of Mediterranean-style and prudent diets. The mean n-6/n-3 fatty acid ratio of the Mediterranean-style diet was 6.7 ± 1.1. The mean n-6/n-3 fatty acid ratio of the prudent-style diet was 11.2 ± 1.9 ($p<0.001$). After two years, the mean change in CRP concentration was -1.1 ± 0.4 mg/L in the Mediterranean diet group and -0.1± 0.3 mg/L in the prudent diet group ($p<0.01$). The mean change in IL-6 concentration was -0.7 ± 0.3 pg/ml in the Mediterranean diet group and -0.1 ± 0.2 pg/ml in the prudent diet group ($p=0.04$). The results from these studies suggest that the impact of low- and high- carbohydrate diets on circulating concentrations of inflammatory markers and fatty acids is not consistent.

Table 8. Studies Reporting Changes in Body Weight and Inflammatory Marker Concentrations

Author, Year (Ref)	Study Design	Subjects/ Intervention	Baseline Weight (kg)	Change in Weight (kg)	P	Baseline CRP (mg/L)	Change in CRP (mg/L)	P	Baseline in IL-6 (pg/ml)	Change in IL-6 (pg/ml)	P
Roytblat et al., 2000 (26)	One time blood draw	23 obese women							7.7 ± 5.1		<0.001
		12 healthy weight women							1.3 ± 0.9		
Esposito et al., 2004 (50)	Randomized Double Blind Study (2 years)	90 Mediterranean Style Diet	78 ± 8	-4 ± 1.1	<0.001	2.8 IQR= 0.7-5.4	-1.1 ± 0.4	0.01	2.1 IQR= 0.5-4.8	-0.7 ± 0.3	0.04
		90 Prudent Diet	77 ± 8	-1.2 ± 0.6		2.9 IQR= 0.5-5.7	-0.1 ± 0.3		1.9 IQR= 0.5-4.7	-0.1 ± 0.2	
Nicklas et al., 2004 (32)	Randomized Single Blinded Study (18 mo)	71 Weight Loss	96 ± 15	-13 ± 19	p<0.001	6.0 ± 6.5	-0.13 ± 0.5	0.01	5 ± 3	-0.7 ± 2.4	0.009
		70 Control	96 ± 19	-2 ± 12		5.9 ± 6.0	0.35 ± 1.9		5 ± 3.2	0.3 ± 2.8	
Dansinger et al., 2005 (61)	Randomized Study (2 mo)	40 Atkins	100 ± 14	-5 ± 3***	NS	4.4 ± 3.8	-0.42± 1.8***	<0.001			
		40 Zone	99 ± 18	-5 ± 3***		3.7 ± 3.4	-0.27 ± 2.1***				

Mean ± SD; BL=Baseline; IQR= Interquartile Range; NS=Not Significant;
P= *Significantly Different from comparison group; **Significantly Different from Baseline p<0.05;
*** Significantly Different from Baseline p<0.01

Table 8. Studies Reporting Changes in Body Weight and Inflammatory Marker Concentrations (Continued)

Author, Year (Ref)	Study Design	Subjects/ Intervention	Baseline Weight (kg)	Change in Weight (kg)	P	Baseline CRP (mg/L)	Change in CRP (mg/L)	P	Baseline in IL-6 (pg/ml)	Change in IL-6 (pg/ml)	P
Noakes et al., 2005 (35)	Randomized Study (12 wks)	52 High- Protein	87 ± 12	-7 ± 4	0.04	6.6 ± 0.7	- 1.7 ± 0.4	<0.001			
		48 High Carbohydrate	86 ± 12	-5 ± 4		4.8 ± 0.5	- 0.8 ± 0.3				
Villareal et al., 2006 (33)	Randomized Controlled Study (6 mo)	17 Weight. Loss Diet + Exercise	103 ± 20	-8.2 ± 5.4	<0.001	6 ± 5	-2.5 ± 4.3	<0.01	4.6 ± 4.5	-2.4 ± 4.7	<0.001
		10 Control Group	100 ± 14	-0.7 ± 2.7		6 ± 5	0.8 ± 2.8		3.3 ± 1.6	1.6 ± 4.3	
Rankin et al., 2007 (36)	Randomized Feeding Study (1 mo)	14 *ad lib.* Low Carbohydrate	87 ± 15	-3.8 ± 1.2**	NS	6 ± 6	1.4 ± 0.6***	<0.001	1.6 ± 0.8	0.18 ± 0.05	NS
		15 Energy-Restricted High Carbohydrate	79 ± 16	-2.6 ± 1.7**		5 ± 4	-2.1 ± 1.3***		1.2 ± 0.8	0.27 ± 0.27	

Mean ± SD; BL=Baseline; IQR= Interquartile Range; NS=Not Significant;
P= *Significantly Different from comparison group; **Significantly Different from Baseline p<0.05;
*** Significantly Different from Baseline p<0.01

Table 8. Studies Reporting Changes in Body Weight and Inflammatory Marker Concentrations (Continued)

Author, Year (Ref)	Study Design	Subjects/ Intervention	Baseline Weight (kg)	Change in Weight (kg)	P	Baseline CRP (mg/L)	Change in CRP (mg/L)	P	Baseline in IL-6 (pg/ml)	Change in IL-6 (pg/ml)	P
Peairs et al., 2008 (34)	Randomized Feeding Double Blinded Study (7 days)	10 LC	97 ± 9	-3.0 ± 1.4	NS	3 ± 1	0.8 ± 0.8	NS	0.9 ± 0.2	0.2 ± 0.03	NS
		8 Control	105 ± 9	-3.6 ± 1.3		3 ± 0.5	1.3 ± 0.7		1.0 ± 0.3	0.2 ± 0.02	
Keogh et al., 2008 (38)	Randomized Parallel Study (8 weeks)	52 LC	94 ± 15	- 7.5 ± 2.6**	<0.05	3 ± 2	0.3 ± 0.2**	<0.05			
		47 HC	97 ± 14	- 6.2 ± 2.9**		4 ± 3	1.1 ± 0.7***				
Podesta et al., 2010	Randomized Feeding Study (6 weeks)	10 LC	103 ± 13	-5 ± 3**	0.05	5 ± 4	-1.3 ± 3	NS	3 ± 4	0.06 ± 1	NS
		13 HC	99 ± 14	-3 ± 2**		5 ± 4	-0.8 ± 2		2 ± 1	0.4 ± 0.7	

Mean ± SD; BL=Baseline; IQR= Interquartile Range; NS=Not Significant;
P= *Significantly Different from comparison group; **Significantly Different from Baseline p<0.05;
*** Significantly Different from Baseline p<0.01

Strengths and Limitations

Strengths

This secondary analysis of samples derived from the Energy Balance Study was performed to determine whether changes in circulating concentrations of fatty acids and inflammatory markers would be different after six weeks of low- or high-carbohydrate dietary intervention. We also determined the relationships between changes in fatty acid and inflammatory marker concentrations after these dietary interventions. We studied healthy obese individuals who underwent extensive screening to rule out the presence of chronic disease. Participants were allocated to intervention groups using a design-adaptive covariate randomization scheme known as the minimization method. This randomized method ensured that groups were balanced for sex, age, BMI, total cholesterol and triglyceride concentrations; factors that could influence changes in circulating fatty acid and inflammatory marker concentrations. A controlled feeding study design was used. This type of methodology allowed us to precisely compute individualized diets and record actual intakes for each participant so we knew, with a high level of confidence, what was eaten throughout the study. Inclusion of a three-weeks standardized, weight-maintenance diet prior to the intervention diet reduced the variation that personal diets may have had on plasma fatty acid and inflammatory marker concentrations at baseline. The hyper-caloric "*ad libitum*" design was unique compared to other studies and allowed participants to select from a variety of foods and to eat until satisfied but to not be forced to eat all of the foods available. This design component contributed to a high level of participant satisfaction throughout the study. Subject completion rate was close to 100%, another indicator of participant satisfaction, which reduced bias associated with differential completion rates between groups. Sample collection and analysis of fatty acid and inflammatory marker concentrations were also 100% so that no bias was introduced by missing data or data that had to be imputed. The method used to measure plasma fatty acid concentrations, GC-MS, was quantitative; so that fatty acid concentrations were analyzed and not relative molar ratios of fatty acids.

Limitations

Despite the many strengths of the design and methodology used, this study also had limitations. Perhaps the most important limitation was the small sample size. The parent study was powered to detect differences in weight loss between groups and was not powered, a priori, to detect differences in changes in fatty acid and inflammatory marker concentrations. Because of this limitation, our results, that there were no differences in changes in circulating fatty acid and inflammatory marker concentrations between groups, should be interpreted with caution.

Based on our results, to detect a difference in mean change in CRP concentration of 0.5 mg/L with a standard deviation of 2 mg/L; a sample size of 566 participants in each group would be needed to provide two-sided power of 80%. To detect a significant difference in the mean change in IL-6 concentration of 0.4 pg/ml with a standard deviation of 0.7 pg/ml, a sample size of at least 99 participants in each group would be needed to provide a two-sided power of

80%. In our opinion, detecting a difference in an average change of 0.5 mg/L in CRP concentrations or a 0.4 pg/ml in IL-6 concentrations is of marginal clinical relevance. It is likely that a greater reduction in these inflammatory marker concentrations is needed to impact disease risk on an individual and group basis.

This study had a low enrollment of men, with one man in the low-carbohydrate diet group and three men in the high-carbohydrate diet group. Therefore the ability to generalize these results to healthy obese men is limited. Also, due to the age of our participants, our results may not apply to younger adults, children or the elderly. Likewise, our study enrolled few individuals of racial and ethnic groups other than non-Hispanic, Caucasians. Therefore generalization of our results to other high-risk ethnic and racial groups is limited.

Of particular importance, participants were allowed to take nonsteroidal anti-inflammatory medications, such as ibuprofen, as needed during the study. Use of these medications could have artificially lowered inflammatory marker concentrations making it more difficult to detect small differences in mean changes associated with diet and weight loss between groups.

Another unanticipated characteristic of the study was that both the low- and high-carbohydrate diets contained relatively high amounts of olive oil, and therefore relatively high amounts of monounsaturated fatty acids (~35% of total fat in both diets; 7 ± 0.5 % of total energy in the low-carbohydrate diet and 13 ± 1 % of energy in the high-carbohydrate diet). Diets high in monounsaturated fatty acids, like the Mediterranean diet, are known to have anti-inflammatory properties and to be cardio-protective (62). This characteristic, too, could have reduced circulating concentrations of inflammatory markers making it more difficult to detect clinically relevant differences in mean changes from baseline between dietary groups.

Clinical Implications

Despite significant differences in dietary fat intake, consumption of the low- and high-carbohydrate weight loss diets did not result in significant differences in circulating concentrations of inflammatory markers. Those in the high-carbohydrate diet group did not demonstrate the expected improvement in inflammatory marker concentrations. And those in the low-carbohydrate diet group did not demonstrate the expected worsening of inflammatory marker concentrations. This lack of significant change may be due at least in part, to two conditions:

1) All but one participant lost weight during the intervention and the state of active weight loss may override the pro-inflammatory factors associated with consuming a low-carbohydrate, high-fat diet.

2) Participants in this study were healthy, despite being obese, and as such, were not in a state of significantly heightened inflammation when they entered the study. The lower mean concentrations of inflammatory markers at baseline may have limited the potential for clinically relevant improvements in inflammatory status, especially among those consuming the high-carbohydrate diet. What is interesting is that there was no apparent worsening of inflammatory status among those consuming the low-carbohydrate diet which is inherently high in fat.

Because this study was not powered to detect differences in changes in inflammatory markers following the low- or high-carbohydrate dietary interventions, these results should be interpreted with caution. In particular, caution should be used when these results are applied to individuals who are not actively losing weight, to those who are known to have chronic inflammatory conditions, and to those who have or other co-morbid conditions. These results suggest, nonetheless, that weight loss, regardless of the composition of the weight-loss diet, may have positive effects on inflammatory marker concentrations.

CHAPTER 6. CONCLUSIONS

This secondary analysis of samples derived from the Energy Balance Study was performed to determine whether changes in circulating concentrations of fatty acids and inflammatory markers would be different after six weeks of low- or high-carbohydrate dietary intervention. In addition, the relationships between changes in fatty acid and inflammatory marker concentrations were assessed. The primary aim of this study was to assess how low- and high-carbohydrate diets impact circulating concentrations of saturated, n-6 and n-3 fatty acids and the n-6/n-3 fatty acid ratio. The secondary aim of this study was to assess how low- and high-carbohydrate diets impact circulating concentrations of IL-6 and CRP. The tertiary aim of this study was to determine how changes in plasma saturated, n-6 and n-3 fatty acid concentrations and the n-6/n-3 fatty acid ratio relate to changes in circulating IL-6 and CRP concentrations.

We hypothesized that participants in the high-carbohydrate diet group would demonstrate a greater reduction from baseline in circulating saturated and n-6 fatty acid concentrations and the n-6/n-3 fatty acid concentration ratio than those in the low-carbohydrate diet group. Based on the results presented here, we reject part of this hypothesis, as there were no differences between groups in the change in plasma saturated and n-6 fatty acid concentrations from baseline. There was, however, a greater reduction in the plasma n-6/n-3 fatty acid concentration ratio in the high-carbohydrate diet group than the low-carbohydrate diet group so we accepted this part of the hypothesis.

We also hypothesized that participants in the low-carbohydrate diet group would demonstrate a greater reduction in plasma n-3 fatty acid concentrations than those in the high-carbohydrate diet group. This hypothesis was also rejected. Omega-3 (n-3) fatty acid concentrations were higher in both groups after the dietary interventions than before; but only significantly higher in the high-carbohydrate diet group. Changes from baseline in plasma n-3 fatty acid concentrations were not significantly different between groups.

Our hypothesis associated with the secondary aim, that the high-carbohydrate diet would result in greater reductions in IL-6 and CRP concentrations than the low-carbohydrate diet, was rejected. There were no differences within or between groups in the changes in circulating IL-6 and CRP concentrations.

Our hypotheses associated with the tertiary aim, that reductions in circulating IL-6 and CRP concentrations would be directly correlated to reductions in plasma saturated and n-6 fatty acid concentrations and the n-6/n-3 fatty acid ratio in the high-carbohydrate diet group, was accepted in part. There was a positive relationship between the change in plasma saturated fatty acid concentrations and the change in plasma CRP concentrations in the high-carbohydrate diet group. However, there were no significant relationships between the changes in n-6 fatty acid concentration or in the n-6/n-3 fatty acid concentration ratio and changes in IL-6 and CRP concentrations. The hypothesis that changes in circulating IL-6 and CRP concentrations would be directly correlated to changes in plasma saturated fatty acid, n-6 fatty acid, and n-6/n-3 fatty acid ratio in the low-carbohydrate diet group was also rejected. The hypothesis that an indirect relationship would exist between changes in n-3 fatty acid concentrations and changes in IL-6 and CRP concentrations in the low-carbohydrate diet group, was accepted in part. A significant indirect relationship was identified between the change in n-3 fatty acid concentrations and the change in CRP concentrations in the low-carbohydrate diet group. The hypothesis that the change in n-3 fatty acid concentrations would be indirectly related to the change in IL-6 and CRP concentrations in the high-carbohydrate diet group was not accepted.

Our findings show that, during active weight loss, changes in circulating concentrations of saturated, n-6 and n-3 fatty acids, and IL-6 and CRP were similar between groups despite extreme differences in the macronutrient content, specifically the fat content, of the low- and high-carbohydrate diets. The results of this preliminary analysis were different from what we anticipated as we hypothesized that the low-carbohydrate diet would increase concentrations of markers of inflammation and the high-carbohydrate diet would reduce concentrations of markers of inflammation, especially with weight loss.

Although a randomized, well controlled, feeding study design was used to test these hypotheses, the study was not powered, a priori, to detect differences in change from baseline of our outcome variables between groups. Therefore, our results should be interpreted with caution due to the risk of committing a type II error; that we would conclude that no differences exist when in fact differences actually exist. To confirm the results described here, additional studies with larger sample sizes that are powered to detect small mean differences in changes in plasma fatty acid and inflammatory marker concentrations after low- and high-carbohydrate dietary interventions should be performed.

Chapter 7. FUTURE DIRECTIONS

The preliminary data derived from this study generated some provocative and unexpected findings. We expected to see a reduction in inflammatory marker concentrations in the high-carbohydrate diet group and an increase in inflammatory marker concentrations in the low-carbohydrate diet group. However, this was not the case. Instead we observed that with weight loss, regardless of dietary macronutrient content, there was no significant change from baseline in IL-6 or CRP concentrations. Despite these findings there are other areas that should be explored including the impact of diet on other markers of inflammation, like pro-inflammatory HDL cholesterol. In 1977, Gordon et al. reported a strong negative association between HDL cholesterol concentrations and the incidence of coronary heart disease in men and women >50 years of age (63). In 1991, Navab et al. reported that human HDL concentrations can induce anti-inflammatory properties associated with the inhibition of low-density lipoprotein-induced monocyte chemotactic activity in human artery wall cocultures (64). However, in 1995, Van Lenten et al. reported that in humans, in an acute phase inflammatory response state, HDL cholesterol had pro-inflammatory properties initiated by low-density lipoprotein-induced monocyte chemotactic activity (65). Some acute phase inflammatory responses are linked to elevated plasma CRP concentrations. At the end of our study, despite significant weight loss, participants consuming the low- and high-carbohydrate diets continued to have mean plasma CRP levels >2 mg/L. To better understand the relationship between obesity, diet, weight loss and markers of inflammation, future studies should explore the relationship between changes in weight and changes in concentration of HDL cholesterol along with other markers of inflammation.

Measuring fasting plasma triglyceride concentrations and relating these values to inflammatory marker concentrations should also be included in future studies. In 2010, a study by Papakonstantinou et al. concluded that obese subjects consuming a diet with a protein to fat ratio of 1:1.5, lowered triglyceride concentrations and blood pressure compared to those consuming a diet higher in fat (66). The protein to fat ratio in our study was 1:1 in the low-carbohydrate diet and 1: 0.73 in the high-carbohydrate diet. It would be interesting to explore the relationship between changes in fasting triglyceride concentrations and changes in CRP and IL-6 concentrations in obese participants consuming a low- or high-carbohydrate diet for six weeks.

Future studies should also measure the effect of low- and high-carbohydrate diets on obese participants with co-morbid conditions, such as the metabolic syndrome. In 2004, Esposito et al. assessed the effect of a Mediterranean diet on endothelial function in patients with the metabolic syndrome (50). After 2 years, participants consuming the Mediterranean diet had lost significantly more weight and had significantly lower CRP and IL-6 concentrations than participants on a prudent diet.

Measurement of erythrocyte fatty acid concentrations should be considered along with or in addition to plasma fatty acids in future studies.

Erythrocyte fatty acid concentrations are considered to be a longer-term marker of dietary intake and may be less volatile to change than plasma fatty acid concentrations. Sun et al. compared plasma and erythrocyte fatty acid concentrations to determine their abilities to reflect usual fatty acid intake in 306 women 43 to 69 years of age (67). Food frequency questionnaires were administered to determine dietary intake of fatty acids. Results found that docosahexaenoic acid in erythrocytes rather than plasma had the strongest correlation with dietary intake (correlation coefficient=0.56). Measuring erythrocyte fatty acid concentrations may be an alternative to measuring plasma fatty acid concentrations in future studies. The disadvantage to measuring erythrocyte fatty acid concentrations is that the duration of the dietary intervention would have to be increased so that changes due to diet could be achieved and detected.

REFERENCES

1. Health FaDANIo. Nutrition and Overweight. 2008.

2. Balkau B, Deanfield JE, Despres JP, Bassand JP, Fox KA, Smith SC, Jr., Barter P, Tan CE, Van Gaal L, Wittchen HU, Massien C, Haffner SM. International Day for the Evaluation of Abdominal Obesity (IDEA): a study of waist circumference, cardiovascular disease, and diabetes mellitus in 168,000 primary care patients in 63 countries. Circulation 2007;116:1942-51.

3. Hubert HB, Feinleib M, McNamara PM, Castelli WP. Obesity as an independent risk factor for cardiovascular disease: a 26-year follow-up of participants in the Framingham Heart Study. Circulation 1983;67:968-77.

4. Li JJ, Fang CH. C-reactive protein is not only an inflammatory marker but also a direct cause of cardiovascular diseases. Med Hypotheses 2004;62:499-506.

5. Danesh J, Kaptoge S, Mann AG, Sarwar N, Wood A, Angleman SB, Wensley F, Higgins JP, Lennon L, Eiriksdottir G, Rumley A, Whincup PH, Lowe GD, Gudnason V. Long-term interleukin-6 levels and subsequent risk of coronary heart disease: two new prospective studies and a systematic review. PLoS Med 2008;5:e78.

6. Nishida M, Moriyama T, Sugita Y, Yamauchi-Takihara K. Abdominal obesity exhibits distinct effect on inflammatory and anti-inflammatory proteins in apparently healthy Japanese men. Cardiovasc Diabetol 2007;6:27.

7. Tay J, Brinkworth GD, Noakes M, Keogh J, Clifton PM. Metabolic effects of weight loss on a very-low-carbohydrate diet compared with an isocaloric high-carbohydrate diet in abdominally obese subjects. J Am Coll Cardiol 2008;51:59-67.

8. Zhao G, Etherton TD, Martin KR, West SG, Gillies PJ, Kris-Etherton PM. Dietary alpha-linolenic acid reduces inflammatory and lipid cardiovascular risk factors in hypercholesterolemic men and women. J Nutr 2004;134:2991-7.

9. Vedin I, Cederholm T, Freund Levi Y, Basun H, Garlind A, Faxen Irving G, Jonhagen ME, Vessby B, Wahlund LO, Palmblad J. Effects of docosahexaenoic acid-rich n-3 fatty acid supplementation on cytokine release from blood mononuclear leukocytes: the OmegAD study. Am J Clin Nutr 2008;87:1616-22.

10. Trebble T, Arden NK, Stroud MA, Wootton SA, Burdge GC, Miles EA, Ballinger AB, Thompson RL, Calder PC. Inhibition of tumour necrosis factor-alpha and interleukin 6 production by mononuclear cells following dietary fish-oil supplementation in healthy men and response to antioxidant co-supplementation. Br J Nutr 2003;90:405-12.

11. Clarke R, Shipley M, Armitage J, Collins R, Harris W. Plasma phospholipid fatty acids and CHD in older men: Whitehall study of London civil servants. Br J Nutr 2008:1-6.

12. Block RC, Harris WS, Reid KJ, Sands SA, Spertus JA. EPA and DHA in blood cell membranes from acute coronary syndrome patients and controls. Atherosclerosis 2008;197:821-8.

13. Kiecolt-Glaser JK, Belury MA, Porter K, Beversdorf DQ, Lemeshow S, Glaser R. Depressive symptoms, omega-6:omega-3 fatty acids, and inflammation in older adults. Psychosom Med 2007;69:217-24.

14. Wang Y, Beydoun MA, Liang L, Caballero B, Kumanyika SK. Will all Americans become overweight or obese? estimating the progression and cost of the US obesity epidemic. Obesity (Silver Spring) 2008;16:2323-30.

15. Ogden CL, Carroll MD, Curtin LR, McDowell MA, Tabak CJ, Flegal KM. Prevalence of overweight and obesity in the United States, 1999-2004. JAMA 2006;295:1549-55.

16. Danaei G, Ding EL, Mozaffarian D, Taylor B, Rehm J, Murray CJ, Ezzati M. The preventable causes of death in the United States: comparative risk assessment of dietary, lifestyle, and metabolic risk factors. PLoS Med 2009;6:e1000058.

17. Lilja M, Eliasson M, Stegmayr B, Olsson T, Soderberg S. Trends in obesity and its distribution: data from the Northern Sweden MONICA Survey, 1986-2004. Obesity (Silver Spring) 2008;16:1120-8.

18. Matsushita Y, Takahashi Y, Mizoue T, Inoue M, Noda M, Tsugane S. Overweight and obesity trends among Japanese adults: a 10-year follow-up of the JPHC Study. Int J Obes (Lond) 2008;32:1861-7.

19. Bahceci M, Gokalp D, Bahceci S, Tuzcu A, Atmaca S, Arikan S. The correlation between adiposity and adiponectin, tumor necrosis factor alpha, interleukin-6 and high sensitivity C-reactive protein levels. Is adipocyte size associated with inflammation in adults? J Endocrinol Invest 2007;30:210-4.

20. Kishimoto T. Interleukin-6: discovery of a pleiotropic cytokine. Arthritis Res Ther 2006;8 Suppl 2:S2.

21. Gabay C. Interleukin-6 and chronic inflammation. Arthritis Res Ther 2006;8 Suppl 2:S3.

22. Weisberg SP, McCann D, Desai M, Rosenbaum M, Leibel RL, Ferrante AW, Jr. Obesity is associated with macrophage accumulation in adipose tissue. J Clin Invest 2003;112:1796-808.

23. Heinrich PC, Behrmann I, Haan S, Hermanns HM, Muller-Newen G, Schaper F. Principles of interleukin (IL)-6-type cytokine signalling and its regulation. Biochem J 2003;374:1-20.

24. Van Snick J. Interleukin-6: an overview. Annu Rev Immunol 1990;8:253-78.

25. Sheu WH, Chang TM, Lee WJ, Ou HC, Wu CM, Tseng LN, Lang HF, Wu CS, Wan CJ, Lee IT. Effect of weight loss on proinflammatory state of mononuclear cells in obese women. Obesity (Silver Spring) 2008;16:1033-8.

26. Roytblat L, Rachinsky M, Fisher A, Greemberg L, Shapira Y, Douvdevani A, Gelman S. Raised interleukin-6 levels in obese patients. Obes Res 2000;8:673-5.

27. Cronstein BN. Interleukin-6--a key mediator of systemic and local symptoms in rheumatoid arthritis. Bull NYU Hosp Jt Dis 2007;65 Suppl 1:S11-5.

28. Zhong W, Zen Q, Tebo J, Schlottmann K, Coggeshall M, Mortensen RF. Effect of human C-reactive protein on chemokine and chemotactic factor-induced neutrophil chemotaxis and signaling. J Immunol 1998;161:2533-40.

29. Woollard KJ, Phillips DC, Griffiths HR. Direct modulatory effect of C-reactive protein on primary human monocyte adhesion to human endothelial cells. Clin Exp Immunol 2002;130:256-62.

30. Shine B, de Beer FC, Pepys MB. Solid phase radioimmunoassays for human C-reactive protein. Clin Chim Acta 1981;117:13-23.

31. Bermudez EA, Rifai N, Buring J, Manson JE, Ridker PM. Interrelationships among circulating interleukin-6, C-reactive protein, and traditional

cardiovascular risk factors in women. Arterioscler Thromb Vasc Biol 2002;22:1668-73.

32. Nicklas BJ, Ambrosius W, Messier SP, Miller GD, Penninx BW, Loeser RF, Palla S, Bleecker E, Pahor M. Diet-induced weight loss, exercise, and chronic inflammation in older, obese adults: a randomized controlled clinical trial. Am J Clin Nutr 2004;79:544-51.

33. Villareal DT, Miller BV, 3rd, Banks M, Fontana L, Sinacore DR, Klein S. Effect of lifestyle intervention on metabolic coronary heart disease risk factors in obese older adults. Am J Clin Nutr 2006;84:1317-23.

34. Peairs AT, Rankin JW. Inflammatory response to a high-fat, low-carbohydrate weight loss diet: effect of antioxidants. Obesity (Silver Spring) 2008;16:1573-8.

35. Noakes M, Keogh JB, Foster PR, Clifton PM. Effect of an energy-restricted, high-protein, low-fat diet relative to a conventional high-carbohydrate, low-fat diet on weight loss, body composition, nutritional status, and markers of cardiovascular health in obese women. Am J Clin Nutr 2005;81:1298-306.

36. Rankin JW, Turpyn AD. Low carbohydrate, high fat diet increases C-reactive protein during weight loss. J Am Coll Nutr 2007;26:163-9.

37. Weigert C, Brodbeck K, Staiger H, Kausch C, Machicao F, Haring HU, Schleicher ED. Palmitate, but not unsaturated fatty acids, induces the expression of interleukin-6 in human myotubes through proteasome-dependent activation of nuclear factor-kappaB. J Biol Chem 2004;279:23942-52.

38. Keogh JB, Brinkworth GD, Noakes M, Belobrajdic DP, Buckley JD, Clifton PM. Effects of weight loss from a very-low-carbohydrate diet on endothelial function and markers of cardiovascular disease risk in subjects with abdominal obesity. Am J Clin Nutr 2008;87:567-76.

39. Funk CD. Prostaglandins and leukotrienes: advances in eicosanoid biology. Science 2001;294:1871-5.

40. Piomelli D. Archadonic Acid. 2000.

41. Sundrarjun T, Komindr S, Archararit N, Dahlan W, Puchaiwatananon O, Angthararak S, Udomsuppayakul U, Chuncharunee S. Effects of n-3 fatty acids on serum interleukin-6, tumour necrosis factor-alpha and soluble

tumour necrosis factor receptor p55 in active rheumatoid arthritis. J Int Med Res 2004;32:443-54.

42. De Caterina R, Zampolli A. n-3 fatty acids: antiatherosclerotic effects. Lipids 2001;36 Suppl:S69-78.

43. Moriguchi EH, Moriguchi Y, Yamori Y. Impact of diet on the cardiovascular risk profile of Japanese immigrants living in Brazil: contributions of World Health Organization CARDIAC and MONALISA studies. Clin Exp Pharmacol Physiol 2004;31 Suppl 2:S5-7.

44. Niu K, Hozawa A, Kuriyama S, Ohmori-Matsuda K, Shimazu T, Nakaya N, Fujita K, Tsuji I, Nagatomi R. Dietary long-chain n-3 fatty acids of marine origin and serum C-reactive protein concentrations are associated in a population with a diet rich in marine products. Am J Clin Nutr 2006;84:223-9.

45. Dyerberg J, Bang HO, Hjorne N. Fatty acid composition of the plasma lipids in Greenland Eskimos. Am J Clin Nutr 1975;28:958-66.

46. Bang HO, Dyerberg J, Sinclair HM. The composition of the Eskimo food in north western Greenland. Am J Clin Nutr 1980;33:2657-61.

47. U.S. Department of Agriculture ARS. Nutrient Intakes from Food: Mean Amounts Consumed per Individual, One Day, 2005-2006. 2008.

48. Simopoulos AP. Importance of the ratio of omega-6/omega-3 essential fatty acids: evolutionary aspects. World Rev Nutr Diet 2003;92:1-22.

49. Yamagishi K, Iso H, Date C, Fukui M, Wakai K, Kikuchi S, Inaba Y, Tanabe N, Tamakoshi A. Fish, omega-3 polyunsaturated fatty acids, and mortality from cardiovascular diseases in a nationwide community-based cohort of Japanese men and women the JACC (Japan Collaborative Cohort Study for Evaluation of Cancer Risk) Study. J Am Coll Cardiol 2008;52:988-96.

50. Esposito K, Marfella R, Ciotola M, Di Palo C, Giugliano F, Giugliano G, D'Armiento M, D'Andrea F, Giugliano D. Effect of a mediterranean-style diet on endothelial dysfunction and markers of vascular inflammation in the metabolic syndrome: a randomized trial. JAMA 2004;292:1440-6.

51. Simopoulos AP. The importance of the omega-6/omega-3 fatty acid ratio in cardiovascular disease and other chronic diseases. Exp Biol Med (Maywood) 2008;233:674-88.

52. Boothby WM BJ, Dunn HL. Studies of the energy of metabolism of normal individuals: A standard for basal metabolism, with a nomogram for clinical application. Am J Physiol 1936;116:468–484.

53. Pocock S. Clinical trails: a practical approach. New York: John Wiley & Sons 1984.

54. Atkins R. Dr. Atkins' New Diet Revolution. New York: HarperCollins, 1992.

55. Your Guide To Lowering Your Blood Pressure with DASH. In: Services USDoHaH, ed. Washington D.C., 1998.

56. Schakel SF, Sievert YA, Buzzard IM. Sources of data for developing and maintaining a nutrient database. J Am Diet Assoc 1988;88:1268-71.

57. Quantikine HS: High Sensitivity ELISAs Human IL-6. In: Systems RD, ed. R& D Systems, Inc. Minneapolis 2008.

58. High Sensitivity CRP. In: Services SHDT, ed. Siemens. New York, 2008.

59. Lagerstedt SA, Hinrichs DR, Batt SM, Magera MJ, Rinaldo P, McConnell JP. Quantitative determination of plasma c8-c26 total fatty acids for the biochemical diagnosis of nutritional and metabolic disorders. Mol Genet Metab 2001;73:38-45.

60. Gardner CD, Kiazand A, Alhassan S, Kim S, Stafford RS, Balise RR, Kraemer HC, King AC. Comparison of the Atkins, Zone, Ornish, and LEARN diets for change in weight and related risk factors among overweight premenopausal women: the A TO Z Weight Loss Study: a randomized trial. JAMA 2007;297:969-77.

61. Dansinger ML, Gleason JA, Griffith JL, Selker HP, Schaefer EJ. Comparison of the Atkins, Ornish, Weight Watchers, and Zone diets for weight loss and heart disease risk reduction: a randomized trial. JAMA 2005;293:43-53.

62. Camargo A, Ruano J, Fernandez JM, Parnell LD, Jimenez A, Santos-Gonzalez M, Marin C, Perez-Martinez P, Uceda M, Lopez-Miranda J, Perez-Jimenez F. Gene expression changes in mononuclear cells from patients with metabolic syndrome after acute intake of phenol-rich virgin olive oil. BMC Genomics;11:253.

63. Gordon T, Castelli WP, Hjortland MC, Kannel WB, Dawber TR. High density lipoprotein as a protective factor against coronary heart disease. The Framingham Study. Am J Med 1977;62:707-14.

64. Navab M, Imes SS, Hama SY, Hough GP, Ross LA, Bork RW, Valente AJ, Berliner JA, Drinkwater DC, Laks H, et al. Monocyte transmigration induced by modification of low density lipoprotein in cocultures of human aortic wall cells is due to induction of monocyte chemotactic protein 1 synthesis and is abolished by high density lipoprotein. J Clin Invest 1991;88:2039-46.

65. Van Lenten BJ, Hama SY, de Beer FC, Stafforini DM, McIntyre TM, Prescott SM, La Du BN, Fogelman AM, Navab M. Anti-inflammatory HDL becomes pro-inflammatory during the acute phase response. Loss of protective effect of HDL against LDL oxidation in aortic wall cell cocultures. J Clin Invest 1995;96:2758-67.

66. Papakonstantinou E, Triantafillidou D, Panagiotakos DB, Koutsovasilis A, Saliaris M, Manolis A, Melidonis A, Zampelas A. A high-protein low-fat diet is more effective in improving blood pressure and triglycerides in calorie-restricted obese individuals with newly diagnosed type 2 diabetes. Eur J Clin Nutr.

67. Sun Q. Comparison between plasma and erythrocyte fatty acid content as biomarkers of fatty acid intake in US women.
. Am J Clin Nutr 2007;86:74-81.