INFLAMMATORY MARKERS AND THEIR CORRELATES IN MORBID OBESITY

BY

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DEDICATION

This thesis is dedicated to the most wonderful parents I could ever ask for, Scott and Margaret Reddan. I have been blessed beyond words throughout my life, especially the past few years. Your unconditional love and support throughout this whole process has been more than I could have ever asked for or imagined. I would not have turned out to be the person I am today without your love, many sacrifices, support and positive attitudes. You two have been my biggest fans in every aspect of life and I can’t possibly think of better role models. Thank you for everything! I love you and know that no matter what path I am on or where I may be, home is where you are…and the beach too!
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ABSTRACT

INFLAMMATORY MARKERS AND THEIR CORRELATES IN MORBID OBESITY

Thesis under the direction of Gary D. Miller, PhD., Associate Professor of Health and Exercise Science.

Morbid obesity has increased at an alarming rate with the number of individuals having a BMI $\geq$40 kg/m$^2$ reaching more than 1 in 20 Americans. In order to understand the biology of obesity, especially morbid obesity, scientists have investigated the physiology of adipose tissue. Adipose tissue has many key functions as an endocrine organ with the secretion of inflammatory biomarkers known as adipokines. Adipokines are associated with negative health consequences and obesity leads to abnormal production of adipokines. As adipose tissue hypertrophy occurs in obese individuals the up-regulation of the pro-inflammatory biomarkers known as leptin, CRP, TNF$\alpha$ and IL-6 occurs while down-regulation of the anti-inflammatory biomarkers known as adiponectin simultaneously occurs. PURPOSE: The primary purpose of this study was to observe the effect of gastric bypass surgery on inflammatory biomarkers, and to examine correlates of baseline biomarker levels with baseline measures of diet, body mass index and visceral fat volume. METHODOLOGY: Participants scheduled to undergo Roux-en-Y gastric bypass surgery at WFUBMC were utilized for the present study, (n=28; age 43.5(9.2) years (values are mean (Standard Deviation)); BMI=53.8 (7.3) kg/m$^2$). Baseline measurements of dietary intake, inflammatory markers (CRP, leptin, IL-6, TNF$\alpha$, adiponectin), body fat indices (visceral fat volume, BMI, weight) and demographics were all taken prior to surgery. Follow-up measurements were taken at 3 weeks, 3 months and 6 months post-surgery for weight, BMI and inflammatory biomarkers. STATISTICAL ANALYSIS: Repeated measures ANOVA assessed
differences for inflammatory biomarkers across 6-months of follow-up. Spearman correlations examined associations between baseline measures of inflammatory biomarkers, diet and body fat estimates, and changes and percent changes between baseline and 6 months for inflammatory biomarkers and body weight. **RESULTS:** Leptin concentration significantly decreased from baseline (57.8 (23.1) ng/ml) to 6 months (19.6 (9.5) ng/ml). Adiponectin concentrations increased from baseline (9.1 (6.6) µg/ml) at 3 months (10.7 (5.5) µg/ml) and 6 months (9.9 (4.6) µg/ml). CRP concentration also significantly decreased from baseline (13.8 (8.5) µg/ml) to 6 months (9.3 (6.5) µg/ml). Neither TNFα nor IL-6 showed changes from baseline. At baseline, leptin was related to body weight (r=.502, p=.024), BMI (r=.550, p=.012) and number of co-morbidities (r=.445, p=.049). Percent change in leptin and body weight from baseline to 6-months were significantly correlated (r=.743, p=.004). At baseline, CRP was significantly correlated with BMI (r=.474, p=.030). The only significant association for baseline measures of diet was with adiponectin as it was indirectly associated with total dietary fiber intake (r=-.578, p=.006). **CONCLUSION:** These findings suggest that weight loss through bariatric surgery in morbidly obese patients has a role in decreasing the obesity-related pro-inflammatory adipokines, while increasing adiponectin, an anti-inflammatory biomarker. Dietary intake showed very few associations with inflammatory biomarkers. Further work is needed in this area to determine sources of variance in the inflammatory biomarkers levels.
MORBID OBESITY PREVALENCE

In 2008 the World Health Organization reported current estimations of more than 1.5 billion adults ages 20+ as being overweight in the world; including 200 million men and 300 million women defined as obese [1]. Overweight is defined as a body mass index (BMI) equal to or greater than 25 kg/m² and less than 30 kg/m², while obesity is defined as a BMI greater than 30 kg/m² which increases the risk of all-cause death [1]. The obesity epidemic has been rising rapidly, but most dramatic has been the rise in morbid obesity (BMI >40 kg/m²) increasing twice as fast as the prevalence of obesity [2]. Between 1986 and 2000, the prevalence of individuals with a BMI of 40 kg/m² or greater quadrupled from about 1 in 200 adult Americans to 1 in 50.[1] Morbid obesity (BMI≥40 kg/m2), which is defined as a main criteria for eligibility for bariatric surgery, is estimated to affect about 5% of the US population [3]. More recent data (2000-2005) confirms this prevalence trend as BMI>30 kg/m² increased by 24%, BMI>40 kg/m² increased by 50% and BMI>50kg/m² increased by 75%. The rates of morbid obesity (BMI>40 kg/m²) and super obesity (BMI>50 kg/m²) increased at rates 2 and 3 times faster than that of the overall obesity epidemic. [2] The growing prevalence of morbid obesity is evident in middle age women (those 40-59) as their prevalence rates from 1999-2004 increased from 7.5% to 7.9%. [4, 5]

Obesity occurs from a multi-factorial interaction between genetics, metabolism, and behaviors, including excess energy intake and/or low energy expenditure. [6,7] Morbid obesity has reached a severe level in our society, resulting in an array of health complications, some of which may be subtle subclinical changes that over time cause
disease conditions. The risk of various co-morbid conditions increases as BMI increases [1] and have been found to be related to many of the leading diseases in our country such as heart disease, diabetes, degenerative joint disease, gallstones, hypertension, colon cancer and stroke. Using the Nurses’ Health Study data, Field et al found that women (mean age of 52.9 years, predominantly white) had an increased risk for a number of these obesity co-morbidities as severity of overweight and obesity increased [8]. After a ten year follow-up, women with a BMI of 35.0 kg/m² or more were about 17 times more likely to develop diabetes when compared to their leaner peers with a BMI of 18.5-24.9 kg/m² (RR=17.0, p<0.05). Overweight women with a BMI between 25.0 and 29.9 kg/m² were also more likely to develop gallstones (RR=1.9, p<0.05), hypertension (RR=1.7, p<0.05), high cholesterol (RR=1.1, p<0.05), and heart disease (RR=1.4, p<0.05) when compared to a cohort of lean individuals with a BMI score between 18.5 to 24.9 kg/m². Therefore, it has been shown that as BMI increases above 25.0 kg/m², so does the risk of various co-morbid conditions.

SURGICAL TREATMENT OF MORBID OBESITY

The National Institutes of Health (NIH) has suggested that bariatric weight loss surgery may be the best treatment option for individuals who are morbidly obese. [9] Many morbidly obese individuals find it difficult to lose weight through diet and exercise alone, leaving bariatric surgery as a last option for leading a healthy life. In the population of morbidly obese individuals, surgical intervention has become the most effective approach in achieving significant weight loss to reduce co-morbidities [7]. Roux-en-Y gastric bypass surgery is commonly used as a treatment method for morbid
obesity (BMI > 40kg/m²) as it helps to bypass a part of the small intestine while restricting the volume of the stomach [10].

INFLAMMATORY BIOMARKERS, ADIPOSE TISSUE, AND OBESITY

Obesity is characterized by an excess of stored energy in adipose tissue known as triglycerides. However, recent research documents that adipose tissue is no longer just a site for energy storage but it is an endocrine organ composed of multiple cell types that expresses and secretes various signaling molecules, known as adipocytokines, or adipokines. These include leptin and adiponectin as well as a number of other cytokines that are involved in inflammation [11-14].

With the onset of obesity, secretions of adipokines from adipose tissue change. The number and localization of immune, vascular, and structural cells within adipose tissue change with weight gain and loss. In addition, the location of the fat depot alters expression and secretion of adipokines with visceral fat generally having a higher secretion of adipokines. Adipose tissue can also be present in organs including the heart, liver, and kidney, as well as in bone marrow and lungs. The ramifications of fat depots in these organs are not entirely known, but they appear to increase an individual’s health risk for morbidity and mortality [11-13].

Obesity results in an increase in these pro-inflammatory adipokines as well as a decrease in the anti-inflammatory adipokine, which are suggested to lead to co-morbidities of obesity [12, 13]. It is the raised levels of circulating hormones and adipocytokines in the body that has lead to the view of obesity being characterized as a state of chronic low-grade inflammation, further linking it to disease etiology [11, 12].

Normal conditions of health consist of a balance between anti- and pro-inflammatory
molecules in various tissues. However, conditions such as obesity result in changes in concentrations leading to problems in function and disease.

INFLAMMATION

The process of inflammation is complex as it involves many cell types and molecules performing various functions, these include initiation, amplification, attenuation and resolution [15]. Inflammation is assessed by biomarkers, with some released in part by adipose tissue, or by other tissues, with their release being stimulated by signaling molecules from adipose tissue. Many inflammatory molecules have multiple functions and contribute to both the increase and decrease of inflammation at various time points. [15] Due to the complexity in biomarkers of inflammation it is understood that it is not just one but many simultaneous effects causing an inflammatory burden [16].

Commonly assessed biomarkers for inflammation include leptin, tumor necrosis factor (TNF)-α, interleukin (IL)-6, adiponectin and C-reactive protein (CRP) [7]. Leptin, TNFα, IL-6 and CRP are known as pro-inflammatory cytokines as they induce inflammation whereas adiponectin is known as an anti-inflammatory cytokine as it acts to help reduce inflammatory pathways [7, 17]. The section below will describe the metabolism of these various biomarkers with regard to obesity and weight loss.
REVIEW OF LITERATURE

The purpose of this chapter is to review the literature pertaining to prevalence and health risks of morbid obesity, inflammatory biomarkers, effect of weight loss interventions on these markers, and current knowledge about the relationships between the inflammatory biomarkers and dietary variables prior to surgery. The contents will focus on the health concerns associated with inflammation, particularly pertaining to morbid obesity. Many studies have found that weight loss alone helps in lowering inflammation, but dietary factors may also play a significant role in decreasing the inflammatory process.

C-REACTIVE PROTEIN (CRP)

CRP PHYSIOLOGY

C-reactive protein (CRP) acts primarily in humans as a pro-inflammatory molecule [18]. CRP may have an active role in disease pathogenesis as a result of its pro-inflammatory process increasing during inflammatory states such as obesity [18]. CRP is part of the global immune response as its synthesis rapidly increases in response to tissue injury or infection, acting as a defense mechanism [18].

CRP production occurs in hepatocytes and is hypothesized to be stimulated by IL-6, which originates in part from adipose tissue [19, 20]. CRP is an important inflammatory biomarker in humans because it is responsible for promoting synthesis of other cytokines [19]. CRP has also been associated with a higher degree of cardiovascular and atherosclerotic risk through several pathways [17, 21]. Suggested pathways include the possibility that CRP levels rise in response to inflammation of coronary vessels related to severity associated with the formation of plaque [22, 23].
Plasma CRP levels are also linked with the amount and activity of other pro-inflammatory cytokines such as Tumor-Necrosis factor-α (TNF-α) and Interleukin-6 (IL-6) [22].

RELATIONSHIP TO ADIPOSITY

The mechanism between the degree of adiposity and circulating CRP levels has yet to be clearly established. It is hypothesized to be mediated by IL-6 concentration, as CRP synthesis in the liver is under the control of IL-6 [20, 22].

As a result of this proposed mechanism, CRP has more recently been hypothesized as a screening tool for identification of cardiovascular events [21]. Both the Centers for Disease Control and the American Heart Association have recommended those individuals at risk for coronary heart disease would benefit from CRP measurement for assessing future cardiovascular events [18]. Guidelines from the American Heart Association (AHA) have designated CRP levels less than 1mg/L as low, 1.0 to 3.0mg/L as average, and >3mg/L as high, increasing the level of disease occurrence, such as cardiovascular disease [24]. As a result, low concentrations of plasma CRP are common in healthy individuals [24]. Many studies stratify CRP levels as high or low with high levels pertaining to CRP concentration >10mg/L and low concentrations <10mg/L [17, 25]. CRP measurements can be reported in different forms, as either milligrams per liter (mg/L) or milligrams per deciliter (mg/dl). The AHA reports CRP concentration as mg/L with less than 1.0mg/L as low. In other studies a low level could be presented as 10mg/dl. Therefore, average values would be represented as 10.0 to 30.0 mg/dl and high would be values greater than 30mg/dl. Concentrations may also be measured and reported in micrograms per milliliter (µg/ml) which is equivalent to milligrams per liter (1
microgram per milliliter equals 1 milligram per liter). For this review, units will be presented as mg/L, consistent with the AHA.

Past research revealed that increased levels of CRP in morbidly obese patients correlated with risk in co-morbidities such as hypertension, insulin resistance and endothelial dysfunction [17, 25]. In a population-based cross-sectional study (n=1929, age=50 years, 63% males, 95% Caucasian) CRP was higher in subjects that were obese compared to their lean counterparts. A CRP concentration greater than 10mg/L was considered high, suggesting a source of inflammation. The percentage of normal weight subjects (BMI<25 kg/m²) with a CRP concentration >10mg/L was 3.4% while the percentage of Class III obese individuals (BMI ≥40kg/m²) had a value of 35.0% suggesting obesity was associated with inflammation. [25]

Associations between obesity class and inflammatory biomarkers were assessed in men and women who participated in the 1999-2004 National Health and Nutrition Examination Survey [26]. Weight classes ranged from normal weight (BMI<25.0), overweight (BMI 25.0-29.9), obesity class 1 (BMI 30-34.9), obesity class 2 (BMI 35.0-39.9) and obesity class 3 (BMI>40.0). CRP was measured and normal weight individuals used as the reference group (reference value=0.5mg/L). At each increase in weight class, CRP levels almost doubled: 1.1mg/L for overweight, 2.1mg/L for class 1 obesity, 4.3mg/L for class 2 obesity, and 7.3mg/L for class 3 obesity (P<0.01). Those categorized as class 3 obesity had the largest increase in CRP concentration. Hypertension and diabetes incidence was also higher in individuals with higher levels of CRP when stratified according to BMI. Therefore, a direct association was seen between higher
levels of obesity class and chance for obesity-related co-morbidities with higher levels of the inflammatory biomarker of CRP. [26]

WEIGHT LOSS INTERVENTIONS

Dynamic weight loss in 83 healthy, obese women (mostly sedentary, mean age 48 years, average BMI 33.8 (0.4) kg/m², range 28.2 to 43.8 kg/m²) was achieved via a 12 week very-low-fat/energy restricted diet [27]. CRP measurement was taken at baseline and 12 weeks post-diet to assess whether CRP could be reduced from weight loss (reported as mean (SEM)). CRP concentration at baseline was at a level of 5.56 (0.36) mg/L and after the 12 week energy restricted diet was reduced to 4.12 (0.36) mg/L (P<0.001). Weight loss during the time period was 7.9kg; the amount of weight lost was positively correlated with CRP (r=0.270, p=0.013). The high baseline CRP measurements in this cohort may be reflective of the obesity status in this study group. This study demonstrates that mild to moderate weight loss can modify CRP levels; however CRP did not reach levels previously quantified as normal in a healthy population, which could be attributed to the subjects BMI still remaining at a level classified as obese. What this study does not assess, however, are the long term effects of weight loss on CRP concentration or how other methods of weight loss, such as bariatric surgery may affect concentrations. [27]

A 12-week weight loss intervention may not be representative of the long-term effects of weight loss on inflammatory biomarkers. A longer term study was conducted in sixty-one obese, white, postmenopausal women (average age of 56.4 years) with a BMI>27 kg/m² as they were recruited into a weight loss program and tested at baseline[22]. Of the cohort, 25 completed a medically supervised weight loss program
with the main goal of reducing body weight to <120% of ideal value. The program lasted an average of 13 months, including weight stabilization before metabolic testing. The dietary intervention consisted of a 1200 kcal/day American Heart Association step 2 diet. It was also encouraged for the women not to change their current physical activity habits.

CRP was assessed at baseline and then again at the end of the weight loss program. At baseline, CRP values were an average 3.06 (+0.69/-1.29) mg/ml. An average 14.5 (6.2) kg of body weight was lost after the weight loss program as CRP concentration was also reduced to 1.63(+0.70/-0.75) mg/ml (P<0.0001). This study helped test the hypothesis that a substantial reduction in body fat induces reduction in plasma CRP levels. This study is concordant with the results of Heilbronn et al, demonstrating weight loss reduction inducing decreases in plasma CRP both in the short term follow-up of 12 weeks and long-term of 13 months. [22]

Weight loss through both short term and long term intervention has had positive relationships to decreasing inflammation, in particular CRP concentration [22, 27]. But, a greater amount of weight loss could be achieved through surgical procedure possibly invoking greater reductions in CRP. A prospective controlled clinical study by Chen et al measured 640 subjects (442 females and 198 males, mean age 31.3 years, mean BMI 41.5 kg/m²), undergoing both gastric bypass and gastric banding, which were measured at baseline before surgical induced weight loss and then 1 year after surgery[28]. In this cohort of patients, 476 (74.4%) had elevated concentrations of CRP (>3.0 mg/L) at baseline. The average pre-surgical CRP concentration was 8.4 mg/L with those having higher BMI subsequently having a higher concentration of CRP. For example, those subjects with a BMI 32-34.9 kg/m² had a 49.5% elevated concentration of CRP, but those
with a BMI greater than 50 kg/m² had a 93.1% elevated concentration of CRP. This finding helps support the previous hypothesis involving adipose tissue as the underlying culprit connecting elevations in CRP with increased inflammatory markers in obese individuals. [28]

Zagorski et al further showed improvement in CRP levels as soon as 3 and 6 months post-gastric bypass surgery[29]. The twenty subjects (19 females and 1 male) had significant reduction in BMI level from 44.5(4.7) kg/m² at baseline to 35.7(5.1) kg/m² at 3 months and finally 31.5(4.3) kg/m² by 6 months. CRP levels also decreased from baseline at each time point (represented as mean (SD)), with no further reduction between 3 and 6 months [10.2 mg/L at baseline, 4.9 mg/L at 3 months (P<0.05) and 3.9 mg/L at 6 months]. This study showed a decrease in CRP level 6 months post-surgery to that below a normal range, which this study defined as a CRP concentration <5mg/L, although the AHA defines this level of that below 3.0mg/L.

Laparoscopic gastric banding (LAGB) surgery reduced CRP concentration when comparing assessments at baseline and 18 months later (n=32 morbid obese females, 34(9) years old, BMI≥40 kg/m²) [17]. CRP level was significantly reduced from a baseline value of 10.3mg/L to 3.5mg/L (P<0.01) 18 months later. Body mass index was also significantly reduced from 43.37(4.39) kg/m² to 30.53(6.11) kg/m² 18 months after surgery. Chronic inflammation appears to be related to BMI as it had a significant correlation with CRP concentration (r=0.431, p=0.009). Therefore, surgical weight loss of morbidly obese women helps to reduce inflammatory biomarkers over a longer time period of 18 months postoperatively, particularly CRP. [17]
In the prospective intervention by Laimer et al, 20 middle-age, morbidly obese women were included with pro-inflammatory cytokines measured at baseline and 1 year after gastric banding surgery to assess the risk between inflammation and obesity levels of CRP, IL-6 and TNFα[30]. The control group consisted of 10 lean females assessed at both time points. Significant changes were seen from baseline before surgery to one year post-surgery. BMI levels decreased from 41.6 (5.4) kg/m² to 30.8 (6.1) kg/m² (P<0.001) and fat mass also decreased from 53.9 (10.3) kg to 29.8 (12.1) kg (P<0.001). These values were still above the control, lean subjects who had an average BMI of 23.1 (2.3) kg/m² and fat mass of 15.6 (4.9) kg. However, these decreases were great enough to invoke significant changes in CRP level. Before surgery, CRP levels were an average 13.3mg/L while one year after surgery the levels decreased to that of 4.0mg/L (P<0.001). Although a significant decrease, most likely due to a decrease in weight, it was still higher than the lean, control subjects CRP level at 1.5mg/L. CRP levels were significantly reduced, invoking a reduction in inflammation but levels of IL-6 and TNFα did not reach significant reduction levels.

It is well known that TNFα induces IL-6 production which in turn induces CRP synthesis. This study shows CRP levels decreasing as a result of proportional decreases in weight loss following gastric banding. The lack of significant reduction in IL-6 and TNF-α could illustrate that mediators affect these cytokines. For example, ingesting an anti-inflammatory diet may help reduce these levels to a greater extent when compared to weight loss alone. Therefore, this study shows weight loss by surgical intervention significantly decreases inflammation by means of decreased CRP but other factors may come into play to help reduce IL-6 and TNFα levels. [30]
The previous studies demonstrate how weight loss of any kind induces decreases in CRP, with long-term studies (greater than 1 year and involving bariatric surgery) having the greatest effect. Moderate weight loss from behavioral interventions decreases CRP concentration too, but using surgical intervention to promote greater weight loss has been shown [17, 28, 30] to have the largest reduction in CRP.

LEPTIN

LEPTIN PHYSIOLOGY

Leptin is a hormone derived from adipocytes and circulates in both the free form and bound to leptin-binding proteins [31]. Leptin is responsible for various functions including appetite and energy balance, and regulation of neuroendocrine and immune function [32]. The level of circulating leptin is influenced by the amount of energy that is stored in adipose tissue as well as acute energy imbalance, i.e. energy intake versus expenditure [31]. The role of leptin in energy intake and expenditure is a result of its binding mechanism to specific receptors in the hypothalamus, altering expression of numerous neuropeptides that are responsible for regulation of neuroendocrine function [31]. Alteration of neuropeptides results in decreased appetite, increased energy expenditure, and change in function of the neuroendocrine system [31]. The major role of leptin is therefore seen in both obesity pathways and eating disorders, acting on the mediation of the neuroendocrine response [31].

Originally, leptin was described as a signal used to help prevent obesity as leptin deficiency was found in obese ob/ob mice [33]. In contrast, humans very rarely have leptin deficiency or leptin receptor defects [33, 34]. Individuals with higher BMI or fat mass exhibit higher levels of leptin, because, on average, the release of leptin per gram of
adipose tissue is two times greater in obese when compared to lean subjects [33]. Caro et al further found that leptin concentration in peripheral circulation is up to four times higher in obese when compared to lean individuals [35].

Other than alterations in the obese state, leptin levels are also altered by the cytokines TNFα, IL-1, and IL-6 through alteration of mRNA levels and circulating levels [31, 36]. Women also seem to have higher leptin levels when compared to men, independent of fat mass, as a possible result of the different distribution of body fat [31, 37] and sex hormones [38].

RELATIONSHIP TO ADIPOSITY

Leptin is secreted from adipocytes directly proportional to the amount of adipose tissue mass as well as nutritional status [14]. Increased fat mass (i.e. energy storage) elevates leptin expression, secretion and serum leptin levels [31, 37]. In fact, when serum concentrations of leptin were compared in obese (n=139) and normal-weight (n=136) subjects those with a BMI>27.3 kg/m² for men and 27.8 kg/m² for women had higher levels of leptin. The normal-weight subjects had an average leptin concentration of 7.5 (9.3) ng/ml while the obese subjects had an average concentration of 31.3 (24.1) ng/ml (P<0.001). There was also a very strong correlation between serum leptin and BMI (r=0.85). [39] Ruhl et al further established this relationship concluding that percentage fat was strongly associated with leptin and BMI in both men and women (r=.68 in men and r=.71 in women, p<0.05) [40].

Energy imbalance is also characterized by leptin levels as prolonged fasting decreases leptin levels and excessive overfeeding greatly increases levels [31, 41]. Circulating leptin levels decline with caloric restriction and weight loss [14]. However,
increased levels of leptin, especially above a serum leptin of 25 to 30 ng/ml could lead to leptin resistance and further exacerbate the obesity process [31, 35].

WEIGHT LOSS INTERVENTIONS

Levels of plasma leptin are increased with weight gain [35] and decreased with weight loss [33]. Therefore, weight loss interventions result in decreased levels of leptin.

An 8 week body weight reduction regimen was implemented in 62 overweight and obese individuals (mean (SD) for age 31.1 (9.5) years for men and 38.0 (10.6) years for women; BMI>27 kg/m²), resulting in decreases in BMI and leptin levels[42]. BMI reduction from 31.5 (4.3) kg/m² at baseline to 29.4 (4.2) kg/m² (P<0.001) at 8 weeks as well as decreased leptin from 31.5 (17.6) ng/ml to 26.5 (17.2) ng/ml (P<0.001) at 8 weeks resulted from an exercise and diet program. This program consisted of an energy-restricted diet of 1200-1600 kcal/day dependent upon dietitian counseling, nutritional counseling, and physical activity of low-impact exercises two to three times per week in one hour sessions. Baseline leptin concentrations (reported as mean (SD)) were significantly higher in women (36.9 (17.1) ng/ml) than in men (19.36 (12.0) ng/ml). After the weight loss program, leptin concentration significantly decreased more in women (by 5.8 (12.4) ng/ml) than in men (by 3.2 (9.2) ng/ml) (P<0.01). Therefore, leptin concentrations decreased in the short-term from both diet and physical activity changes suggesting that decreases in body weight and fat may play a role in this. [42]

A longer term randomized trial in 42 obese (mean (SD)) (BMI 33.6 (0.3) kg/m²) female volunteers was implemented to induce weight loss and plasma leptin levels, as measured at baseline, 3 months and 6 months of the diet[43]. Subjects were randomly assigned to either a very low carbohydrate diet or a low fat diet to induce weight loss as it
successfully did (average of 7.6 (0.7) kg at 3 months and 8.5 (1.0) kg at 6 months in the low carbohydrate group and 4.2 (0.8) kg at 3 months and 3.9 (1.0) kg at 6 months in the low fat group). Leptin concentration decreased throughout with the greatest decrease occurring from baseline to 3 months followed by a reversal towards normal by 6 months. For those in the very low carbohydrate diet, leptin was 25.43 (1.49) ng/ml at baseline and decreased to 16.23 (1.09) ng/ml at 3 months and then increased to 21.68 (1.49) ng/ml at 6 months. The low fat diet group had similar trends with baseline leptin levels at 30.08 (1.88) ng/ml, which decreased to 25.35 (1.82) ng/ml at 3 months and reverted back to baseline to 29.40 (2.58) ng/ml at 6 months. The results indicate that the greatest amount of weight was lost by 3 months as it also showed the greatest decrease in leptin concentration. But, as weight was either stabilized or only slightly decreased by 6 months the concentrations of leptin reverted back towards baseline. This reveals the need for either more severe weight loss over a longer time point or ingesting a certain diet to help consistently decrease levels. Also, the diet with the greatest weight loss (low carbohydrate) had the greatest reduction in leptin concentration illustrating that the greater the weight loss the greater the decrease in leptin. [43]

Plasma leptin levels were measured in a study comparing lean to morbidly obese individuals (21 lean and 30 obese, BMI=46.1 (5.1), age=37.2 (8.2) in obese and 33.2 years in lean, both male and female-majority female) before, 3, 6, and 12 months post-gastric restrictive surgery[44]. Long-term effects of weight loss in the morbidly obese population were shown to linearly correlate with rapid decreases in leptin concentration. Before surgery, leptin concentrations correlated significantly with level of body mass index (BMI) ($r=0.796$, $P<0.001$) with significantly higher levels of leptin concentration
seen in morbidly obese subjects (95.0 (53.2) ng/ml) when compared to lean individuals (15.5 (21.3) ng/ml). Gastric restrictive surgery in the morbidly obese individuals reduced BMI (from a level of 46.1 (5.8) kg/m² at baseline to 39.6 (5.5) kg/m² at 3 months, 35.5 (5.0) kg/m² at 6 months and 33.2 (5.6) kg/m² at 12 months) while concomitantly decreasing leptin concentrations. Leptin levels dropped quickly within the first 3 months after surgery (from 95.0 (53.2) ng/ml to 44.5 (28.2) ng/ml at 3 months), probably as a result of severely decreased food intake, but did not remain this low throughout the time point of 12 months. A high level of leptin concentration before surgery was most likely a cause of increased leptin release by adipocytes, a possible result of morbid obesity. The rapid decrease in leptin from baseline to 3 months post-operatively was a result of either rapid weight loss or energy restriction. Overabundance of food intake is most likely responsible for increased leptin release by adipocytes. [44]

Examination of both leptin and CRP levels at baseline and one year after RYGB surgery showed significant decreases in these inflammatory markers[45]. RYGB induced significant reductions in plasma CRP and leptin concentrations, beginning with a CRP level of 1.23 (0.15) ng/ml pre-surgery to 0.46 (0.08) ng/ml one year post-surgery (P<0.001). The concentration of leptin also decreased from 110.2 (7.3) ng/ml pre-surgery to 36.4 (4.9) ng/ml 1 year post-surgery (P<0.001). Significant reductions in concentrations of CRP and leptin as a result of RYGB surgery supports the use of surgically induced weight loss on the level of inflammation in the body. [45]

Faraj et al measured inflammatory biomarkers at baseline and 15(6) months post-surgery in 50 (39 women, 11 men) morbidly obese subjects (BMI in men= 50.7 (14.9) kg/m² and women=50.2 (8.1) kg/m²) undergoing Roux-en-Y gastric bypass surgery[10].
Concentrations of leptin at baseline were 48.9 (19.3) ng/ml in women and 27.6 (24.4) ng/ml in men and after weight loss (16-55%) postoperative levels decreased to 11.1 (8.2) ng/ml in women and 5.1 (2.9) ng/ml in men. At baseline, correlations between leptin and BMI existed ($r=.60$, $p<0.0005$). Decreases in leptin levels in this study stay consistent with the known effects of negative energy balance on leptin production. [10]

Significant decreases in leptin have been shown in these previous studies [44, 45] to occur with weight loss one year and greater than one year [10] after weight loss surgery. The longitudinal study by Lin et al looked at changes after just 6 months[46]. Subjects included 28 morbidly obese females (average BMI is 48.2 kg/m², average age 36.1 years) who had inflammatory biomarkers assessed at baseline and then 1 and 6 months postoperatively. Leptin concentrations significantly dropped from a baseline value of 159.3 (11.1) ng/ml to 86.1 (6.0) (P<0.001) at one month and even greater decreases by 6 months with an average concentration of 53.5 (6.6) ng/ml (P<0.001). Therefore, decreases in leptin occur with weight loss alone and as early as 1-6 months and continue to decrease through 12 months postoperatively. What has yet to be looked at is whether dietary factors can also contribute to or mediate these effects.

INTERLEUKIN-6 (IL-6)

IL-6 PHYSIOLOGY

As much as one-third of circulating IL-6 originates from adipose tissue [14]. Synthesis is regulated in part by TNFα as it is released in large amounts by adipose tissue in vivo [11, 47]. IL-6 is expressed in and secreted by adipocytes leading to local action within the tissue as well as release into circulation [11]. Concentrations of circulating IL-6 are positively correlated with obesity, as levels decrease in response to weight loss [14].
Likewise, it also appears to mediate weight-regulating processes as its production and systemic concentration increase linearly with increasing adiposity levels [47].

IL-6 determines the acute-phase response [19, 48] and also controls hepatic CRP production [19, 49]. Elevations of IL-6 occur as a result of systemic infection or inflammation and may induce a rise in CRP levels [20]. The main contribution of IL-6 regulating the hepatic acute phase response is hypothesized to be caused by obesity and the promotion of a low-grade inflammatory state [20].

RELATIONSHIP TO ADIPOITY

Elevated levels of IL-6 have been found concurrently with increased production of the hormone corticosterone by abdominal adipose tissue [7]. There is a balance maintained between pro-inflammatory and anti-inflammatory molecules under normal conditions[7]. Under abnormal conditions, such as obesity, the concentrations of pro-inflammatory cytokines (TNFα, IL-6) are elevated which causes a reciprocal rise in corticosterone[7]. The anti-inflammatory effects of corticosterone work by suppressing the production of IL-6, therefore decreasing inflammation[7]. The balance between corticosterone and IL-6 determines the level of inflammation in adipose tissue[7]. This would be problematic in morbid obesity as the rate of pro-inflammatory effects from IL-6 would be too rapid for corticosterone production and leads to a pro-inflammatory state.

Increased IL-6 levels in an obese state could be explained by adipose tissue’s ability to secrete and express IL-6, as it is higher in obese subjects [50]. What is presently unknown is the molecular mechanism for increases in expression of IL-6 in adipocytes from obese subjects [50].
In a study of 29 subjects (both diabetic and non-diabetic) plasma IL-6 concentration, TNFα and leptin were all significantly associated with BMI level [50]. Associations between BMI and concentrations of inflammatory markers suggest increased circulating concentrations produced by adipose tissue and therefore the need for weight loss interventions in those with a high BMI.

**WEIGHT LOSS INTERVENTIONS**

Excess adipose tissue could have major effects on concentrations of inflammatory markers, particularly IL-6. In a study by Ziccardi et al in 2001 they compared 56 healthy, obese (BMI 37.2 (2.2), ages 25-44 years) premenopausal women to 40 age-matched controls of normal weight[51]. Concentrations of IL-6 were compared, as well as other inflammatory markers. Levels of IL-6 were found to be significantly higher in the obese group when compared to the normal weight group. The cohort of obese women was enrolled in a 12 month program to induce weight reduction through diet, exercise and behavioral and nutritional counseling. An average 10% reduction of weight was achieved when compared to baseline values. After 12 months, weight loss in obese women resulted in significant reductions in concentrations of IL-6; 3.18 (0.9) pg/ml at baseline reduced to 1.7 (0.5) pg/ml at 12 months (P<0.01). The level of reduction in IL-6 concentrations in obese women after 12 months of weight loss resulted in values similar to the cohort of healthy, non-obese women with an IL-6 concentration of 1.4 (0.5) pg/ml[51]. In obese women, weight reduction represented a safe method for decreasing the inflammatory state back to a normal level; a level similar to the non-obese women.

In studies using bariatric surgery (RYGB) as a means for weight loss, IL-6 levels were reduced post-surgery. Statistically significant changes were concluded in the study
by Gletsu et al after 6 months of follow-up (n=15, mean BMI 48.5 (0.9) kg/m², ages 18-
65) in post-RYGB surgery patients[52]. IL-6 concentration decreased from 4.92 (0.67)
pg/ml at baseline to 3.47 (0.37) pg/ml (P<0.005) 6 months post-surgery. Reductions
from baseline to 6 months post-surgery suggest a significant reduction in subcutaneous
and visceral adipose tissue, as well as total body weight and BMI; supporting the
hypothesis that excess adipose tissue contributes to elevated circulating IL-6
concentrations which could be reversed in as little as 6 months. [52]

Emery et al measured the inflammatory biomarker IL-6 in 13 Caucasian women
(mean age 46.9 (5.7)) at baseline and 12 months post-RYGB surgery[53]. IL-6
concentration was significantly reduced from a baseline value of 5.41 (1.91) pg/ml to
2.24 (0.86) pg/ml (P<0.01). Although a small sample size in this observational study, a
significant reduction of inflammatory markers resulted[53]. The significant reduction
seen here could be attributed to a longer follow-up of 12 months.

The previous two studies[52, 53] showed significant changes in IL-6 both 6
months and 12 months post-surgery. Holdstock et al measured IL-6 at baseline, 6 months
and 12 months post-RYGB surgery, giving a better indication of the effects of weight
loss and decreased BMI on levels of inflammation at each time point[54]. The study
included 66 individuals (12 males, 54 females), average age 39 years, and average BMI
45 kg/m² (range 33-64). As BMI decreased at each time point, from 44.7 (6.4) kg/m² at
baseline to 34.7 (5.7) kg/m² at 6 months and finally 31.5 (6.1) kg/m² at 12 months
(P<0.01) so did levels of IL-6 concentration, from 3.5 (42.5) ng/L preoperatively to 2.7
(15.2) ng/L at 6 months and finally 1.7 (12.5) ng/L at 12 months (P<0.01). IL-6 was
reduced by 30% at 6 months and 50% by 12 months post-surgery[54].
Changes at 6 months were shown by Emery et al, Gletsu et al and Holdstock et al [52-54]. This is an important time period because of the length of the current study. Lin et al used a longitudinal study to measure the inflammatory markers in 28 morbidly obese females (mean age was 36.1 years, mean BMI=48.1 kg/m²) both 1 month and 6 months post-RYGB surgery and compared the concentrations to those taken at baseline[46]. IL-6 concentration from baseline to 1 month was not decreased, but changes by 6 months were statistically significant (P<0.001) from baseline values of 3.93 (0.42) ng/ml to 2.96 (0.31) ng/ml at 6 months. This study shows that improvement in inflammation occurs in morbidly obese females concomitantly with decreased weight (by 25%) from RYGB surgery, with the time point of 6 months being the main period where significant changes begin to occur.

In conclusion, weight loss from RYGB surgery results in decreases in the inflammatory marker IL-6 beginning at 6 months and lasting as long as 12 months post-surgery. However, what these previous studies did not report is the effect of dietary factors and how they may also affect IL-6 concentrations to help attenuate inflammation.

TUMOR NECROSIS FACTOR-ALPHA (TNFα)

TNFα PHYSIOLOGY

TNF-α is a regulatory cytokine responsible for many functions such as inflammation, cell apoptosis and survival, cytotoxicity, insulin resistance and the production of other inflammatory cytokines such as IL-6 [55]. TNFα has a range of processes acting in both paracrine and autocrine manners as it is a powerful local regulator. Within white adipose tissue there seems to be a hierarchy of cytokines with TNFα playing a central role in the production of other cytokines and adipokines [11].
Adipose tissue is composed of many cell types, one in particular being adipocytes, which are the primary source of TNFα [55]. TNFα is the first step in the inflammatory cascade as it is the main cytokine that induces IL-6 production [19, 33].

RELATIONSHIP TO ADIPOSITY

TNFα is a cytokine that has been found to play a role in the pathogenesis of obesity and insulin resistance [14, 55]. TNFα is increased in obese humans as it is positively correlated to adiposity and insulin resistance [14, 50, 55]. Its expression is dependent on total and regional fat mass as it influences gene expression in tissues such as adipose tissue and the liver [14]. Adipose tissue metabolism may be regulated in part by TNFα as it is over-expressed in adipose tissue when compared to the tissue from lean individuals [56].

WEIGHT LOSS INTERVENTIONS

TNFα concentrations are also affected by excess adipose tissue. In the previously mentioned study by Ziccardi et al, significant changes in IL-6 occurred along with weight reduction[51]. TNF-α concentration was also significantly decreased as levels were higher in the obese group when compared to the normal weight group. The TNFα concentration of 5.8 (1.5) pg/ml at baseline was reduced to 4.0 (1.1) pg/ml at 12 months (P<0.01). Twelve months of weight loss in obese women resulted in decreases of TNFα to values similar to the cohort of healthy, non-obese women with a TNFα concentration of 3.6 (0.9) pg/ml. In obese women, weight reduction resulted in a safe method for decreasing the inflammatory state back to a normal level, a level similar to women who are not obese.
In studies using bariatric surgery (RYGB) as a means for weight loss, TNFα levels did not always decrease to a statistically significant level. In those studies, serum CRP was reduced suggesting some markers may be less sensitive in identifying a clinically significant effect from bariatric surgery alone. For example, in the study by Vasquez et al, 26 morbidly obese patients (23 women, 3 men; age 39.0 (10.0); average BMI 46.2 kg/m²) were evaluated before and 4.2 (0.8) months after bariatric surgery[56]. Inflammatory marker levels were compared to a cohort of 26 normal, healthy weight controls (average BMI 23 kg/m²; matched for age and sex). The obese individuals had slight decreases in TNFα and IL-6 but none of statistical significance. CRP was decreased from baseline to four months post-surgery from 0.63 (0.10) mg/dl to 0.40 (0.06) mg/dl (p<0.05) but was still higher than the healthy controls with a value of 0.15 (0.04)mg/dl. There are a few possible reasons for this randomized controlled trial failing to show changes in IL-6 and TNFα, including the short follow-up period of four months, the low amount of lost adiposity (mean=26.7 kg) or the lack of introducing any dietary factors to aid in weight loss post-surgery. [56] Therefore, more research is needed to see effects of weight loss surgery on TNFα concentration particularly with a longer follow-up period.

ADIPONECTIN

ADIPONECTIN PHYSIOLOGY

Regulation of adiponectin is similar to that of TNFα and leptin; hormones are responsible for its expression and secretion [57]. It is a secreted protein of 247 amino acids, expressed primarily in an individual’s adipose tissue acting as an anti-inflammatory biomarker [57, 58]. Numerous pro-inflammatory adipokines are secreted
by adipose tissue as described above but the anti-inflammatory factor known as adiponectin is also secreted [58]. Levels of adiponectin in plasma and adipose tissue are decreased in obese individuals when compared to lean individuals [59]. Along with this finding, pro-inflammatory factors such as TNFα and IL-6 inhibit the production of adiponectin by adipocytes [59].

Clinical observations linking associations between adiponectin and metabolic dysfunction in obese individuals has lead to a few known disease-related problems. First, the levels of plasma adiponectin are negatively correlated with visceral fat accumulation. Next, individuals with type 2 diabetes have decreased levels of plasma adiponectin concentration and those with higher levels of adiponectin are associated with lower risk in developing type 2 diabetes. [59] In contrast to pro-inflammatory adipokines, adiponectin is therefore higher in functional adipocytes for lean individuals but down-regulated in dysfunctional adipocytes associated with obesity leading to adverse metabolic dysfunction [59].

RELATIONSHIP TO ADIPOSITY

Most proteins produced by adipose tissue are elevated in the obese state while adiponectin concentration has an inverse association with obesity as levels decrease in response to weight gain [13, 57, 58]. When adiponectin levels are decreased, it cannot inhibit inflammatory pathways of other biomarkers such as TNFα, resulting in increased inflammation [57, 58]. Arita et al compared the concentrations of adiponectin in both obese (n=57) and non-obese (n=87) subjects[60]. The plasma of non-obese subjects had an abundance of adiponectin (8.9 (5.4) µg/ml; ranging from 1.7 to 17.0 µg/ml), in contrast levels were lower in obese subjects (3.7 (3.2) µg/ml) (P<0.0001). The specific
mechanism between obesity and decreased plasma adiponectin has yet to be clarified but associations between obesity and lowering of adiponectin have been measured, suggesting a need for weight loss interventions.

WEIGHT LOSS INTERVENTIONS

A short term weight loss intervention by very low energy diet has shown inverse associations in adiponectin over 8 weeks. But, when followed for 3 years, concentrations tended to revert back to baseline, independent of orlistat/placebo treatment, gender, age, site and fasting plasma glucose (n=93 females, ages 18-65, BMI between 30-45 kg/m²) (p<0.01) [13]. Concentrations of adiponectin showed significant (p<0.05) positive correlations with weight loss (22% increase) after 8 weeks of very low energy diet (VLED, 800kcal/day) and remained high at 6 months (24% increase) but returned to baseline after 3 years [13]. The full study time period encompassed three years of measurements in adiponectin concentration but long-term continuous significant changes did not occur. In the group of placebo subjects, 8 week VLED resulted in decreases in weight (from about 109.9 to 95.6 kg) and increases in adiponectin (from about 0.44 to 0.50 µg/ml). After 3 years these values reverted back to baseline with body weight measuring 105kg and a mean adiponectin concentration of 0.42 µg/ml[13].

There are many hypotheses for this normalizing back to baseline such as a reduction in amount of continual weight loss over a longer time period or changes in dietary intake when anti-inflammatory vitamins are not ingested. Also, weight loss through very low energy diet (VLED=800kcal/day) may not be the best means for increasing this anti-inflammatory biomarker for the long-term, but rather weight loss through surgery. The study by Madsen et al showed that weight loss greater than 12% of
initial body weight results in improvement in inflammatory markers like adiponectin, while weight loss below this level may not induce increases [13].

Morinigo et al measured 36 Caucasian severely obese patients about to undergo surgery and evaluated levels of inflammatory markers at baseline, 6 weeks and 52 weeks post-surgery, comparing them to 20 normal-weight healthy controls[61]. Many inflammatory markers were measured, including adiponectin and TNFR2. In those who had undergone surgery, a significant decline was observed in the pro-inflammatory marker TNFR2 and increases in the anti-inflammatory marker adiponectin, relative to baseline. Adiponectin, which has anti-inflammatory effects, increased from 8.83 µg/L at baseline to 11.72 µg/L at six weeks and 13.16 µg/L at 52 weeks post-surgery. The control subjects had adiponectin levels of an average 20.74 µg/L, correlating with higher levels in non-obese individuals. Most inflammatory markers were altered from baseline to 52 weeks, especially when compared to the normal weight subjects, suggesting beneficial effects of weight loss. The data here support our view of obesity contributing to elevations of plasma concentration of inflammatory biomarkers with weight loss through surgical intervention improving concentrations a significant amount. [61]

Lin et al measured inflammatory biomarker concentrations both pre-RYGB surgery and again 1 month and 6 months postoperatively. In the study of 28 severely obese women, weight loss at both 1 and 6 months revealed statistical significance relating to adiponectin concentration[46]. As weight decreased, the concentration of adiponectin inversely increased. Concentrations of adiponectin at baseline averaged 5.82 (0.51) µg/ml and by one month it had risen to 6.72 (0.66) µg/ml (p<0.05) while after 6 months an increase resulted by an average 8.04 (0.65) µg/ml (p<0.001). As weight continually
decreased at each time point (127.7 kg at baseline to 115.2 kg at one month and finally 92.4 kg at 6 months) the rise in adiponectin increased[62].

The previously mentioned study by Faraj et al (50 participants, mean BMI of 50.7 (14.9) kg/m² in 11 men and 50.2 (8.1) kg/m² in 39 women) adiponectin levels increased from 2.5 µg/ml to 9.0 µg/ml (p<0.02) with approximately 20% weight loss when comparing baseline measures to 15(6) months post-RYGB surgery[10].

Increases in adiponectin were seen in various weight loss interventions of time periods ranging from 1-6 months to 52+ weeks post-weight loss. The measurements of the influence dietary factors may have on adiponectin concentration encompasses one of the main things not assessed in these studies.

DIET, NUTRIENT INTAKE, AND INFLAMMATORY BIOMARKERS

Chronic inflammation is regulated in part by a person’s diet [63]. Consistent results in dietary variables have shown mediation of inflammatory pathways as a result of certain dietary intakes, in particular energy intake, total fat, total carbohydrates, total protein, Vitamin C, Vitamin E, omega-3 polyunsaturated fatty acids, total dietary fiber and beta-carotene. Furthermore, types of diets vs. nutrient intake are described below.

Higher levels of CRP, TNF-α and IL-6 have been found in the Western-type diet when compared to a healthier diet including more fruits, vegetables and whole grains [63, 64]. Mean scores of biomarkers, based on dietary pattern scores (measured by Food Frequency Questionnaire) in a population of 486 Tehrani women between the ages of 40 and 60 years, showed that those in the highest quintile for a healthy diet had CRP levels of 1.7 (1.8) mg/L while CRP levels for those in the highest quintile for the western diet were higher at 2.6 (2.2) mg/L. Levels of TNFα were higher for those in the highest
quintile for the western diet with a value of 4.7 (2.7 ng/L) when compared to those in the highest quintile of healthy pattern scores having a mean TNFα concentration at 4.4 (1.7) ng/L. IL-6 levels were also higher for those in the western diet with an IL-6 concentration at 2.3 (2.0) ng/L when compared the healthy diet group having lower concentrations of IL-6 at 1.7 (1.9) ng/L. A healthy dietary intake, when compared to a more Western diet, showed positive relationships to plasma concentrations of the inflammatory markers IL-6, TNF-α and CRP. The Western-diet is higher in red meat, high-fat dairy products, refined grains and simple carbohydrates while the healthier (often known as Mediterranean) diet focuses more on whole grains, fish, fruit, olive oil, moderate alcohol intake and green vegetables, suggesting a healthier dietary intake as a possible result for lower levels of inflammation [63, 64]. After adjustments for smoking, physical activity, current estrogen use, menopausal status, family history of diabetes and stroke, and energy intake differences between diet groups still remained; further suggesting the main interaction between diet and the ability to reduce inflammation through fruit, vegetable and whole grain intake [64].

Lower levels of inflammation have been found in diets that include a high fruit and vegetable intake [65]. CRP levels have been significantly reduced in a group of both Hispanic and non-Hispanic individuals (≥60 years, n=1030). After adjusting for potential confounders, a significant inverse dose-response association was seen between fruit and vegetable intake and plasma CRP. A greater prevalence of CRP above 10 mg/L was seen in those in the lowest quartile for fruit and vegetable intake (17.9%) versus the highest quartile for fruit and vegetable intake (9.1%). [65] A more frequent intake of fruits and
vegetables was associated with a significantly lower CRP concentration; 21% reduction in the likelihood of a high CRP concentration with each increase in serving. [65]

It is hypothesized that the reduction of inflammation and therefore disease as a result of fruit and vegetable intake is from a combination of antioxidants, fiber, potassium, magnesium and various other phytochemical reactions [64]. Lowering concentrations of CRP and therefore inflammation may significantly help to reduce the onset of disease. This study supports the notion for increased fruit and vegetable intake as a primary preventive measure against inflammation and therefore potential risk for chronic disease. The highest quintile (5 servings) showed the greatest reduction of plasma-CRP, therefore recommendations for ingesting 5+ servings of fruit and vegetables daily to reduce the risk of chronic diseases. Wannamethee et al further supported these results as a fruit intake of more than 7 servings per week were associated with a 24% lower risk of elevated CRP concentration when compared to those consuming less than 3 servings per week (p<.007)[66].

A more direct effect from increased fruit and vegetable (F+V) intake was tested in 63 healthy, non-smoking men (average age 32 years, average BMI 23-24 kg/m²)[67]. This randomized controlled trial involved participants consuming ≤2 servings per day of fruit or vegetables for 4 weeks. After the 4 week period subjects were randomized to one of three groups (n=21 each): consuming 2 servings/day, 5 servings/day or 8 servings/day of fruit or vegetables for another 4 weeks. As levels increased in the group from 2 servings/day of F+V to 5 servings/day and lastly 8 servings/day, a reduction in plasma CRP concentration by the 8th week resulted. Mean (SEM) plasma CRP concentration changed between weeks 4 and 8 in those consuming greater levels of fruits and
vegetables. The level of CRP concentration was reduced from 1.51 (0.39) mg/L (2 servings/day group) to 0.85 (0.21) mg/L (5 servings/day group) and then to 1.41 (0.40) mg/L (8 servings/day group). Therefore, consuming 5 servings/day and up to 8 servings/day of fruits or vegetables resulted in reductions of the inflammatory biomarkers, with a greater decrease at 5 servings/day. [67] Body weight, body mass index, total energy and macronutrient intakes did not significantly change over the 4 week intervention suggesting that it is not only weight loss that can help decrease CRP levels but a healthy dietary intake of fruits and vegetables. Therefore, reduction in CRP concentration may also occur independent of weight loss.

The role of inflammation in the disease state was seen in the study by Esposito et al (n=180, average ages 43-44 years) when measuring dietary intake (Mediterranean diet) and the role of decreased inflammation on metabolic syndrome[68]. This randomized single-blind study included half of the cohort (n=90) to an intervention group and the other half (n=90) as controls. Two years of adherence to the Mediterranean style diet (mostly whole grains, fruits, vegetables, nuts and olive oil) resulted in decreased levels of inflammatory biomarkers, particularly CRP and IL-6, when compared to the prudent diet of the control group (50-60% carbohydrates; 15-20% protein; total fat<30%). Results were adjusted for body weight changes, suggesting this diet may play a role in reduction of the inflammatory state and endothelial dysfunction associated with the metabolic syndrome. At the two year follow-up, significant changes in CRP (p<0.01) and IL-6 resulted (p<0.02). CRP concentration in the intervention group after two years was 1.7 mg/L (0.4-4.9) with a change of -1.1 (0.4). The control diet had an average CRP concentration of 2.8 mg/L (0.5-5.5) with a change of -0.1 (0.3). IL-6 concentration for
the intervention group was 1.4 pg/ml (0.4-3.8) with an average change of -0.7 (0.3) while the control group had a level of 1.8 pg/ml (0.5-4.5) with an average change of -0.1 (0.2). Adiponectin was increased from 5.6 (2.2) µg/ml at baseline to 8.3 (2.9) µg/ml (p<0.02) after two years. The control groups remained about the same starting at 5.4 (2.1) µg/ml for baseline to 5.8 (2.1) µg/ml (p<0.13) after two years. After two years of follow-up the level of metabolic syndrome was also assessed according to the number of components of the disease (3, 4, or 5), with only 42 individuals in the intervention group still having features and 78 individuals in the control group[68]. Based on these results, individuals adhering to the Mediterranean diet for two years can expect a significant reduction in CRP and IL-6 concentration further reducing the prevalence of metabolic syndrome. [68]. Therefore, adiponectin, IL-6 and CRP levels were increased or decreased, respectively, as loss of both weight and BMI occurred along with dietary changes. However, it is unclear whether the levels changed from weight loss alone or in combination with exercise and dietary intake.

Another study [69] looking at dietary weight loss, but over a two year time period, was conducted in the Dietary Intervention Randomized Controlled Trial (DIRECT) which compared the effectiveness of three different nutritional protocols: a low-fat, restricted-calorie diet; a Mediterranean, restricted-calorie diet; and a low-carbohydrate, non-restricted-calorie diet. Eligible participants (n=322) were between the ages of 40 and 65 years (mean age of 52 years), a BMI>27 kg/m² (mean BMI of 31 kg/m²), or the presence of type 2 diabetes or coronary heart disease (regardless of age or BMI). The low-fat, restricted-calorie diet was defined as an average 1500 kcal/day for women and 1800 kcal/day for men; 30% of calories from fat, 10% of calories from saturated fat, and
300mg of cholesterol per day. The Mediterranean diet focused on vegetables, poultry and fish instead of red meat, beef and lamb. Energy intake was the same at 1500 kcal/day for women and 1800 kcal/day for men with no more than 35% of calories from fat. The low-carbohydrate diet was modeled after the Atkins diet with a non-restricted-calorie diet aimed to provide 20g of CHO for the first 2 months and up to 120g of CHO for the remainder of the study. Blood samples were taken after a 12 hour fast each time at 6, 12, and 24 months to assess inflammatory biomarkers[69].

Results indicated most of the cohort was made up of males (86%) with a high rate of adherence, 95.4% at 12 months and 84.6% at 24 months. The greatest time period of weight loss occurred from 1-6 months with maintenance following through 24 months as it was achieved in both the low-carbohydrate and the Mediterranean diet groups. The levels of adiponectin increased from baseline (7.3 (2.8) mg/dl) to 24 months, with the greatest increase seen in the low-carbohydrate diet. Low-carbohydrate diet group resulting in the greatest amount of weight loss suggests it may be the quantity of weight loss (in kg) versus the quality of the diet used. As adiponectin levels were significantly increased (p<0.05) by 1.3 mg/dl in the low-carbohydrate diet, and 0.8 mg/dl in both the low-fat and Mediterranean diet. Leptin levels were also reduced by 24 months, with the greatest reduction seen at 6 months. At 6 months, changes in all diets ranged from 2.6 mg/dl to 3.5 mg/dl (greatest decrease in the low-CHO diet) and then at 24 months the changes were not as high with values ranging from 2.1 mg/dl to 2.9 mg/dl. This further suggests that circulating leptin reflects the amount of body-fat mass instead of a particular diet because weight stabilized after 6 months and that is where the increase towards baseline in leptin occurred. It was the changes in CRP that most reflected the
type of diet. For example, although the low-fat and Mediterranean diets had similar amount of weight loss at 6 months, the Mediterranean diet had a greater decrease in CRP levels. Both diets saw a decrease in fat mass of about 5.4 kg but CRP level decreased by 0.5 mg/L in the Mediterranean diet group while decreases in the low-fat group were only by 0.2 mg/L. The low-carbohydrate diet saw the greatest decrease in CRP as it also had the greatest decrease in weight. This suggests CRP reduction is mainly regulated by the amount of weight loss but is also influenced by the type of diet, particularly when comparing a low-fat diet and a Mediterranean diet. In conclusion, both leptin and adiponectin changes may be more influenced by the greater amount of weight loss while CRP may be influenced by weight loss as well as dietary composition. [69]

Although this study enrolled very few women, significant results were seen overall. In fact, when measuring women separately, they tended to lose more weight on the Mediterranean diet suggesting that diet composition may affect women to a greater degree. Overall, this study and many more are beginning to show that improvement in biomarkers over a longer period of time (24 months) can be achieved with a diet composed of healthy foods, despite the maximal weight loss at 6 months. Therefore, a healthy diet can achieve benefits of inflammatory biomarkers beyond weight reduction.

FAT/CARBOHYDRATE INTAKE

Sharman et al used a randomized cross-over design of 15 overweight men (mean (SD)) (BMI>34 (5.6) kg/m², age 34.3 (11.3) years, otherwise healthy) to compare a very-low-carbohydrate diet (<10% energy from CHO) and a low-fat weight loss diet (<30% energy from fat) on inflammatory markers[70]. These two different diets were each consumed for 6 week periods both resulting in significant decreases from baseline to end...
of diet in the biomarkers IL-6, TNF-α and CRP. Baseline values for CRP averaged 2.9 (1.5) mg/L while 6 weeks of a very-low carbohydrate diet reduced levels to 1.3 (0.9) mg/L (p<0.00005) and six weeks of a low-fat diet resulted in similar reductions to 1.5 (0.8) mg/L (p<0.00001). Reductions were also seen in TNFα from a baseline value of 3.3 (1.0) pg/ml to a value of 1.8 (0.4) pg/ml (p<0.00001) post-very-low carbohydrate diet and a value of 1.9 (0.4) pg/ml (p<0.00001) post-low fat diet. Concentrations of IL-6 were also significantly reduced from a baseline value of 3.9 (1.4) pg/ml to 1.9 (0.6) pg/ml (p<0.00000) post-low-carbohydrate diet and then 2.1 (0.6) pg/ml (p<0.00000) post-low fat diet. Both dietary changes consisted of extreme energy-restriction resulting in similar reductions of inflammatory biomarkers. Therefore, overweight individuals can use various modes of diet composition to achieve improvements in inflammation, as long as weight is lost. Short-term weight reduction in this study suggests it is the actual amount of weight lost that is the driving force of inflammatory reduction rather than the specific composition of the diet. [70]

A slightly longer intervention was conducted by Arvidsson et al in 40 women aged 21-49 years, BMI of 30.9-47.7 kg/m², randomly assigned a 10-week hypo-energetic (600 kcal/day lower than their estimated energy requirement measured via indirect calorimetry) diet of either moderate fat/moderate carbohydrate (fat=40-45% of energy, n=20) or low fat/high carbohydrate (fat=20-25% of energy, n=20) content[71]. Both groups resulted in similar findings that as body weight decreased by approximately 7.5%; and the levels of leptin, TNFα and IL-6 also decreased as adiponectin increased (mean (SD)). Leptin decreased in the moderate fat group from 38.8 (3.1) ng/ml to 29.9 (2.6) ng/ml (p<.008) and in the low fat group from 36.5 (4.0) ng/ml to 24.4 (2.5) ng/ml
(p<.0012). TNFα did not decrease in the moderate fat group from 4.8 (1.6) pg/ml at baseline to 4.84 (1.5) pg/ml (p<.65) at 10 weeks while the low fat group resulted in decreases from 2.79 (0.72) pg/ml to 2.16 (0.53) pg/ml (p<.059). IL-6 decreased in the moderate fat diet from 3.82 (0.41) pg/ml to 3.54 (0.48) pg/ml (p<.064) and in the low fat group from 3.56 (0.22) pg/ml to 3.38 (0.37) pg/ml (p<.31). Adiponectin increased from 16.4 (1.7) µg/ml to 16.9 (1.6) µg/ml (p<0.88) in the moderate fat diet while the low fat diet resulted in increases from 18.3 (1.8) µg/ml to 20.6 (1.9) µg/ml (p<.30[71]. These studies suggest caloric restriction (of either type measured) influenced the production and secretion of adipose tissue derived proteins during weight loss. Macronutrient composition of fat and carbohydrate intake in hypoenergetic diets was not of significant importance for the outcome of dietary treatment influencing TNFα, IL-6, and adiponectin but does have influences on leptin concentration. CRP concentration was not assessed here but Heilbronn et al measured the inflammation in 83 healthy females (mean age=48 (0.9) years, mean BMI=33.8 (0.4) kg/m²) ingesting a low fat diet (61.4 (0.3)%CHO, 14.2 (0.2)% fat, and 22.8 (0.2)% protein), which led to reductions in CRP concentration (mean (SEM)) from 5.56 (0.36) mg/L at baseline to 4.12 (0.36) mg/L (p<0.001) after 12 weeks of the diet[27]. Although results seem to be due to lower energy intake instead of fat or carbohydrate intake, this could be a result of short-term follow-up lasting as long as 6 to 10 weeks instead of longer-term intervention studies.

PROTEIN INTAKE

Brinkworth et al assessed a total of 43 obese, non-diabetic subjects (mean BMI 34.0 kg/m², mean age 50.2 years, mostly female) who were randomized to one of two groups, a standard protein diet (15% protein, 55% CHO) or a high protein diet (30%
protein, 40% CHO) during a 68-week study period: 12 weeks of energy restriction followed by 4 weeks of energy balance and then dietary maintenance for 52 weeks[72]. Energy intake did not differ between groups during any of the time points. CRP concentration began at a mean value of 4.0 (0.5) mg/L in the standard protein diet and 6.7 (1.0) mg/L in the high protein diet. After 16 weeks CRP was reduced to 3.4 (0.6) mg/L (p<0.05) in the standard protein diet and 5.7 (1.0) mg/L in the high protein diet. At the end of the 68 week intervention, CRP concentration was reduced to 3.4 (0.6) mg/L (p<0.05) in the standard protein diet and 5.4 (1.0) mg/L (p<0.05) in the high protein diet[72]. Similar reductions in both inflammation and weight were seen in both types of diets suggesting a high protein diet offers no greater advantages or disadvantages.

High protein diets are not often used to induce reduction in inflammatory processes. Therefore, not much research has been conducted on the use of this dietary factor even though high protein diets are often used to lose weight and those undergoing surgical weight loss are encouraged to ingest protein post-surgery.

**DIETARY FIBER INTAKE**

Inflammation has been reduced with increasing levels of fiber intake. King et al obtained dietary information from 4,900 adult participants in the NHANES study from 1999 to 2000[73]. The relationship of dietary fiber to the levels of CRP in this cross-sectional study of adult US residents showed that those in the higher quartiles of fiber intake had lower risk of elevated CRP levels. Comparing individuals in the highest quartile of fiber intake to those in the lowest quartile, a 23% reduction in mean CRP level was seen in those with a greater fiber intake (p<0.05). Risk of elevated CRP level was reduced by 2% for each additional gram of fiber consumed per day. Dividing fiber intake
into quartiles and relating this to the likelihood of elevated CRP revealed those in the higher quartiles had lower odds of having elevated CRP concentrations. Using the lowest quartile as a reference group (Q1<8.4 g/day) of 1.0 there was a reduced odds of exposure to higher CRP concentration as dietary fiber/day in grams increased. Quartile 2 (8.4-13.3 g/day) had an OR of 0.75 (CI 0.53-1.07), Quartile 3 (13.3-19.5 g/day) had an odds ratio of 0.64 (CI 0.43-0.96) and Quartile 4 (>19.5 g/day) had an odds ratio of 0.58 (CI 0.38-0.88) in the adjusted model and a 95% confidence interval[74]. High levels of CRP were considered above 3.0 mg/L, as this matches the definition from the American Heart Association [24]. According to the Dietary Guidelines for Americans set forth by the USDA, 14g of fiber per day is recommended per 1,000 calories. Therefore, as individuals meet the requirements of fiber intake their odds of increased CRP level decreased by 42% [74].

Qi and associates looked at whole-grain, bran and cereal fiber intakes through a food frequency questionnaire, as well as markers of systemic inflammation in women (n=902) with diabetes[75]. Those in higher quintiles for cereal fiber intake had 18% lower concentrations of CRP. Whole grain and bran fiber intakes were measured across quintiles with higher quintiles representing higher intake. In those consuming whole grain foods, CRP concentrations decreased as quintiles increased from Q1-Q5 with CRP reduction from 6.60 mg/L to 5.52 mg/L (P<0.03). Bran intake was also similar in overall CRP concentration reduction across quintiles as Q1-Q5 resulted in CRP reduction from 6.29 mg/L to 4.96 mg/L (P<0.007). As an observational study using the Nurses’ Health Study, there is no claim for causality between fiber intake and reduced inflammation but
cereal fiber intake, whole grain and bran fiber intakes were associated with lower inflammatory concentrations of CRP. [75]

Yunsheng Ma et al in 2008 examined the associations between dietary fiber and the inflammatory markers CRP, IL-6, and TNF-α-R2[76]. Baseline data in 1,953 racially and ethnically diverse post-menopausal (50-79 years, average BMI 28.8 kg/m²) women in the Women’s Health Initiative Observational Study was used. Significant associations were seen as inverse relationships between total fiber, IL-6 (p=0.01) and TNF-α-R2 (p=0.002) after adjustments for covariates. As quintiles increased so also did fiber intake and reductions were significantly seen in IL-6; quintile 1 of total fiber having a concentration of 2.16 (1.99-2.35) pg/ml to a concentration of 1.68 (1.55-1.83) pg/ml in quintile 5 (p<0.01). No significant associations could be concluded among dietary fiber and CRP, however. But, IL-6 and TNFα are inflammatory cytokines that are responsible for regulating the marker CRP and any dietary influence would first act on IL-6 and TNFα, indirectly affecting CRP over a longer period of time. This research could help draw conclusions based on hypotheses that ingestion of a high fiber diet may lower inflammation through reduction of IL-6, CRP and TNF-α-R2. [76]

Increased dietary fiber intake was associated with lower leptin concentrations in 424 (ages 18-22) female Japanese dietetic students[77]. As quintile of serum leptin increased the concentration of dietary fiber decreased. Quintile 1 of leptin concentration (4.2 ng/ml) had a dietary fiber level of 7.5 (2.5) g/1000kcal where Quintile 5 of leptin concentration (14.5 ng/ml) had a dietary fiber level of 6.6 (1.8) g/1000kcal (p<.0009). Although the cohort was young and healthy Japanese women, conclusions can be made
supporting decreased inflammation in a population consuming higher dietary fiber intakes. [77]

A cross-sectional analysis from the Nurses’ Health Study assessed 902 women with type 2 diabetes comparing dietary fiber and adiponectin concentration using food frequency questionnaires[78]. Increased intake of cereal fiber and fruit fiber were found to have statistical associations with increased levels of adiponectin. After adjustment for BMI as well as other covariates, adiponectin concentration increased in the higher quintile of cereal and fruit fiber. Increasing levels of plasma adiponectin was found to be associated with a higher level of mean cereal fiber in quintile 5 (8.3g/day) versus quintile 1 (6.7g/day) (p<0.002). The same results existed for those in quintile 5 of fruit fiber (8.3g/day) when compared to those consuming fruit fiber in quintile 1 (6.7g/day) (p<0.036). [78]

Therefore, dietary fiber has direct associations with adiponectin, leptin, CRP, IL-6 and TNF-α, as evidenced by the previous studies.

ANTIOXIDANTS (VITAMIN C, VITAMIN E, BETA CAROTENE)

It has been suggested that antioxidants may help protect against inflammation and therefore help prevent disease. A cross-sectional study encompassing men from the British Regional Heart Study (ages 60-79 and free of cardiovascular disease) was conducted to examine the associations between dietary and plasma vitamin C concentrations, fruit and vegetable intake and markers of inflammation[66]. Vitamin C concentration was obtained from a 7-day recall food-frequency questionnaire. As dietary vitamin C was stratified by quartiles, a reduction in CRP concentration was seen with increasing levels of Vitamin C. Using quartile 1 as the reference group (1.0)
(<54.6mg/day), the levels of CRP decreased concomitantly to the 2nd quartile (dietary vitamin C value of 54.6 to <77.2 mg/day) with an OR of 0.99 (0.79, 1.24), to the 3rd quartile (77.2 to <103.7 mg/day) with an OR of 0.77 (0.61, 0.97) and lastly to the 4th quartile (≥103.7 mg/day) with an OR of 0.81 (0.64, 1.03) (p<0.02). Therefore, the odds for greater CRP level were significantly reduced by 13% (looking at Q3 with a statistically significant CI) in those with vitamin C concentrations greater than 54.6mg/day. Mean concentration of CRP was found to be inversely related to vitamin C concentration after adjustment for confounders. [66]

A representative random selection of individuals (n=359, 172 women, 187 men; mean age 42) was taken from 2 Catalanian villages from the town hall population registry[79]. Dietary intake was assessed by a 3-day estimated food record and one week after a fasting blood sample was obtained. Vitamin E intake was measured, as it exists in different forms, one of them known as tocopherol; as well as energy intake (kcal/day), carbohydrate intake (g/day), protein intake (g/day), total lipids (g/day) and ascorbate (Vitamin C) in (mg/day). Lower daily intake of energy, carbohydrates, protein, total lipids, ascorbate and tocopherol were associated with increased levels of CRP concentration. As each quartile of CRP increased from lower to higher concentrations the level of each of these daily nutrient intake categories increased; the first quartile had values ranging from <0.1-0.44 mg/L whereas the range in the fourth quartile was 3.13-47.47 mg/L. Tocopherol (in mg/day, represented as mean (SD)) decreased (p<0.002) from 13.9 (7.3) mg/day to 10.6 (3.8) mg/day; energy intake decreased (p<0.001) from 2398 (710) kcal/day to 1992 (605) kcal/day; carbohydrate intake decreased (p=0.001) from 226.3 (75.6) g/day to 186.8 (69.4) g/day; protein intake decreased (p=0.001) from
99.4 (30.7) g/day to 83.8 (24.3) g/day; daily total lipid intake decreased (p<0.001) from 114.1 (36.1) g/day to 90.8 (28.2) g/day; and daily ascorbate (Vitamin C) decreased (p=0.163) from 115.2 (79.1) mg/day to 99.8 (59.8) mg/day. This suggests that increased levels of CRP concentration were associated with decreased levels of tocopherol (vitamin E), ascorbate (Vitamin C), energy intake, carbohydrate intake, protein and total lipid (or fat) intake. [79]

Most studies focus on decreased energy/caloric intake diets in helping to reduce inflammation. What is not as commonly studied are the effects particular micro/macronutrients may have on decreasing levels of inflammation. For example, although it is difficult to draw associations between naturally occurring Vitamin E and inflammatory responses because of its many forms [80], alpha-tocopherol is the most commonly assessed form of Vitamin E, as it is the most bio-available form [81]. The recommended dietary intake of Vitamin E is 15mg per day and this value is often not reached, resulting in immune alterations [81]. This could result in over expression in pro-inflammatory cytokines such as TNFα and IL-6 [81, 82]. The study by Capuron et al looked at Vitamin E ingestion and measures of biomarkers (CRP and IL-6) in 69 subjects (46 females, 23 males, mean age=78.9 years, mean BMI=27.7 kg/m²). Higher IL-6 levels corresponded to lower concentrations of Vitamin E (r=0.277, p<0.01). Although this cohort consisted of elderly subjects, an association can still be seen between lower vitamin E levels and inflammation. Therefore, intake of vitamin E may produce anti-inflammatory processes; as alpha-tocopherol is responsible for modulating immune function and regulation [81, 83].
High-pressurized orange juice was used to assess levels of vitamin C and associations with CRP concentration in the study by Sanchez-Moreno et al[84]. Subjects included 6 men and 6 women consuming 500 ml/day for 14 days, corresponding to a 250mg intake of vitamin C. The concentrations of plasma CRP was lower at day 14 when compared to baseline (p=0.317 in men and 0.235 in women). CRP concentrations were also found to be lower in a cohort of 3,015 men aged 60-79 years whose Vitamin C and fruit and vegetable intakes were assessed using a food-frequency questionnaire [66]. Those in higher quartiles of plasma Vitamin C (Q4≥40.25 µmol/L versus Q1≤14.44 µmol/L) had lower concentrations of CRP at 1.34 (1.23, 1.44) mg/L in Q 4 and 1.88 (1.73, 2.03) mg/L in Q1 (p<0.0001) [66]. Although only CRP is assessed along with Vitamin C concentration, inflammation in general may be decreased with this anti-inflammatory vitamin leading to healthier endothelial function.

BETA-CAROTENE

Erlinger et al looked at the associations between CRP concentration and serum beta-carotene finding that in NHANES III data (in non-smokers) those with higher beta-carotene levels have lower CRP concentrations[85]. Those with a level of beta-carotene at 18.9 mg/dl had a CRP concentration less than 2.2 mg/L. However, those with a beta-carotene level of 11.0 mg/dl had a clinically elevated CRP concentration above 10.0 mg/L. Hu et al found serum beta-carotene concentrations to be associated with not only CRP concentration but IL-6 as well in 672 (mean age=72.2, mean BMI=26.8 in high B-carotene levels and 25.4 kg/m² in the high B-carotene levels) individuals[86]. Those with decreased levels of beta carotene (<0.17 µmol/L) had increased CRP concentrations (mean (SD)) at 3.5(5.5) mg/L and increased IL-6 concentrations at 5.2 (6.3) pg/ml versus
those with increased beta-carotene levels ($\geq 0.17 \, \mu \text{mol/L}$) having a decreased CRP concentration at 2.9 (4.3) mg/L ($p<0.003$) and decreased IL-6 concentration at 3.9 (4.6) pg/ml ($p<.001$). CRP and IL-6 are the biomarkers commonly used to assess inflammation along with vitamin C, vitamin E and beta-carotene and future studies are needed to look at the direct effects on leptin, adiponectin and TNF$\alpha$.

**OMEGA-3 POLYUNSATURATED FATTY ACID**

It has been hypothesized that an intake of (n-3) fatty acids can improve inflammation. A cross-sectional study of 727 women from the Nurses’ Health Study I cohort was used to test this[87]. Both CRP and IL-6 were assessed from blood drawn along with dietary information obtained from a food frequency questionnaire from 1986-1990 in women ages 43-69 years. Both IL-6 and CRP levels decreased with increased dietary intake of (n-3) fatty acids but showed insignificant relationships. The EPA + DHA (Eicosapentaenoic acid and Docosahexaenoic acid) quintile resulted in similar reductions in concentration of CRP but still remained insignificant. Although reductions were not significant, an inverse relation between (n-3) fatty acids (ALA, EPA, DHA) and plasma concentrations of CRP and IL-6 resulted. Individuals in the highest quintile of total (n-3) fatty acid intake had a 29% lower level of CRP concentration and 23% lower level of IL-6 concentration. Due to the fact that this study is cross-sectional, causality cannot be inferred but the associations that dietary (n-3) fatty acids has with lower levels of the biomarkers IL-6 and CRP may help explain healthier endothelial function and the chance for reduced risk of cardiovascular disease with lower inflammation. [87]

Circulating leptin concentrations were shown in a recent population study to be reduced from dietary omega-3 fatty acids independent of body fat [88]. The 63
participants were enrolled in a 16-week factorial randomized controlled trial in one of four groups: control group (weight maintaining diet); a fish group (weight-maintaining diet incorporating a daily fish meal); a weight loss group (energy-restricted diet); or a fish-weight-loss group (energy-restricted diet incorporating a daily fish meal). Baseline leptin values were not statistically different between groups and were as follows (mean (SEM)): 19.72 (3.72) ng/ml in the control group (n=16); 15.52 (2.88) ng/ml in the fish group (n=17); 15.69 (2.45) ng/ml in the weight loss group (n=16); and 18.39 (3.19) ng/ml in the fish plus weight loss group. Post-16 week intervention serum leptin concentrations were changed as follows: an increase to 20.32 (4.08) ng/ml in the control without significance; an increase to 16.72 (3.07) ng/ml (p=0.001) in the fish group; a decrease to 14.29 (2.42) ng/ml (p<0.126) in the weight loss group; and a decrease to 13.31 (2.92) ng/ml (p=0.003) in the fish plus weight loss group[88]. Serum leptin concentrations were decreased the most in the group following a weight reduction program with a daily serving of fish. Previous studies have shown that weight loss alone can help reduce the level of leptin in [42-44] adipose tissue but an even greater decrease was seen in those also consuming fish with their weight loss (0.98 ng/ml). Therefore, it is both the interaction of omega-3 fatty acid intake and energy restriction which helps to reduce leptin concentration in adipose tissue.

Intake of omega-3 PUFA in 405 healthy men and 454 healthy women was found to be inversely related to TNFα (p=.03) concentration, somewhat lower in CRP (p=.08) and not significantly changed in IL-6 concentration. The lowest level of inflammation for individuals in the study was associated with higher levels of omega-3 and omega-6 intake [89]. A cross-sectional study of 727 women from the Nurses’ Health Study (aged
43-69 years) looked at omega-3 intake to evaluate its associations with biomarkers of inflammation. Quintiles of omega-3 intake were used to compare dietary intake and found that those in the highest quintile of omega-3 intake were associated with 29% lower levels of CRP (p=.007) and 23% lower levels of IL-6 (p=.009) [87].

Therefore, intake of omega-3 polyunsaturated fatty acids may help reduce leptin, CRP and TNF\(\alpha\) and IL-6; while adiponectin is not commonly assessed to look at the associations.

CONCLUSION

The prevalence of morbid obesity is on the rise and is rapidly increasing such that in some populations, nearly 1 in 6 has a BMI> 40.0 kg/m\(^2\). Accompanying this rise in obesity is an increase in diseases associated with this energy imbalance. Many of these co-morbidities are related to the pro-inflammatory state of obesity. The increase in circulating pro-inflammatory and the reduction in anti-inflammatory biomarkers, especially those that originate or affected by adipose tissue, is thought to underlie the inflammatory state[59]. A number of studies have examined changes in adipose tissue through weight loss interventions and have shown that the inflammatory state improves with weight loss. Additionally, many dietary components have been associated with a number of inflammatory markers, including the levels of macronutrients and micronutrients present in an individual’s diet. Specifically, these include fat, carbohydrate, protein, antioxidants, dietary fiber and omega-3 fatty acids.

LIMITATIONS OF THE PREVIOUS RESEARCH

Only a limited number of studies have examined gastric bariatric surgeries as the mode for weight loss in the context of inflammation. Furthermore, those studies have
generally only looked at the long-term effect (> 1 to 2 years), and not during the acute weight loss period of the first 6-months. Examination of only one to two biomarkers within one study also has limited the earlier work. Finally, a correlation of dietary components with inflammation in the morbidly obese population prior to surgery has not been demonstrated previously.

SPECIFIC AIMS OF THE STUDY

Therefore, the specific aims of the present study were to examine the effect of weight loss surgery on a number of pro- and anti-inflammatory biomarkers at several time points during a 6-month follow-up period, and to study the relationships between baseline measures of inflammatory biomarkers, body fat, and dietary variables.

HYPOTHESES

Supported by the described research and based on the development of the aims of the study, the main hypothesis is that pro-inflammatory biomarker concentrations (TNF$\alpha$, IL-6, CRP and leptin) will significantly decrease and the concentration of the anti-inflammatory biomarker, adiponectin, will significantly increase from baseline to 6-months after surgery. Two other hypotheses have been formed from these aims as well and are as follows: changes in weight from baseline to 6-months will be significantly correlated with the changes in inflammatory biomarkers; and significant correlations will exist between baseline inflammatory biomarkers and a number of dietary variables obtained at baseline.
METHODOLOGY

STUDY POPULATION

Data for this investigation were obtained from 28 patients recruited before laparoscopic Roux-en-Y gastric bypass surgery. Men (n=2) and women (n=26) already scheduled to undergo the weight loss surgery at Wake Forest University Baptist Medical Center (WFUBMC) were consented if they met the inclusion criteria. Recruitment occurred over one year and follow-up was for 6 months.

Recruitment was completed by their surgeon and co-investigator of this study, Dr. Fernandez, as well as his nurse (Susan Butler, R.N.). Patient’s eligibility for enrollment was assessed at their first screening visit. Eligibility included BMI level above 40.0 kg/m² or greater than 35.0 kg/m² with an obesity related co-morbidity, reported sedentary lifestyle, and self-reported difficulty in completing at least one of the following activities associated with back, hip, knee, or ankle pain: lifting and carrying groceries, walking one-quarter mile, getting in and out of a chair, or walking up and down stairs. If patient’s appeared to be eligible based on this criteria, Dr. Fernandez informed the patient of this study and obtained informed consent. Once obtained, clinic staff gathered health history, current medications, and demographic information. Baseline measurements prior to surgery were also scheduled.

STUDY DESIGN

This observational study provided baseline (pre-surgery) measures of 28 individuals that underwent surgery. Follow-up measures at 3 weeks, 3 months, and 6 months were obtained from a total of 13 participants. The primary outcome variable for
this analysis was inflammatory biomarkers at each of the measurement periods, including adipose tissue metabolic hormones (adipokines) and inflammatory cytokines. Other variables of interest for this analysis included demographics, nutrient intake, and visceral fat volume, which were only collected at baseline, as well as body weight and body mass index.

PROCEDURES

Upon meeting eligibility criteria, participants were invited into the study and began the study testing visits. Participants reported to the Geriatric Research Center (GRC) and the Geriatric General Clinical Research Center (GGCRC) for testing. The following assessments were obtained at this baseline visit: demographics, a 12-hour fasting blood sample to measure hormones and inflammatory biomarkers, anthropometrics, and dietary intake.

Whole blood samples were collected in EDTA-treated vacutainers via venipuncture in the early morning (between 7-9AM) after a 12-hr fast. Prior to the blood sampling, participants were queried about their medication use and health status. Any participant who reported currently taking any antibiotic medication, or having an overt infection (e.g., urinary tract, respiratory, etc.) or fever (>99.0°F) were rescheduled. Samples were put immediately on ice and separated by centrifugation for 20 minutes at 4°C within 30 minutes of collection. After separation, specimens were stored in 1ml aliquots at -80°C until analyses.

Fasting plasma concentrations of leptin, adiponectin, IL-6 and TNFα were determined by enzyme-linked immunosorbent assay (ELISA) kits (high-sensitivity for
IL-6 and TNFα) from R&D Systems, Minneapolis, MN. CRP was measured using an automated immunoanalyzer (Immulite, Diagnostics Products Corp., Los Angeles, CA). All samples were measured in duplicate and the average of the two values was used for data analyses. Samples that were above the maximum detection limit were diluted (1:2) and re-analyzed. Duplicate samples that did not provide a coefficient of variation<15% were re-analyzed. The intra-assay and inter-assay coefficients of variation (CV) for IL-6 were 7% and 16%, respectively, for TNFα were 8% and 23%, respectively. The inter-assay and intra-assay CV’s for the CRP assay (ALPCO, Windham, NH) were 8% and 7%, respectively.

Nutritionists from the GCRC instructed participants on recording 4 day food records, which included at least one weekend day. Food records were analyzed for both total energy and micro-and macronutrient content by the Nutrition Data System for Research (NDS-R) software version 4.05-33. The average across the 4 day record was used for analysis.

Visceral fat volume was measured from a computer tomography (CT) scan (Lightspeed Plus, General Electric Medical Systems, Milwaukee, WI, USA). Abdominal scan parameters were set at helical mode, 120kVp, 250mA, 4x25mm collimation, standard reconstruction kernel, and a display field-of-view of 500mm. CT slices within 15mm centered at the L4-5 level were used to calculate volume. Adipose tissue volume was quantified [GE Healthcare, Advantage Windows 4.2 Volume Viewer (Waukesha, WI, USA). Muscle volume (cm³) was non-adipose, non-bone tissue within the deep fascia plane. The inner and outer aspects of the abdominal wall were traced and visceral fat was defined as fat enclosed by the inner aspect of the abdominal wall.
**STATISTICAL ANALYSIS**

All data were checked for normality using histograms to show frequencies. Concentrations of leptin, adiponectin, CRP, IL-6 and TNFα were not normally distributed and were transformed to the log values for subsequent analyses and calculated. For ease of interpretation, figures contain non-transformed values, but analyses and significance for the skewed variables are based on log transformed values. Descriptive statistics (including means, standard deviations, and frequency) were determined for age, gender (% female), race (% Caucasian), total number of medications, visceral fat volume, and total number of co-morbidities at baseline only. Descriptive statistics were run for those individuals who completed all aspects of the study (n=13) and those who did not (n=15). Mean values (SD) were calculated at each time point for inflammatory biomarkers and adipokines, body weight, and BMI, as well as weight loss from baseline for completers. Repeated measures ANOVA assessed differences for these variables across the follow-up period. Changes and percent changes from baseline in weight and inflammatory biomarkers were also calculated. These analyses were from participants that completed all 4 visits. T-tests were performed to compare baseline data for those who completed all 4 visits (n=13) vs. those who did not complete all 4 visits (n=15).

Spearman correlations were used to determine the relationships between inflammatory biomarkers, diet, and body fat estimates at baseline, as well as the changes and percent changes in body weight between baseline and 6-months. All participants with baseline data were included for determining correlations.
Statistical significance was set at p<0.05 for all statistical tests. All analyses were performed using SPSS 18.0.
RESULTS

DEMOGRAPHICS

Baseline characteristics for the 28 individuals in the study are shown in Table 1. Participants included 26 females and 2 males with race being predominantly white (89.3%), and the remainder African-American. The mean age of the group was 43.5 years. The average number of medications was 1.4 and co-morbidities ranged from 0 to 8 with an average of 4.8. A total of 13 participants completed all of 3 follow-up time points and had blood drawn and stored for analysis of inflammatory biomarkers. A total of 15 individuals either missed a follow-up period appointment, dropped out of the study (n=3), or were not able to obtain blood for biomarker analysis at each time point. The demographic information for both those who completed all aspects of the study versus those who did are shown in Table 2. Individuals who completed all aspects of the study were older, had a higher BMI at baseline and had a greater volume of visceral fat.

Independent samples t-tests were performed to compare various demographic and outcome measures at baseline for completers vs. non-completers. There was no significant difference between weight, BMI, age, total co-morbidities, medication, visceral fat volume, the inflammatory markers of CRP, IL-6, TNFα, and leptin. The dietary variables of energy, fat, carbohydrate, protein, dietary fiber, vitamin E, vitamin C and beta-carotene intake were also not different between groups. The only significant differences existed between baseline adiponectin (p=.049) and omega-3 fatty acids (p=.007) in those who completed all aspects of baseline dietary intake and blood biomarker measurements.
BASELINE WEIGHT AND BODY MASS INDEX

Baseline, 3 week, 3 month and 6 month body weight and BMI data measured as means with standard deviation are presented for those that completed all aspects of the measured variables for this study in Table 3. The initial weight was an average 149(21.4) kg while the baseline body mass index (BMI) was 54.7(6.0) kg/m². The average weight loss of the subjects over the 6 month time period was 38.7 kg while the average reduction in BMI was 14.7 kg/m² with significant changes from one to the next time point. Percent weight loss from baseline was assessed and achieved statistical significance at each time point with an average loss of 26.2% from baseline to 6 months.

HORMONE CONCENTRATIONS

Table 4 presents the natural log transformed values for the inflammatory biomarkers. Figures 1-3 show the non-transformed values for ease of interpretation. Statistical analysis was performed on the transformed values only, and these results are presented in both the table and the figures. Values for the inflammatory biomarkers are presented as means (S.D.).

Leptin decreased between time points from 57.8 (23.1) ng/ml at baseline to 34.9 (12.0) ng/ml at 3 weeks, 27.7 (13.4) ng/ml at 3 months and 19.6 (9.5) ng/ml at 6 months, with significant decreases achieved from each succeeding time point (Figure 1). For TNFα, there were no significant differences across the follow-up (Figure 2). IL-6 levels did not show significant differences between any of the time points [8.6 (6.9) pg/ml at baseline, 8.5 (6.8) pg/ml at 3 weeks, 10.9 (7.7) pg/ml at 3 months and 7.4 (4.7) pg/ml at 6 months] (Figure 2). Adiponectin concentrations were significantly higher for 3 and 6
months compared to baseline and 3 weeks (Figure 3). CRP concentrations decreased from a baseline value of 13.8 (8.5) µg/ml to 9.6 (10.5) µg/ml at 3 weeks, rose back to a value of 13.2 (10.4) µg/ml by 3 months and then decreased again to 9.3 (6.5) µg/ml at 6 months (Figure 3). Statistically significant differences were seen for CRP between baseline and 6 months.

BASELINE DIETARY VARIABLES

Total calories (energy, kcals), fat, carbohydrates (CHO), protein, dietary fiber, Vitamin E (α-tocopherol), Vitamin C, beta-carotene, and omega-3 polyunsaturated fatty acid (PUFA) were all assessed through 4-day food record at baseline (Table 5). As shown in the table, the mean energy intake was 2132 kcals with nearly 93 grams of fat or nearly 40% of total energy from fat. Carbohydrates represented about 45% of total daily energy (240 grams of carbohydrates). Protein intake was ~90 grams per day or about 17% of total daily energy intake.

WEIGHT CHANGE AND INFLAMMATORY MARKERS

Table 6 shows Spearman correlations (and respective p values) between baseline inflammatory markers with baseline body weight, BMI, number of co-morbidities, age, number of medications, and volume of visceral fat. Statistical significant correlations were seen between leptin and body weight (r=.561, p=.010), BMI (r=.628, p=.003), and co-morbidities (r=.444, p=.050). Adiponectin was significantly correlated with age (r=.547, p=.010). CRP had trends toward significance with BMI (r=.421, p=.058) and age (r=.414, p=.062). IL-6 correlations revealed a trend towards significance with BMI (r=.380, p=.098).
Correlations between percent change from baseline to 6-months in weight and inflammatory markers were assessed and are presented in Table 7. Trends toward significance were seen between the percent change in leptin with the percent change in weight (r=.495, p=.086). Correlations were also computed between absolute changes in inflammation and weight with similar results, Table 8; leptin had a trend toward significance with changes in weight (r=.484, p=.094).

Correlations were also assessed between the various inflammatory biomarkers and are expressed in Table 9. The only significant correlation existed between adiponectin and CRP (r=.468, p=.033). Adiponectin and IL-6 were not significantly correlated but correlations resulted in a trend toward significance (r=-.406, p=.076).

ASSOCIATIONS BETWEEN BASELINE VALUES OF INFLAMMATORY BIOMARKERS AND DIETARY MEASURES

A secondary objective of this analysis was to determine the relationships at baseline between dietary and inflammatory biomarkers variables (Table 10). Preliminary analyses were performed to ensure no violation of the assumptions of normality, linearity, and homoscedasticity.

There was an inverse correlation found between higher total dietary fiber intake and lower adiponectin concentration, (r=-.460, p=.036). The relationship between IL-6 and total energy intake resulted in a trend towards significance (r=.389, p=.090), and the relationship between leptin and vitamin C intake also resulted in an inverse trend towards significance as well (r=-.380, p=.089). No other significant correlations were found between these variables.
# TABLES AND FIGURES

## Table 1. BASELINE PARTICIPANT CHARACTERISTICS

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean (Std Deviation)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>27.1</td>
<td>59.1</td>
<td>43.5(9.2)</td>
</tr>
<tr>
<td>Body Weight (kg)</td>
<td>98.3</td>
<td>207.4</td>
<td>150.7(25.2)</td>
</tr>
<tr>
<td>Body Mass Index (kg/m²)</td>
<td>36.3</td>
<td>70.4</td>
<td>53.8(7.3)</td>
</tr>
<tr>
<td>Visceral Fat (cm³)</td>
<td>573.7</td>
<td>2877.5</td>
<td>1468.2(597.7)</td>
</tr>
<tr>
<td>Number of Medications (n)</td>
<td>0</td>
<td>7</td>
<td>1.4(1.4)</td>
</tr>
<tr>
<td>Total Comorbidities (n)</td>
<td>0</td>
<td>8</td>
<td>4.8(2.0)</td>
</tr>
<tr>
<td>DJD (n)</td>
<td></td>
<td></td>
<td>26</td>
</tr>
<tr>
<td>Hypertension (n)</td>
<td></td>
<td></td>
<td>19</td>
</tr>
<tr>
<td>GERD (n)</td>
<td></td>
<td></td>
<td>19</td>
</tr>
<tr>
<td>Dyslipidemia (n)</td>
<td></td>
<td></td>
<td>15</td>
</tr>
<tr>
<td>Depression (n)</td>
<td></td>
<td></td>
<td>15</td>
</tr>
<tr>
<td>Sleep Apnea (n)</td>
<td></td>
<td></td>
<td>14</td>
</tr>
</tbody>
</table>

DJD: Degenerative Joint Disease; GERD: Gastroesophageal Reflux Disease
Table 2. BASELINE PARTICIPANT CHARACTERISTICS FOR THOSE COMPLETING ALL ASPECTS OF STUDY VERSUS THOSE WHO DID NOT

<table>
<thead>
<tr>
<th>Variables</th>
<th>Completers (n=13)</th>
<th></th>
<th>Non-Completers (n=15)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (SD)</td>
<td>Mean (SD)</td>
<td>Mean (SD)</td>
<td>Mean (SD)</td>
</tr>
<tr>
<td></td>
<td>Min-Maximum Range</td>
<td>Min-Maximum Range</td>
<td>Min-Maximum Range</td>
<td>Min-Maximum Range</td>
</tr>
<tr>
<td>Age (Years)</td>
<td>45.5(9.3)</td>
<td>41.0(8.8)</td>
<td>27.1-59.1</td>
<td>28.8-55.6</td>
</tr>
<tr>
<td>Gender</td>
<td>F=13, M=0</td>
<td>F=13, M=2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Race</td>
<td>Caucasian=11, African American =2</td>
<td>Caucasian=14, African American=1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body Mass Index (kg/m²)</td>
<td>55.1(5.9)</td>
<td>52.7(8.4)</td>
<td>45.4-65.9</td>
<td>36.3-70.4</td>
</tr>
<tr>
<td>Visceral Fat (cm³)</td>
<td>1363.4(513.0)</td>
<td>1565.5(671.2)</td>
<td>573.7-2133.1</td>
<td>591.8-2877.5</td>
</tr>
<tr>
<td>Body Weight (kg)</td>
<td>149.0(21.4)</td>
<td>152.3(28.7)</td>
<td>120.1-187.7</td>
<td>98.3-207.4</td>
</tr>
<tr>
<td>Number of Medications</td>
<td>1.9(1.8)</td>
<td>1.0(0.65)</td>
<td>0-7</td>
<td>0-2</td>
</tr>
<tr>
<td>Total Co-morbidities</td>
<td>4.7(1.8)</td>
<td>4.8(2.2)</td>
<td>1-8</td>
<td>0-8</td>
</tr>
</tbody>
</table>
Table 3. BODY WEIGHT AND BODY MASS INDEX AT BASELINE AND FOLLOW-UP

<table>
<thead>
<tr>
<th></th>
<th>Mean(SD)</th>
<th>Baseline</th>
<th>3 Weeks</th>
<th>3 Months</th>
<th>6 Months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight(kg)</td>
<td>149.0 (21.4)a</td>
<td>136.8 (19.3)b</td>
<td>123.2 (17.2)c</td>
<td>110.3 (15.9)d</td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>54.7 (6.0)a</td>
<td>50.3 (5.8)b</td>
<td>44.6 (5.6)c</td>
<td>40.0 (5.5)d</td>
<td></td>
</tr>
<tr>
<td>Weight Loss (% from BL)</td>
<td>_</td>
<td>-8.0(1.5)a</td>
<td>-17.0(2.9)b</td>
<td>-26.2(4.6)c</td>
<td></td>
</tr>
</tbody>
</table>

Mean (SD) with different letters denote significant differences between each other (p<0.05)

Table 4. Ln VALUES IN BLOOD BIOMARKERS OVER TIME POINTS [Mean (SD)]

<table>
<thead>
<tr>
<th></th>
<th>Mean (SD)</th>
<th>Baseline</th>
<th>3 Weeks</th>
<th>3 Months</th>
<th>6 Months</th>
</tr>
</thead>
<tbody>
<tr>
<td>lnLeptin (ng/ml)</td>
<td>4.0 (0.6)a</td>
<td>3.6 (0.6)b</td>
<td>3.2 (0.7)c</td>
<td>2.9 (0.7)d</td>
<td></td>
</tr>
<tr>
<td>lnAdiponectin(µg/ml)</td>
<td>2.0 (0.7)a</td>
<td>2.2 (0.6)a</td>
<td>2.3 (0.6)b</td>
<td>2.2 (0.5)b</td>
<td></td>
</tr>
<tr>
<td>lnTNF-α (pg/ml)</td>
<td>0.7 (0.7)a</td>
<td>0.6 (0.8)a</td>
<td>0.5 (0.6)a</td>
<td>0.5 (0.6)a</td>
<td></td>
</tr>
<tr>
<td>lnIL-6 (pg/ml)</td>
<td>2.0 (0.7)a</td>
<td>1.9 (1.0)a</td>
<td>2.1 (1.0)a</td>
<td>1.7 (0.8a)</td>
<td></td>
</tr>
<tr>
<td>lnCRP (µg/ml)</td>
<td>2.6 (0.7)a</td>
<td>2.1 (0.8)b</td>
<td>2.5 (0.8)b</td>
<td>2.1 (0.7)c</td>
<td></td>
</tr>
</tbody>
</table>

Mean (SD) with different letters denote significant differences between each other (p<0.05)
Figure 1. Measures of Leptin Concentration at Baseline, 3 Weeks, 3 Months and 6 Months (Mean(SD)) Post-surgery

Mean (SD) with different letters denote significant differences between each other (p<0.05)
Figure 2. Measures of TNFα and IL-6 Concentrations at Baseline, 3 Weeks, 3 Months, and 6 Months (Mean(SD)) Post-surgery
Figure 3. Measures of Adiponectin and CRP Concentrations at Baseline, 3 Weeks, 3 Months and 6 Months (Mean(SD)) Post-surgery

Mean (SD) with different letters denote significant differences between each other (p<0.05)
Table 5. DIETARY VARIABLES AT BASELINE

<table>
<thead>
<tr>
<th>Baseline Diet Variable</th>
<th>Mean (SD)</th>
<th>Baseline Diet Variable</th>
<th>Mean (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy (kilocalories)</td>
<td>2132.1 (806.1)</td>
<td>Vit E, (mg)</td>
<td>8.4 (6.2)</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>92.9 (45.1)</td>
<td>Vitamin C (mg)</td>
<td>69.6 (57.5)</td>
</tr>
<tr>
<td>CHO (g)</td>
<td>240.6 (104.3)</td>
<td>Beta-Carotene (mg)</td>
<td>3656.7 (7949.1)</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>89.8 (32.4)</td>
<td>Omega-3 FA (g)</td>
<td>1.8 (1.3)</td>
</tr>
<tr>
<td>Dietary Fiber (g)</td>
<td>16.3 (9.7)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 6. BASELINE CORRELATIONS OF INFLAMMATION WITH BODY WEIGHT, BMI, CO-MORBIDITIES, MEDICATION, VISCERAL FAT, AGE

<table>
<thead>
<tr>
<th></th>
<th>LnLeptin</th>
<th>LnAdiponectin</th>
<th>LnCRP</th>
<th>LnIL-6</th>
<th>LnTNFα</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Body Weight</strong></td>
<td>r = .561*</td>
<td>-.158</td>
<td>.299</td>
<td>.273</td>
<td>.087</td>
</tr>
<tr>
<td></td>
<td>p = .010</td>
<td>.494</td>
<td>.188</td>
<td>.244</td>
<td>.681</td>
</tr>
<tr>
<td><strong>BMI</strong></td>
<td>.628**</td>
<td>-.119</td>
<td>.421#</td>
<td>.380#</td>
<td>.002</td>
</tr>
<tr>
<td></td>
<td>.003</td>
<td>.608</td>
<td>.058</td>
<td>.098</td>
<td>.991</td>
</tr>
<tr>
<td><strong>Co-Morbidities</strong></td>
<td>.444*</td>
<td>.184</td>
<td>-.023</td>
<td>.173</td>
<td>-.178</td>
</tr>
<tr>
<td></td>
<td>.050</td>
<td>.425</td>
<td>.920</td>
<td>.467</td>
<td>.396</td>
</tr>
<tr>
<td><strong>Age</strong></td>
<td>.005</td>
<td>.547*</td>
<td>-.414#</td>
<td>.129</td>
<td>.013</td>
</tr>
<tr>
<td></td>
<td>.982</td>
<td>.010</td>
<td>.062</td>
<td>.587</td>
<td>.949</td>
</tr>
<tr>
<td><strong>Total Medication</strong></td>
<td>.205</td>
<td>.411#</td>
<td>.131</td>
<td>-.105</td>
<td>-.020</td>
</tr>
<tr>
<td></td>
<td>.385</td>
<td>.064</td>
<td>.571</td>
<td>.660</td>
<td>.925</td>
</tr>
<tr>
<td><strong>Visceral Fat Volume</strong></td>
<td>.150</td>
<td>.225</td>
<td>-.306</td>
<td>.156</td>
<td>-.264</td>
</tr>
<tr>
<td></td>
<td>.527</td>
<td>.327</td>
<td>.177</td>
<td>.510</td>
<td>.203</td>
</tr>
</tbody>
</table>

**Correlation is significant at the 0.01 level (2-tailed).**

* Correlation is significant at the 0.05 level (2-tailed).

# Trend towards significance (p<0.10)
<table>
<thead>
<tr>
<th>Percent Change in LnLeptin</th>
<th>Percent Change in Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percent Change in LnAdiponectin</td>
<td>.027</td>
</tr>
<tr>
<td>Percent Change in LnTNFα</td>
<td>-.335</td>
</tr>
<tr>
<td>Percent Change in LnIL-6</td>
<td>.294</td>
</tr>
<tr>
<td>Percent Change in LnCRP</td>
<td>.170</td>
</tr>
</tbody>
</table>

**Correlation is significant at the 0.01 level (2-tailed).**

*. Correlation is significant at the 0.05 level (2-tailed).

#. Trend towards significance (p<0.10)
Table 8. CORRELATIONS OF CHANGE IN INFLAMMATION WITH CHANGE IN WEIGHT FROM BASELINE TO 6 MONTHS

<table>
<thead>
<tr>
<th>Change in LnLeptin</th>
<th>Change in Weight</th>
<th>( r = .484 )</th>
<th>( p = .094 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Change in LnAdiponectin</td>
<td></td>
<td>.022</td>
<td>.943</td>
</tr>
<tr>
<td>Change in LnTNF(\alpha)</td>
<td></td>
<td>.016</td>
<td>.957</td>
</tr>
<tr>
<td>Change in LnIL-6</td>
<td></td>
<td>.329</td>
<td>.297</td>
</tr>
<tr>
<td>Change in LnCRP</td>
<td></td>
<td>.399</td>
<td>.177</td>
</tr>
</tbody>
</table>

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

#. Trend towards significance (p<0.10)
Table 9. BASELINE CORRELATIONS OF INFLAMMATORY BIOMARKERS WITH THEMSELVES

<table>
<thead>
<tr>
<th></th>
<th>LnLeptin</th>
<th>LnCRP</th>
<th>LnTNFα</th>
<th>LnAdiponectin</th>
<th>LnIL-6</th>
</tr>
</thead>
<tbody>
<tr>
<td>LnCRP</td>
<td>r=.263</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>p=.264</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LnTNFα</td>
<td>.055</td>
<td>.296</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>.818</td>
<td>.192</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LnAdiponectin</td>
<td>-.212</td>
<td>.468*</td>
<td>-.208</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>.369</td>
<td>.033</td>
<td>.366</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LnIL-6</td>
<td>.211</td>
<td>.239</td>
<td>.195</td>
<td>-.406#</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>.385</td>
<td>.310</td>
<td>.410</td>
<td>.076</td>
<td></td>
</tr>
</tbody>
</table>

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

#. Trend towards significance (p<0.10)
Table 10. BASELINE CORRELATIONS OF DIET AND INFLAMMATORY BIOMARKERS

<table>
<thead>
<tr>
<th></th>
<th>lnLeptin</th>
<th>lnAdiponectin</th>
<th>lnCRP</th>
<th>lnIL-6</th>
<th>lnTNFα</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total Energy</strong></td>
<td>r=-.165</td>
<td>-.314</td>
<td>.208</td>
<td>.389#</td>
<td>-.017</td>
</tr>
<tr>
<td></td>
<td>p=.488</td>
<td>.166</td>
<td>.366</td>
<td>.090</td>
<td>.935</td>
</tr>
<tr>
<td><strong>Total Fat</strong></td>
<td>-.229</td>
<td>-.101</td>
<td>.132</td>
<td>.275</td>
<td>.087</td>
</tr>
<tr>
<td></td>
<td>.331</td>
<td>.664</td>
<td>.567</td>
<td>.240</td>
<td>.678</td>
</tr>
<tr>
<td><strong>Total CHO</strong></td>
<td>-.120</td>
<td>-.362</td>
<td>.229</td>
<td>.335</td>
<td>-.153</td>
</tr>
<tr>
<td></td>
<td>.613</td>
<td>.106</td>
<td>.319</td>
<td>.148</td>
<td>.466</td>
</tr>
<tr>
<td><strong>Total Protein</strong></td>
<td>-.130</td>
<td>-.144</td>
<td>-.213</td>
<td>.152</td>
<td>-.078</td>
</tr>
<tr>
<td></td>
<td>.585</td>
<td>.535</td>
<td>.354</td>
<td>.523</td>
<td>.709</td>
</tr>
<tr>
<td><strong>Total Diet. Fiber</strong></td>
<td>.008</td>
<td>-.460*</td>
<td>-.040</td>
<td>.153</td>
<td>.057</td>
</tr>
<tr>
<td></td>
<td>.975</td>
<td>.036</td>
<td>.862</td>
<td>.519</td>
<td>.788</td>
</tr>
<tr>
<td><strong>Vitamin E</strong></td>
<td>-.196</td>
<td>-.247</td>
<td>-.243</td>
<td>.322</td>
<td>-.049</td>
</tr>
<tr>
<td></td>
<td>.407</td>
<td>.279</td>
<td>.289</td>
<td>.166</td>
<td>.815</td>
</tr>
<tr>
<td><strong>Vitamin C</strong></td>
<td>.399#</td>
<td>-.218</td>
<td>.074</td>
<td>.206</td>
<td>.096</td>
</tr>
<tr>
<td></td>
<td>.081</td>
<td>.343</td>
<td>.750</td>
<td>.384</td>
<td>.647</td>
</tr>
<tr>
<td><strong>Beta-Carotene</strong></td>
<td>-.164</td>
<td>.155</td>
<td>-.105</td>
<td>-.262</td>
<td>.007</td>
</tr>
<tr>
<td></td>
<td>.490</td>
<td>.502</td>
<td>.650</td>
<td>.265</td>
<td>.972</td>
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<tr>
<td><strong>Omega-3 FA</strong></td>
<td>-.315</td>
<td>-.007</td>
<td>-.305</td>
<td>.059</td>
<td>-.036</td>
</tr>
<tr>
<td></td>
<td>.177</td>
<td>.977</td>
<td>.179</td>
<td>.806</td>
<td>.865</td>
</tr>
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</table>

**Correlation is significant at the 0.01 level (2-tailed).**

*. Correlation is significant at the 0.05 level (2-tailed).
#. Trend towards significance (p<0.10)
DISCUSSION

The primary purpose of this study was to examine the effect of RYGB on inflammatory markers and adipokines in morbid obesity. Additionally, the analysis studied correlations of baseline, pre-surgical values of the inflammatory biomarkers with dietary intake and regional body composition in this population. There has been limited information on the kinetics of changes in inflammation during the acute weight loss period following bypass surgery. Overall, this study found improvements in several markers of inflammation for up to 6-months following surgery. Furthermore, since earlier work in other populations had shown that specific dietary components were related to inflammation, it seemed reasonable to investigate these relationships in this population. Few significant correlations between baseline values of inflammatory biomarkers and intakes of nutrients were observed. The significance of these results is discussed below.

LEPTIN

Leptin concentration at baseline was 57.8 ng/ml, which is similar to levels found in morbid obesity from others [10, 44, 45]. In comparison, leptin levels in healthy lean individuals are well below 30 ng/ml and can reach <10 ng/ml in certain populations [44]. The greater than 50% reduction in leptin at 6-months (57.8 to 19.6 ng/ml) is similar to that found by others following weight loss surgeries [44-46]. As expected, baseline levels of leptin were positively correlated with body weight $(r=.561, p=.010)$, and BMI $(r=.628, p=.003)$ supporting a number of other studies with this finding. This relationship is observed across a spectrum of body fat levels, from lean to morbid obesity [10, 37, 40, 44, 50]. Leptin was the only biomarker with trends toward significance with percent
change in weight and change in weight, further illustrating the effect of weight and weight loss on leptin [39, 40]. Interestingly, leptin was also associated with the number of co-morbidities (r=.444, p=.050).

Leptin was not correlated with any of the 9 diet variables; this discrepancy with earlier findings may be due to a small number of subjects, and higher BMI levels in our population [69, 77, 88]. Since diet was not related to BMI, it demonstrates the main role of adiposity level on leptin concentration, at least in the morbidly obese population. In past research, decreases in leptin have been correlated with severe caloric restriction [37, 69], higher dietary fiber intake [77], and omega-3 fatty acid ingestion [88]. The impact of caloric restriction on leptin metabolism is not surprising as the functional changes of leptin occur in the neuroendocrine system where it binds to specific receptors in the hypothalamus, causing changes in appetite and/or energy expenditure [31]. Shai et al also saw decreased leptin with a caloric-restricted, low-carbohydrate or low-fat diet but the cohort was not morbidly obese and 86% males, where morbidly obese females tend to have a higher leptin concentration resulting from a different distribution of body fat and sex hormone levels [31, 37, 69]. Prior associations between higher dietary fiber intake and lower leptin concentration were only seen in young (18-22 years old) Japanese females with a normal BMI [77]. Leptin reductions during weight loss combined with high fish-intake showed the possible effects omega-3 fatty acid may have on inflammation, particularly leptin concentration; however this was not prevalent in this study [88]. It is uncertain why we did not see this effect, but it may be related to examination of cross-sectional data in the literature and not longitudinal analysis during
active weight loss. Additionally, the BMI level of this cohort was larger than others examining dietary relationships with this biomarker.

ADIPONECTIN

Consistent with earlier work, adiponectin increased as weight loss decreased following surgery. There was an approximately 10% increase from baseline to 3-6 months. Levels in the current study observed at baseline were at concentrations typically found in non-obese subjects, and nearly 3 fold higher than in obese subjects [60]. Adiponectin was significantly positively correlated with age ($r=.547$, $p=.010$); which is consistent with another [10]. It is difficult to conclude why such correlations exist but in both studies mean ages were in the range of 39-43 years. The lack of correlation between body weight loss and adiponectin changes may be a result of a short follow-up period as Faraj et al found adiponectin changes to be significantly correlated with weight loss ($r=-.59$, $p=.02$) but this study lasted 15 months post-surgery[10]. Overall, adiponectin was significantly increased in studies lasting longer than 6 months (1+ year) suggesting levels rise continuously with weight loss [10, 61].

Inverse correlations were found with dietary fiber intake ($r=-.460$, $p=.036$). Increased dietary fiber intake correlated with decreased adiponectin concentration is not consistent with others as Qi et al found adiponectin increases with higher intakes of cereal fiber after adjustment for BMI and other co-variates in diabetic women[75]. It is important to note that adiponectin circulates at high levels in the blood after it is secreted by adipose tissue, helping to improve insulin sensitivity and reduce blood free fatty acids in this population. BMI was controlled for in Qi et al, suggesting associations between
dietary factors and adiponectin are more of a source of variance for adiponectin than obesity status.

CRP

Baseline CRP concentrations decreased by 33%, which is supported by Chen et al (n=640) and Zagorski et al (n=20) who reported decreases by 64% and 62%, respectively [28, 29]. Reductions in Chen et al were to a concentration of 3.0 µg/ml, where <3.0 is the cutoff by the American Heart Association for elevated CRP concentration. Noteworthy, the degree of CRP concentration from the current study was less than seen by others with weight loss. This is not surprising considering Chen et al had greater decreases in BMI and achieved a desired level (BMI < 25.0 kg/m²) and Zagorski et al had reductions in BMI to a level considered obese (BMI 30.0-30.39 kg/m²)[28, 29]. In contrast, the mean BMI across subjects in the current study were still considered morbidly obese (40.0 kg/m²).

Consistent with others, correlations found trends toward significance between baseline measures of CRP and BMI (r=.421, p=.058). CRP has been found in other studies to have correlations with BMI (r=.431, p=.009) [17, 30] and fat mass [30]; others have also found trends towards a significant relationship between CRP and volume of visceral fat (r=-.408, p=.066) as well. A trend toward significance was also found between CRP and age (r=-.414, p=.062). In contrast to Ramalho et al[17], the current analysis did not find correlations between change in body weight with change in CRP even though baseline BMI was correlated with CRP concentration.
CRP was not found to be correlated with any dietary variables at baseline, which is in contrast to other findings where correlations were found between CRP and omega-3 fatty acid intake in the Nurses’ Health Study [87]. Higher quintiles of omega-3 (which included an intake of 0.45 g/day) were associated with 29% lower levels of CRP (p=.007). In this study, however, the average baseline intake of omega-3 was greater at 1.8 g/day. Both studies report higher omega-3 intake decreasing CRP concentration with differences in the health of each population as the cohort of women in Lopez-Garcia were all healthy while this study involves morbidly obese subjects.

TNF\(\alpha\)

TNF\(\alpha\) level was lower at 3 and 6 months compared to baseline. There are discrepant findings for the effect of surgical induced weight loss on TNF\(\alpha\); some studies show no change and others have slight reductions post-surgery [51, 56, 61]. Study findings differ for a few possible reasons including amount of body weight lost and length of follow-up period. The fact that most of these studies resulted in reduction of BMI but persons still remained relatively obese suggests perhaps a threshold where a certain amount of adiposity must be lost before an effect on this cytokine could be seen [51].

Baseline TNF\(\alpha\) concentrations were not correlated with any of the baseline variables including body weight, BMI, co-morbidities, age, total number of medications and visceral fat volume nor was it correlated with any dietary variables. However, past research has shown significant dietary influences on TNF\(\alpha\) concentration in studies.
looking at both low-carbohydrate and low-fat diets with extreme energy restriction [70, 71] and omega-3 fatty acid ingestion [89].

IL-6

IL-6 concentration decreased by about 15% from baseline to 6 months; however this did not reach statistical significance. In contrast, Gletsu et al, Emery et al, Holdstock et al, and Lin et al saw significant decreases in IL-6 post-RYGB surgery [46, 52-54]. Interestingly, the level of IL-6 was significantly lower in these other studies than what was observed in this study. For example, Gletsu and coworkers presented IL-6 levels at baseline of 4.92 pg/ml, then decreased to 3.47 pg/ml by 6 months[52]. The differences between the earlier studies and the current results may be based on initial BMI and reduction in BMI [52-54] or length of follow-up [53]. Significant correlations between IL-6 and BMI concentration in obese individuals is supported by Bastard et al (r=.602, p<.005) suggesting circulating concentrations are reflected in part by adipose tissue[50].

IL-6 had a trend toward significance with total energy intake (r=.389, p=.090) but no statistical significance resulted between IL-6 and dietary variables, which is in contrast to the literature.

The aforementioned inflammatory biomarkers of leptin, adiponectin, CRP, TNFα and IL-6 have critical roles in the regulation of many obesity related processes. These processes have been shown to be altered through weight loss and possibly dietary variables. This study contributes to the literature in that it measures a number of pro- and anti-inflammatory factors within the same investigation, and it also looks at acute changes in these markers soon after surgery. Some obvious differences were not seen
with the current study when compared to others, possibly due in large part to a small study sample, a BMI level that remained in the morbid obese category after the 6-month follow-up period, and the length of the follow-up period being of shorter duration (only 6-months). Visceral fat volume was not correlated with any of the inflammatory biomarkers. This was surprising considering previous work demonstrating the high metabolic activity of this fat depot. Dysfunctional adipokine secretion in obese women supports the role for visceral fat as a predisposition to disease. After body weight reduction in Ziccardi et al, women with the greatest amount of visceral obesity saw the greatest decrease in cytokine levels and improvement in endothelial function[51].

Although this study resulted in weight loss, the participants remained in the category of morbid obesity suggesting if correlations between inflammation and visceral fat are evident, then a greater reduction in fat mass must be achieved. The small sample size in the study was also a cause for lack of statistical power to determine statistical significance. Further studies are needed to examine changes in all biomarkers across a longer follow-up period as control groups are added and differences in dietary variables over time period are assessed.


SCHOLASTIC VITA

PERSONAL INFORMATION

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EDUCATION

Graduate
2009-2011 Wake Forest University, Master of Science in Health and Exercise Science
Winston-Salem, NC

Undergraduate
2005-2009 Virginia Polytechnic Institute and State University (Virginia Tech)
Bachelor of Science in Human Nutrition, Foods and Exercise
Blacksburg, VA

RESEARCH EXPERIENCE

2010-Present Department of Health and Exercise Science at Wake Forest University
Research Assistant
Dr. Gary Miller, Supervisor

Pilot Exercise Training Following Weight Loss Surgery
The purpose of this randomized trial is to test the feasibility of a 6-month exercise training program for post-surgery patients and to determine the impact of the intervention on physical function, body composition, and muscle function. This study also aims to assess recruitment feasibility, participant adherence to the intervention, and the safety of the exercise intervention. Secondary outcomes are the short physical performance battery (physical function, SPPB), 400 meter walk time, leg muscle strength, lateral mobility, and self-reported disability.
Responsibilities: Project coordination; protocol development and revisions; IRB submission; data collection, management, and analyses; management other research staff; lead exercise interventionist.
PI: Gary Miller, PhD, Wake Forest University; Health and Exercise Science
Co-PI: Aldolfo Fernandez, MD, Wake Forest University Baptist Medical Center; Dept of Surgery

2010-Present **FIT Comparison Study**

**Childhood Obesity Epidemiology Study at WFUBMC**
The purpose of this study is to recruit and collect information to inform programs and interventions to prevent or treat childhood obesity and mental illness.
Responsibilities: Participant recruitment; Survey implementation; Data collection
PI: Ronny A. Bell, PhD, MS; Department of Epidemiology and Prevention, Division of Public Health Sciences, Wake Forest University

**IDEA**

**Intensive Diet and Exercise for Osteoarthritis**
2010-Present The purpose of this 18-month RCT is to investigate the effects of an exercise program and healthy dietary choices on weight loss and pain in overweight individuals with knee osteoarthritis.
Responsibilities: exercise intervention assistant; data entry
PI: Dr. Stephen Messier, PhD; Health and Exercise Science Wake Forest University

**Nitrates**

**Dietary Nitrates and Cognition in Older Adults**
2009-2010 The purpose of this study was to assess the memory and cognition of older individuals undergoing a 2 day low-nitrate diet versus a 2 day high nitrate diet and whether dietary choices have an effect on cognition.
Responsibilities: Food preparation and dissemination; data collection
PI: Dr. Gary Miller, Professor; Wake Forest University

**PROFESSIONAL EXPERIENCE**

2010-Present **Wake Forest Baptist Hospital ActionHealth, PLAN-B (Physical Activity, Lifestyle And Nutrition) Weight Loss Program**
Exercise Supervisor
Responsibilities: Supervise exercise sessions for employees of the hospital and implementation of particular behavior change strategies

2009-2010 **Healthy Exercise and Lifestyle ProgramS (HELPS)**
Responsibilities: Exercise physiologist (cardiovascular and muscular strength training); EKG operator; GXT; introduce and teach program protocols; monitor blood pressure and vital signs
Wake Forest University, Clinical Research Center, Winston-Salem, NC
WORK EXPERIENCE

2010-Present  Wake Forest University Student Athlete Services
  Tutor
  Classes: Health and Exercise Science 100, Undergraduate Statistics

2008-2009  Blacksburg Township Recreation Departments
  Responsibilities: Fitness orientation leader; personal trainer; fitness center scheduling, front desk operator, summer sports camp leader
  Blacksburg, VA

TEACHING EXPERIENCE

2010-Present  Wake Forest University Department of Health and Exercise Science

  HES 101: Exercise for Health
  Graduate Teaching Assistant, Course Instructor
  Responsibilities: Lecture/Lab instructor, exercise lab assessments

  HES 354 Assessment Techniques in Health Sciences undergraduate senior level lecture/lab course
  Exercise Lab Instructor
  Responsibilities: Metabolic cart operation for VO2 max tests; skin fold measurement, pulmonary function testing, blood pressure and heart rate testing, physical function testing, EKG instruction.

VOLUNTEER WORK

2010  Salem Towne Fitness Day Volunteer, April 2010
  Exercise Physiologist
  Responsibilities: Assessment of fitness levels in senior residents; lifestyle change counseling

2010  Crazy Running Club Volunteer Coach
  Responsibilities: Coach elementary and middle school track athletes

PROFESSIONAL EXPERIENCE

2010-2011  HES representative for Graduate Student Association

2010-2011  Translational Science Center (TSC) student-member, Wake Forest University
2010-2011  Member of Southeast American College of Sports Medicine (SEACSM)
2007-2009  Student Athlete Advisory Committee (SAAC) member, Virginia Tech

CERTIFICATIONS

2009  CPR and AED certified, American Heart Association
2009-present  Collaborative Institutional Training Initiative (CITI) Training in Human Subjects Research