Effects of food elimination, based on ALCAT testing, on inflammatory markers, body composition, and medical symptoms

Jodi Marie Hoppensteadt

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ABSTRACT

EFFECTS OF FOOD ELIMINATION, BASED ON ALCAT TESTING, ON INFLAMMATORY MARKERS, BODY COMPOSITION, AND MEDICAL SYMPTOMS

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Northern Illinois University, 2015
Judith Lukaszuk, Thesis Director

Background: Growing evidence suggests that systemic inflammation and obesity are associated. Lifestyle changes such as dietary modification may be beneficial in reducing obesity-related chronic inflammation and its related disease states. Conversely, inflammation may be exacerbated by food intolerance(s). Therefore, it is important to determine if dietary modifications have an effect on inflammatory markers, body composition or medical symptoms.

Objective: The purpose of the study was to determine whether an elimination diet plan, based on ALCAT testing, influenced inflammatory response, body composition and medical symptoms.

Methods: In this pre- and post-test double-blind experimental study, subjects were randomly assigned to the treatment group (n=87) or the control group (n=46). All participants followed an elimination diet for four weeks based on ALCAT testing protocol; treatment group eliminated foods to which they tested to be sensitive and the placebo group eliminated a random list of foods to which they tested not to be sensitive. Participants had pre- and post-test blood drawn to measure effects of elimination diet on inflammatory markers, anthropometric measurements were taken to measure body composition, and they completed medical symptom questionnaires.
In addition, participants kept food and exercise logs throughout the study. All hypotheses were tested using repeated-measures ANOVA.

**Results:** The results of this study found that there were significant differences over time between pre- and post-test values for both the treatment and control groups for the following measures: MPO \((p < .001)\), IL-6 \((p < .03)\), fat percent \((p < .001)\), BMI \((p < .001)\), and MSQ \((p < .001)\) values. Significant interaction effects between group assignment and testing time were also found on measures of SAA \((p < .035)\), LBM \((p < .004)\), BMI \((p < .004)\), and MSQ \((p < .001)\). However, there was not a significant difference between the treatment and placebo groups for the following indices: hs-CRP, MPO, SAA, TNF-α, GM-CSF, IFN-γ, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, LBM, fat percent, BMI, and MSQ scores.

**Conclusions:** These results suggest that the elimination of inflammatory foods had a positive impact on SAA, LBM, BMI, and MSQ.
EFFECTS OF FOOD ELIMINATION, BASED ON ALCAT TESTING,
ON INFLAMMATORY MARKERS, BODY COMPOSITION,
AND MEDICAL SYMPTOMS

BY

JODI MARIE HOPPENSTEADT
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A THESIS SUBMITTED TO THE GRADUATE SCHOOL
IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE
MASTER OF SCIENCE

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Thesis Director
Judith Lukaszuk
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CHAPTER 1

INTRODUCTION

Background

The prevalence of obesity has reached epidemic proportions worldwide.\textsuperscript{1,2} It affects not only the obese individual’s health but it has become a public health issue.\textsuperscript{3} Obesity, which is recognized as a disease, represents a bundle of co-morbidities, including Type 2 diabetes, insulin resistance, coronary heart disease, and nonalcoholic fatty liver disease.\textsuperscript{3-5} The cause(s) of obesity are still poorly defined, although diet and lifestyle appear to be among the primary driving forces, with inflammation as the most common indicator of obesity-related disorders.\textsuperscript{6}

Inflammation is a necessary part of the body’s response system to maintain homeostasis. When inflammation goes awry it continues past the point of homeostasis, creating an imbalance of inflammatory mediators. Consequently, when neutralization of the noxious insult fails, the inflammatory process persists, creating a state of chronic inflammation.\textsuperscript{7} The imbalance of inflammatory mediators being released from the injured cells produces an excess of pro-inflammatory cytokines, resulting in reduced anti-inflammatory cytokines and continued inflammation. This systemic inflammation can be partly attributed to the effects of dietary patterns and nutrient intake and the subsequent interaction of these nutrients on the various components of the inflammatory response system.\textsuperscript{6} On the other hand, chronic systemic inflammation may also result in impaired digestion and malabsorption of nutrients, which may
lead to decreased lean body mass and a compromised nutritional status. The role of inflammation in several disease conditions has been identified, particularly for rheumatoid arthritis (RA), inflammatory bowel disease (IBD), and asthma. The role of inflammation, especially when concurrent with obesity, in the development of non-communicable modifiable chronic diseases as well as in nutrition is not yet entirely understood.

According to one study, visceral adipose tissue increases as excess weight is gained. Circulating levels of many inflammatory cytokines including tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), high-sensitivity C-reactive protein (hs-CRP), myeloperoxidase (MPO), serum amyloid A (SAA), interleukin-8 (IL-8), interleukin-1-beta (IL-1β), and interleukin-10 (IL-10) change as visceral adipose tissue and obesity levels increase. Insulin resistance is strongly associated with the cascading events of pro-inflammatory cytokines hs-CRP, IL-6 and TNFα and may increase obesity status and fat production. It is suspected that this cycle becomes self-sustaining via a positive feedback loop in which increasingly greater levels of insulin resistance and adipose tissue are developed. Malnutrition, which can occur due to impaired digestion and absorption, metabolic disorder, and inadequate or excess intake of food, is also believed to facilitate the release of inflammatory cytokines; this can result in multiple deleterious events including protein deficiency (for those undernourished), diabetes, cardiovascular diseases, metabolic syndrome and some cancers.

Modification of dietary composition has been shown to have a modest effect on some inflammatory markers, although the markers do not change at the same rate or in the same direction with such modification. Consequently, lifestyle changes such as dietary and physical activity may be beneficial in reducing obesity-related inflammation and its related disease states. Conversely, inflammation may be exacerbated by food intolerance(s). In the presence
of an undiagnosed food intolerance, especially one with an undefined physiological mechanism, consumption of the unknown offending food will likely continue. This will inevitably perpetuate the inflammatory cascade cycle, leading to increased inflammation and the further release of pro-inflammatory cytokines.\textsuperscript{15}

**Statement of the Problem**

Evidence indicates that inflammatory markers, body composition values, and subjective ratings of well-being may be linked with dietary intake. However, this link has not been adequately explored in the current research. This study investigated the effect of food elimination on inflammatory markers, body composition values, and medical symptom questionnaire (MSQ) scores.

**Independent Variables**

Participants followed a four-week food elimination diet based on either a list derived from ALCAT test results (treatment group) or an alternative list of food excluding foods identified by ALCAT test results for those in the (placebo group).

**Dependent Variables**

Body composition was measured pre- and post-test for this four-week study to determine differences in lean body mass (LBM), body fat percent, and body mass index (BMI). Total body inflammatory markers such as high-sensitivity C-reactive protein (hs-CRP), myeloperoxidase (MPO), serum amyloid A (SAA), tumor necrosis factor-alpha (TNF-\(\alpha\)), granulocyte macrophage colony-stimulating factor (GM-CSF), interferon gamma (IFN-\(\gamma\)), interleukin-1-beta (IL-1\(\beta\)),
interleukin-2 (IL-2), interleukin-4 (IL-4), interleukin-5 (IL-5), interleukin-6 (IL-6), interleukin-8 (IL-8), and interleukin-10 (IL-10) were measured by drawing blood at Northern Illinois University nutrition lab and were sent to Cell Science Systems for testing these markers. All subjects filled out the MSQ pre- and post-study on the same day body composition was measured.

**Hypotheses**

The following hypotheses were investigated during the four-week elimination program:

1. Subjects randomly assigned to the ALCAT treatment group will have lower hs-CRP at the end of the four-week study compared to those receiving the placebo.

   *Independent Variable:* Food elimination plan

   *Attributes:* Elimination of foods from the diet determined by ALCAT blood testing; varies by person

   *Control:* Placebo group will receive an alternative food elimination plan excluding foods identified by ALCAT blood testing

   *Dependent Variable:* Total body inflammatory markers

   *Attribute:* Comparison of hs-CRP blood levels pre- and post-study

2. Subjects randomly assigned to the ALCAT treatment group will have lower MPO at the end of the four-week study period compared to those receiving the placebo.

   *Independent Variable:* Food elimination plan

   *Attributes:* Elimination of foods from the diet determined by ALCAT blood testing; varies by person
Control: Placebo group will receive an alternative food elimination plan excluding foods identified by ALCAT blood testing

*Dependent Variable:* Total body inflammatory markers

*Attribute:* Comparison of MPO blood levels pre- and post-study

3. Subjects randomly assigned to the ALCAT treatment group will have lower SAA at the end of the four-week study period compared to those receiving the placebo.

*Independent Variable:* Food elimination plan

*Attributes:* Elimination of foods from the diet determined by ALCAT blood testing; varies by person

Control: Placebo group will receive an alternative food elimination plan excluding foods identified by ALCAT blood testing

*Dependent Variable:* Total body inflammatory markers

*Attribute:* Comparison of blood SAA levels pre- and post-study

4. Subjects randomly assigned to the ALCAT treatment group will have lower TNF-α at the end of the four-week study period compared to those receiving the placebo.

*Independent Variable:* Food elimination plan

*Attributes:* Elimination of foods from the diet determined by ALCAT blood testing; varies by person

Control: Placebo group will receive an alternative food elimination plan excluding foods identified by ALCAT blood testing

*Dependent Variable:* Total body inflammatory markers

*Attribute:* Comparison of blood TNF-α levels pre- and post-study
5. Subjects randomly assigned to the ALCAT treatment group will have lower GM-CSF at the end of the four-week study period compared to those receiving the placebo.

*Independent Variable:* Food elimination plan

*Attributes:* Elimination of foods from the diet determined by ALCAT blood testing; varies by person

*Control:* Placebo group will receive an alternative food elimination plan excluding foods identified by ALCAT blood testing

*Dependent Variable:* Total body inflammatory markers

*Attribute:* Comparison of blood GM-CSF levels pre- and post-study

6. Subjects randomly assigned to the ALCAT treatment group will have lower IFN-γ at the end of the four-week study period compared to those receiving the placebo.

*Independent Variable:* Food elimination plan

*Attributes:* Elimination of foods from the diet determined by ALCAT blood testing; varies by person

*Control:* Placebo group will receive an alternative food elimination plan excluding foods identified by ALCAT blood testing

*Dependent Variable:* Total body inflammatory markers

*Attribute:* Comparison of blood levels IFN-γ pre- and post-study

7. Subjects randomly assigned to the ALCAT treatment group will have lower IL-1β at the end of the four-week study period compared to those receiving the placebo.

*Independent Variable:* Food elimination plan

*Attributes:* Elimination of foods from the diet determined by ALCAT blood testing; varies by person
Control: Placebo group will receive an alternative food elimination plan excluding food identified by ALCAT blood testing

*Dependent Variable:* Total body inflammatory markers

*Attribute:* Comparison of blood levels IL-1β pre- and post-study

8. Subjects randomly assigned to the ALCAT treatment group will have lower IL-2 at the end of the four-week study period compared to those receiving the placebo.

*Independent Variable:* Food elimination plan

*Attributes:* Elimination of foods from the diet determined by ALCAT blood testing; varies by person

Control: Placebo group will receive an alternative food elimination plan excluding foods identified by ALCAT blood testing

*Dependent Variable:* Total body inflammatory markers

*Attribute:* Comparison of blood IL-2 levels pre- and post-study

9. Subjects randomly assigned to the ALCAT treatment group will have lower IL-4 at the end of the four-week study period compared to those receiving the placebo.

*Independent Variable:* Food elimination plan

*Attributes:* Elimination of foods from the diet determined by ALCAT blood testing; varies by person

Control: Placebo group will receive an alternative food elimination plan excluding foods identified by ALCAT blood testing

*Dependent Variable:* Total body inflammatory markers

*Attribute:* Comparison of blood IL-4 levels pre- and post-study
10. Subjects randomly assigned to the ALCAT treatment group will have lower IL-5 at the end of the four-week study period compared to those receiving the placebo.

*Independent Variable*: Food elimination plan

*Attributes*: Elimination of foods from the diet determined by ALCAT blood testing; varies by person

Control: Placebo group will receive an alternative food elimination plan excluding foods identified by ALCAT blood testing

*Dependent Variable*: Total body inflammatory markers

*Attribute*: Comparison of blood IL-5 levels pre- and post-study

11. Subjects randomly assigned to the ALCAT treatment group will have lower IL-6 at the end of the four-week study period compared to those receiving the placebo.

*Independent Variable*: Food elimination plan

*Attributes*: Elimination of foods from the diet determined by ALCAT blood testing; varies by person

Control: Placebo group will receive an alternative food elimination plan excluding foods identified by ALCAT blood testing

*Dependent Variable*: Total body inflammatory markers

*Attribute*: Comparison of blood IL-6 levels pre- and post-study

12. Subjects randomly assigned to the ALCAT treatment group will have lower IL-8 at the end of the four-week study period compared to those receiving the placebo.

*Independent Variable*: Food elimination plan

*Attributes*: Elimination of foods from the diet determined by ALCAT blood testing; varies by person
Control: Placebo group will receive an alternative food elimination plan excluding foods identified by ALCAT blood testing

**Dependent Variable:** Total body inflammatory markers

**Attribute:** Comparison of blood IL-8 levels pre- and post-study

13. Subjects randomly assigned to the ALCAT treatment group will have higher IL-10 at the end of the four-week study period compared to those receiving the placebo.

**Independent Variable:** Food elimination plan

**Attributes:** Elimination of foods from the diet determined by ALCAT blood testing; varies by person

Control: Placebo group will receive an alternative food elimination plan excluding foods identified by ALCAT blood testing

**Dependent Variable:** Total body inflammatory markers

**Attribute:** Comparison of blood IL-10 levels pre- and post-study

14. Subjects randomly assigned to the ALCAT treatment group will have higher lean body mass at the end of the four-week study period compared to those receiving the placebo.

**Independent Variable:** Food elimination plan

**Attributes:** Elimination of foods from the diet determined by ALCAT blood testing; varies by person

Control: Placebo group will receive an alternative food elimination plan excluding foods identified by ALCAT blood testing

**Dependent Variable:** Fat mass

**Attribute:** LBM measurements assessment using bioelectrical impedance (Biospace)
15. Subjects randomly assigned to the ALCAT treatment group will have lower fat percent at the end of the four-week study period compared to those receiving the placebo.

*Independent Variable:* Food elimination plan

*Attributes:* Elimination of foods from the diet determined by ALCAT blood testing; varies by person

*Control:* Placebo group will receive an alternative food elimination plan excluding foods identified by ALCAT blood testing

*Dependent Variable:* Fat percent

*Attribute:* Fat percent measurement assessed using bioelectrical impedance (Biospace)

16. Subjects randomly assigned to the ALCAT treatment group will have lower BMI at the end of the four-week study period compared to those receiving the placebo.

*Independent Variable:* Food elimination plan

*Attribute:* Elimination of foods from the diet determined by ALCAT blood testing; varies by person

*Control:* Placebo group will receive an alternative food elimination plan excluding foods identified by ALCAT blood testing

*Dependent Variable:* BMI

*Attribute:* Comparison of BMI pre- and post-study

17. Subjects randomly assigned to the ALCAT treatment group will have lower MSQ scores at the end of the four-week study period compared to those receiving the placebo.

*Independent Variable:* Elimination of foods from the diet determined by ALCAT blood testing; varies by person
Control: Placebo group will receive an alternative food elimination plan excluding foods identified by ALCAT blood testing

*Dependent Variable:* MSQ

*Attribute:* Comparison of MSQ scores pre- and post-study
CHAPTER 2

REVIEW OF LITERATURE

Introduction

The prevalence of obesity has increased worldwide; in fact, it has almost doubled since 1980.\textsuperscript{16,17} More than 1.4 billion adults over the age of twenty are overweight, and more than 10% of the world’s and 30% of the U.S. population is obese.\textsuperscript{16} According to the World Health Organization, 65% of the world’s population lives in countries in which more deaths are linked to obesity than to malnourishment due to being underweight.\textsuperscript{16} Obesity’s burden on health spans multiple organ systems and diseases, including inflammatory disorders such as coronary heart disease, insulin resistance, and non-alcoholic fatty liver disease.\textsuperscript{18} A preponderance of research has demonstrated a clear link between inflammation and the occurrence of obesity and disease.\textsuperscript{19}

Inflammation is a naturally occurring, short-term, and protective response of the immune system to harmful stimuli, whether physical, chemical, or biological, in an effort to re-establish homeostasis.\textsuperscript{7} If homeostasis is not reached, chronic inflammation will persist. In obese individuals, it has been demonstrated that chronic inflammation leads to systemic inflammation accompanied by a continuous activation of the innate immune system.\textsuperscript{7} Simultaneous healing and destruction of tissue from the inflammatory process occurs as a result of this continuous, chronic inflammation. Uncontrolled inflammation may result in amplification of underlying disease states.\textsuperscript{20}
Adipocytes have the ability to secrete more than 75 adipokines including pro-inflammatory cytokines.\textsuperscript{21} It has been well established by numerous studies that as adipose tissue increases so do the markers of inflammation, including hs-CRP, IL-1\(\beta\), IL-6, TNF-\(\alpha\), and MPO. The cascade effect of increased pro-inflammatory cytokines leads to insulin resistance, resulting in increased adipose tissue, which then releases more pro-inflammatory cytokines and continues in this positive feedback loop, increasing adipose tissue.\textsuperscript{20,21} It remains unclear as to the original trigger for this feedback loop that occurs with obesity.\textsuperscript{20} It has been theorized that an imbalanced dietary intake may be the origin of this cascading effect.\textsuperscript{20,22}

The link between diet quality and inflammatory markers is beginning to emerge in current research and further study is needed, especially those investigating changes in dietary markers over time.\textsuperscript{22} In numerous studies, improved diet quality was inversely associated with hs-CRP and IL-6.\textsuperscript{6,22} Several studies have found a statistically significant association between red meat consumption and hs-CRP and IL-6.\textsuperscript{22} One study of particular significance, the WOMAN (Women on the Move Through Activity and Nutrition) study, concluded that levels of IL-6 were predictive of abdominal obesity and insulin resistance while TNF-\(\alpha\) and hs-CRP were predictive of triglyceride levels and insulin resistance.\textsuperscript{23} Diets rich in fiber, fruits, vegetables, \(\omega\)-3 polyunsaturated fatty acids, and magnesium are associated with decreased serum inflammatory markers, while diets low in these foods correlated with increased serum inflammatory markers.\textsuperscript{6} According to Galland, any attempt to create a diet for optimal health must consider how the diet would impact systemic inflammation.\textsuperscript{6}

The use of exercise along with diet to reduce inflammation has not been clearly illustrated, especially in obese individuals.\textsuperscript{24-26} Nonetheless, it is well known that consumption of diets high in fruits, and vegetables, and fiber and low in refined sugars, and simple
carbohydrates, and saturated and trans fats can reduce levels of inflammatory cytokines. In a study conducted by Fisher and colleagues, it was shown that weight loss was associated with significant reduction of inflammatory markers and that when aerobic or resistance exercise was added to the diet regimen, the outcomes were not altered. On the contrary, another study detected a reduction in circulating pro-inflammatory monocytes following exercise. What is not known at this time is if removal of food intolerances will have any effect on levels of inflammatory markers. It is thought that the levels of inflammatory markers hs-CRP, TNF-α, MPO, SAA, IL-1β, IL-6, GM-CSF, IFN-γ, IL-2, IL-4, IL-5, and IL-8 will decrease and IL-10 will increase, thereby reducing systemic inflammation, insulin resistance, and obesity.

Inflammation can be exacerbated by food intolerances. It has been well established that individuals with lactose intolerance or celiac disease can become asymptomatic with strict avoidance of intolerable foods. Research has shown that removing food intolerances from the diets of patients with RA, IBD, and Crohn’s disease will result in a reduction of inflammation and may attenuate many of the symptoms. If food intolerance remains undiagnosed, the inflammatory cascade may continue, leading to increased inflammation, amplified levels of pro-inflammatory cytokines and increased visceral fat tissue.

**Food Intolerances**

Adverse food reaction is a broad phrase that describes any abnormal clinical response associated with the ingestion of a food or food additive. An adverse food reaction can be classified as either food allergy or food intolerance. Boyce and colleagues define a food allergy as “an adverse health effect arising from a specific immune response that occurs reproducibly on exposure to a given food”. (p. 11) Food allergy reactions are immunoglobulin E
(IgE) mediated, but cell-mediated mechanisms may also be involved in these reactions.\textsuperscript{28} Conversely, food intolerance refers to a large variety of non-immunologic reactions to ingested foods.\textsuperscript{28} These non-immune-mediated reactions are due to a variety of mechanisms including metabolic, pharmacologic, toxic, and undefined mechanisms.\textsuperscript{28} The symptoms of food intolerance are varied; they may be delayed, may or may not be reproducible, and are usually quantity dependent.\textsuperscript{28,29}

**Prevalence of Food Allergies and Food Intolerance in the US**

Food allergy affects approximately 5% of adults and 8% of children and appears to be increasing in prevalence in industrialized nations.\textsuperscript{28,30,31} In the ten-year period leading up to 2007, the prevalence of reported food allergy increased 18% among the population under eighteen in the US.\textsuperscript{31} There are eight foods that are responsible for 90% of all allergic reactions: eggs, milk, soy, peanut, tree nut, fish, shellfish and wheat.\textsuperscript{31}

Food intolerance is more common than food allergy; at least 25% of people in industrialized nations believe that they have a food intolerance. Many more cases of food intolerance go undiagnosed due to delayed symptoms and improper testing. Research shows that up to 80% of the US population report symptoms associated with food intolerances.\textsuperscript{28,32}

**Cause of Food Intolerance**

Recent evidence has pointed to several primary reasons for food intolerances, including fatty acid imbalance, inability of liver to detoxify chemicals in foods, inability of the body to process pharmacologic agents, gut membrane dysfunction, enzyme deficiencies, and metabolic disorders.\textsuperscript{15,28} Enzyme deficiencies and metabolic disorders that cause food intolerances such as
lactose intolerances are likely to be lifelong and persistent, although these intolerances may be modified or even attenuated by addressing underlying issues and other food intolerances. The remaining triggers are likely linked to unhealthy food in Western diets, and symptoms can be significantly reduced by removing foods that elicit a response. In one study, removing the offending food resulted in weight normalization, reduction in inflammation, and resolution of inflammation-associated health risks. Fatty acid imbalances appear to be caused by an excess of corn and soy in the diet, which are high in omega-6 fatty acids and lead to increased inflammation. Additionally, the liver cannot easily detoxify over-consumption of naturally occurring and man-made chemicals found in today’s foods, and the immune system is then activated to compensate. The human body is unable to fully process large quantities of tyramine, caffeine, histamine, and alcohol; this can result in pharmacological reactions. Furthermore, the lining of the digestive system forms a natural selective barrier; when poor diet, antibiotics, steroids, or even infection compromise this lining it results in altered permeability, allowing substances that normally cannot leave the digestive tract to exit the digestive system. This “leaky gut syndrome” has been shown to correlate with food intolerance; however, the exact mechanisms by which this phenomenon occurs remain unclear.

**Symptoms of Food Intolerances**

The symptoms of food intolerances are many and varied. The symptoms may mimic that of IgE-mediated food allergies but may not be reproducible and usually have a delayed onset. Common symptoms include, but are not limited to, bloating, flatulence, diarrhea, abdominal pain, loose stools, malabsorption, nausea, dysgeusia, hives, lethargy, failure to thrive, migraine headaches, urticaria, eczema, rash, and inflammation. Inflammation plays a key role in the
intolerance symptoms listed above as well as in the development of autoimmune disease.\textsuperscript{5} Inflammation associated with food intolerance reactions may lead to increased weight.\textsuperscript{27} This inflammation is due to several factors, particularly the release of some hormones and cytokines including IL-6 and TNF–\(\alpha\), which are associated with weight gain and obesity.\textsuperscript{34}

Decreased absorption of nutrients due to poor digestion results in impaired nutritional status and symptoms of food intolerance. For instance, research has shown that inflammation occurring in the small intestine impairs the digestive and absorptive functions, creating altered nutritional outcomes.\textsuperscript{35} This phenomenon is exemplified by various disorders of the digestive tract such as ulcerative colitis, Crohn’s disease, and celiac disease. All three of these disorders reduce absorption by inflammation of the whole or part of the digestive tract resulting in increased movement of food and nutrients and decreased absorption.\textsuperscript{5} All of these disorders are affected by food intolerances and exhibit increased pro-inflammatory cytokine activity.\textsuperscript{5,36}

**Inflammatory Markers**

There are numerous cytokines that are responsible for the inflammatory cascades. Cytokines are produced by many types of cells and are responsible for diverse responses; they may also exhibit overlapping actions. Elevated levels of pro-inflammatory cytokines and a reduction of anti-inflammatory cytokines are traits of chronic low-grade inflammation; such low-grade inflammation is evidenced in obese patients.\textsuperscript{13} The cytokines IL-6, IL-1, and TNF-\(\alpha\) are elevated in most inflammatory states and thus are useful biomarkers of inflammation.\textsuperscript{37} Pro-inflammatory and anti-inflammatory cytokines are responsible for the regulation of adipose tissue, which is found in excess in obese patients.\textsuperscript{13} When homeostasis is disrupted in adipose
tissue, this results in an increase of pro-inflammatory cytokines and reduced anti-inflammatory cytokines and, consequently, increased production of adipose tissue.\textsuperscript{7}

**High-Sensitivity C-Reactive Protein**

Hs-CRP is a protein that is synthesized by hepatocytes in the presence of inflammation due to many factors, including obesity, and is regulated by a number of cytokines (primarily IL-6).\textsuperscript{38,39} Hs-CRP is a marker of systemic inflammation and is associated with coronary heart disease and metabolic syndrome; it is also known to rapidly increase within hours of exposure to inflammatory stimulants.\textsuperscript{18,39} Hs-CRP is highly positively associated with BMI; it is reflective of the inflammation process caused by excessive body fat and is directly correlated with multiple features of metabolic syndrome.\textsuperscript{18,40,41} Fat accumulation in the liver leads to overproduction of low-density lipoprotein (LDL) and in association with IL-6 results in the overproduction of hs-CRP.\textsuperscript{7} Elevated levels of hs-CRP are a risk factor for several non-communicable chronic diseases such as diabetes, cardiovascular disease, cancer, and metabolic syndrome.\textsuperscript{42} Hs-CRP appears to be one of the most studied biomarkers of inflammation. Nguyen and colleagues examined hs-CRP across BMI weight categories and found hs-CRP levels almost doubled with each increase in weight category.\textsuperscript{38} One study found inflammation and metabolic mechanisms were associated with heart failure, diabetes mellitus and/or metabolic syndrome as well increased levels of hs-CRP.\textsuperscript{39} Another study found that a pro-inflammatory diet was associated with elevated levels of hs-CRP and glucose intolerance.\textsuperscript{42} Several studies have consistently shown that females have higher levels of hs-CRP compared with males, and they also have higher percentage body fat.\textsuperscript{39} While hs-CRP is strongly associated with obesity, the exact link between obesity and inflammation remains unclear.\textsuperscript{39}
Myeloperoxidase

MPO is a heme-enzyme that is one of the major neutrophil proteins.\textsuperscript{11,43} MPO is stored in large amounts inside granules of neutrophils; upon activation, MPO is released into extracellular spaces. MPO exhibits strong oxidative activity; it interferes with cellular function and contributes to tissue injury.\textsuperscript{11,43} Kothari and colleagues demonstrated that sepsis patients had increased levels of MPO when compared with the placebo group. This extracellular MPO has been detected in a variety of acute and chronic inflammatory conditions and is used as a biomarker of chronic and acute inflammation as well as of sepsis.\textsuperscript{11,43}

Serum Amyloid A

SAA is an acute-phase protein predominantly produced by the liver and mainly associated with high-density lipoproteins (HDL). HDL is the main transporter of SAA, and it is proffered that SAA is delivered to infection sites where it expresses cytokine–like properties.\textsuperscript{12} In immune cells, SAA induces inflammatory responses.\textsuperscript{12} In the plasma, elevated levels of SAA are present in chronic inflammatory disorders such as atherosclerosis, diabetes mellitus, obesity and rheumatic diseases.\textsuperscript{12,44} One study concluded that SAA has direct effect on adipocytes in humans and is associated with characteristics of inflammation and insulin resistance in adipose tissues in obese patients.\textsuperscript{12} Research has shown that diet quality is associated with SAA and hs-CRP; healthy diets correlate with decreased SAA plasma and lower hs-CRP levels.\textsuperscript{45} In other words, a healthier diet may result in reduced insulin resistance and adipose tissue and, therefore, a reduction in inflammatory markers, specifically SAA and hs-CRP.
**Tumor Necrosis Factor-Alpha**

It has been suggested that TNF-α is one of the most important facilitators of inflammation and is secreted by macrophages that are infiltrating adipose tissue. Adiponectin, an anti-inflammatory protein that increases insulin sensitivity and protects from chronic inflammation, is known to have an inverse association with TNF-α. When TNF-α levels increase, adiponectin levels decrease, increasing inflammation, glucose intolerance, and obesity. According to Mirza et al., increased levels of TNF-α have historically been correlated in numerous studies with obesity, increased insulin resistance, and clinical diagnosis of diabetes mellitus. In one study investigating the relationship of low-grade inflammation and levels of glucose control, levels of TNF-α, as well as IL-6 and IL-8, were most significantly elevated when hemoglobin A1c values were >6.5%. In another study, Wang and colleagues examined the interaction of TNF-α and adiponectin and found that in obese patients the pro-inflammatory response of TNF-α served as feedback control of these energy stores. Furthermore, these researchers posited that chronic inflammation may signify a body’s attempt to motivate energy expenditure in order to control adiposity.

**Granulocyte Macrophage Colony-Stimulating Factor**

GM-CSF is a cytokine that stimulates stem cells to produce granulocytes and monocytes, which, when migrating into tissue, have the ability to mature into macrophages and become a part of the inflammation cascade. GM-CSF is known to be strong instigator of mucosal inflammation and inflammatory cell recruitment in many disease settings. Elevated tissue levels of GM-CSF have been identified in many inflammatory conditions, including obesity. Results of recent trials suggest that inhibiting GM-CSF may hold promise as a therapeutic...
treatment for many autoimmune and inflammatory conditions.\textsuperscript{49} A study by Parks et al. demonstrated that GM-CSF, as a monocyte activator, is in part responsible for the increased levels of IL-6 in vitro; moreover, elevated levels of IL-6 are characteristic of chronic inflammatory diseases.\textsuperscript{1,50}

Interferon-Gamma

IFN-\(\gamma\) is a cytokine that is involved in immune and inflammatory responses. IFN-\(\gamma\) is reported at highly elevated levels in patients with active celiac disease.\textsuperscript{36} In a study assessing immune alterations in obese children, IFN-\(\gamma\) was utilized as a marker of the pro-inflammatory state of pediatric obesity.\textsuperscript{51} Thus, IFN-\(\gamma\) may be a useful marker of systemic inflammation, as elevated levels of IFN-\(\gamma\) appear to be characteristic of chronic inflammatory disease.\textsuperscript{1}

Interleukin-1-Beta

IL-1\(\beta\) is a cytokine produced by activated macrophages and fibroblasts. Parks et al. found that IL-1\(\beta\) induces inflammation by activating the production of IL-6 and IL-8. One study has demonstrated that in the case of non-allergic asthma, the inflammatory response activities included elevated levels of IL-1\(\beta\).\textsuperscript{52} Another study examining inflammatory response to biomaterial reported increased amounts of IL-1\(\beta\) in a co-culture that replicated soft tissues of inflammatory host materials.\textsuperscript{50}

Interleukin-2

Sharma et al.\textsuperscript{53} regard pro-inflammatory cytokine and immune regulator IL-2 as “a two-faced master regulator of autoimmunity” (p. 91). Recent research has demonstrated that IL-2 has
the ability to react as both a pro- and an anti-inflammatory agent. Furthermore, IL-2 has proven to be effective in reversing the effects of diabetes mellitus in non-obese diabetic mice. In another study, IL-2 therapy was shown to effectively induce regression of thyroid cancer, making this a potentially therapeutic instrument. IL-2 has also been proven to function as a pro-inflammatory cytokine by regulating a whole host of genes involved in the inflammation processes. While the exact roles of IL-2 in regulating levels of inflammation are unclear, elevated levels of IL-2 may be a useful indicator of the existence of inflammation.

**Interleukin-4**

IL-4 is a multifunctional cytokine that is produced mainly by activated T-cells. IL-4 has been shown to cause inflammation and fibrosis independently or in combination with other processes. Conversely, a variant of IL-4 called IL-4δ2 is independently active in causing immune inflammation. Both variants are detectable at elevated levels in asthma patients, although the levels of each variant peak at differing times; this phenomenon suggests that these two proteins act independently and could be used as possible markers for inflammation, especially in asthma.

**Interleukin-5**

Research has demonstrated that IL-5 regulates differentiation, maturation, activation, tissue recruitment, and survival of eosinophils. It appears to be up-regulated in cases of ulcerative colitis. In a study assessing IL-5 and submucosa in obese asthma patients, IL-5 levels were found to be elevated. Furthermore, anti-IL-5 therapies have also successfully been utilized to prevent asthma exacerbations by reducing airway inflammation. Therefore, IL-5 could
possibly be used as a marker of systemic inflammation as well as in therapeutic ways to improve the status of inflammatory diseases.

**Interleukin-6**

IL-6 is a pro-inflammatory cytokine and is produced by various cells, including muscle cells, white blood cell, hepatocytes, and adipocytes. Elevated levels of IL-6 are characteristic of chronic inflammatory conditions. A recent study has described an inverse association between plasma concentrations of IL-6 and α-linolenic acid intake. IL-6, like several other cytokines, has the ability to exhibit anti-inflammatory activities. It is involved in the regulation of metabolism and bone maintenance and has multiple neurological roles.

**Interleukin-8**

IL-8 is a chemokine produced by endothelial cells responding to inflammatory stimuli. Recent studies have shown that chemokines such as IL-8 are also secreted by adipocytes and adipose tissue; as adipose tissue increases, more IL-8 is secreted. IL-8 is positively correlated with obesity, BMI, and LDL cholesterol and is negatively correlated with HDL cholesterol, thus making it a predictor of atherosclerosis and chronic inflammatory diseases.

**Interleukin-10**

IL-10 is an anti-inflammatory cytokine that is produced primarily by monocytes and is capable of inhibiting IFN-γ, TNF-α, IL-2, IL-3, and GM-CSF. Regulatory T-cells release IL-10 in an effort to suppress hypersensitivity to antigens found in foods. When IL-10 is present in low levels, it is associated with metabolic syndrome and diabetes mellitus; it is also negatively
associated with BMI percentage of body fat mass.\textsuperscript{1} In addition, IL-10 appears to limit atherosclerosis.\textsuperscript{62} IL-10, unlike other interleukins previously discussed here, tends to down-regulate inflammation and immune responses in order to preserve homeostasis.\textsuperscript{1}

**Body Composition**

Body composition is used to describe percentages of water, fat, muscle and bone in human bodies and is used with several other assessment factors to describe one’s overall health.\textsuperscript{5} Anthropometric methods are used to assess body composition and measure the body based on two components: fat mass and fat free mass.\textsuperscript{63} Hs-CRP and IL-6 have been shown to be independently associated with BMI, and participants with higher BMIs had significantly higher levels of hs-CRP and IL-6.\textsuperscript{64} There are multiple tools used to determine body composition, but for the purpose of this study, measurements will focus on fat percent, lean body mass, and BMI.

**Percent Body Fat**

Body fat percent is the total mass of fat in the body divided by the total mass of the body and is expressed as a percentage.\textsuperscript{65} Body fat is essential to life and is the way in which the body stores energy. BF is usually considered a more accurate measurement of adiposity.\textsuperscript{65}

**Lean Body Mass**

Lean body mass (LBM) is calculated by subtracting body fat weight from total body weight and is expressed in kg.\textsuperscript{5} It is most commonly used to assess metabolic disorders and is useful for determining pharmaceutical dosing.\textsuperscript{27} Research shows that LBM is a more useful measure of body composition as LBM is more relevant than body fat in metabolic reactions.\textsuperscript{65}
Body Mass Index

Body mass index (BMI) is weight (in kilograms) divided by height squared (in meters) and is used in the healthy population to measure fatness, in the undernourished population as an indicator of malnutrition and in the over-nourished populations as a measurement and classification for obesity. The relationship between fatness and BMI is less accurate in individuals with an increased muscle mass and in individuals with edema or ascites.

Bioelectrical Impedance Analysis

Bioelectrical impedance analysis (BIA) assesses body composition based upon the principle that lean tissue, as opposed to fatty tissue, has a higher electrical conductivity and lower impedance. BIA can be affected by dehydration and electrolyte imbalances and is best used on healthy, hydrated individuals. Benefits of BIA are that it is safe, noninvasive, portable, and results are almost immediate. For most accurate results, individuals should abstain from alcohol for 24 hours and exercise for 6 hours before assessment; extreme obesity may also affect the reliability of BIA.

Medical Symptom Questionnaire

The MSQ is a questionnaire assessing 71 cognitive and physiological systems correlated with general health and wellness that uses a 5-point Likert scale to indicate severity of symptoms. The broad range of symptoms that are associated with food intolerances highly correspond to the symptoms listed on the MSQ. The MSQ is a subjective, self-report of symptoms that was used to compare pre- and post-test medical symptoms in this study.
**ALCAT**

ALCAT, or antigen leukocyte antibody test, is a test developed by Cell Science Systems of Deerfield Beach, Florida, to measure adverse reactions to dietary substances (non-IgE-mediated reactions). It has been available for more than 25 years and has the ability to identify non-IgE reactions to over 350 foods, chemicals, and other substances. It is a functional response test and captures reactions of many substances in their final pathway. It frequently reveals significant reactions that are not typical of allergy but are representative of food intolerances. It measures cellular inflammation as a result of exposure to substances that are consumed in most diets. This cellular inflammation is linked to chronic health issues including obesity, diabetes, and skin, heart, joint, and digestive conditions.

**History and Purpose**

ALCAT testing uses blood samples to measure cellular sensitivity reactions to more than 350 foods, chemicals, and herbs. The ALCAT test is used to identify delayed inflammatory reactions related to food and cell-mediated food intolerances. These inflammatory reactions have been linked to chronic health issues like gastrointestinal disorders, obesity, diabetes, and cardiovascular disease, as well as to migraines, aching joints, fatigue, and eczema. ALCAT testing recommends the elimination of foods specific to test results indicating sensitivity reactions as well as to clinical observation of reactivity. The removal of the problematic food should result in improvements in body composition and weight as well reduced systemic inflammation.
ALCAT Testing

ALCAT testing is used in the identification of substances that trigger cellular inflammation. It is a functional cellular test that measures reactions of the innate immune system to food, chemicals, herbs and other substances. This test uses the ROBOCat II, an instrument that is designed to measure the size of leukocytes by determining changes in electrical resistance produced by suspending cells in a conductive liquid. The level of food intolerance is determined by comparing the size and volume of the leukocytes before testing to the size and volume of the leukocytes after post testing. Determined by the level of reaction of the leukocytes, the identified food items are categorized into four distinct areas: no reaction, mild reaction, moderate reaction, and severe reaction. Several studies have provided compelling evidence that following a diet based on ALCAT results improves body composition and reduces self-reported disease symptoms.

Food Elimination

The purpose of food elimination is to remove food items from the diet that are suspected to cause adverse reactions for a specific period of time. This time period is usually 4-12 weeks in order to allow the body to recover from symptoms that are caused by the adverse reactions. It is recommended that food elimination diets be planned on an individual basis. The goal of this study is to eliminate food based on the results of individual ALCAT testing. It is hypothesized that the removal of problematic food from the diet should result in improved gastrointestinal function, nutritional status, and increased liver detoxification.
Conclusion

Research shows that obesity is associated with inflammation, but the exact mechanism linking them remains to be elucidated.\textsuperscript{38,39,59} Numerous studies report that quality of food plays a significant role in diet-related inflammation.\textsuperscript{6,42} It is estimated that almost 80% of Americans have food intolerances, many of which go undiagnosed due to delayed onset of symptoms or lack of recognition of food intolerance symptoms.\textsuperscript{32} Pursuing this further, it is important to determine if dietary composition is the missing link connecting obesity with an inflammatory response. The purpose of this study was to determine what effects food elimination has on inflammation markers, body composition, and MSQ scores using ALCAT testing.

Specifically, this study was designed to answer the following research questions: 1) Is there a significant difference between pre-test and post-test values in the area of inflammatory markers, body composition values and MSQ scores? 2) Is there a significant difference between the experimental and placebo groups in the areas of inflammatory markers, body composition values and MSQ scores? 3) Is there a relationship between group assignment and testing time in the areas of inflammatory markers, body composition values and MSQ scores?
CHAPTER 3

METHODS

Participants

One hundred fifty subjects were selected to take part in this study. Subjects were recruited through social media, by posting flyers and advertisements in local newspapers, on campus, and in fitness centers within a 50 mile radius of the university’s campus (Appendix A). Individuals interested in participating in the study were given a Disease Symptom Inventory (DSI) for eligibility and met the study criteria by rating at least two or more of the symptoms listed on the DSI as “somewhat severe effect” to participate in the study. All subjects completed a DSI (Appendix B), a Medical Symptom Questionnaire (MSQ) (Appendix C), and an ALCAT screening form (Appendix D). They also completed a three-day food record that includes two weekdays and one weekend day (Appendix E), kept an exercise log (Appendix F), and had 25 mL of blood drawn, without fasting, to assess food intolerances and the baseline inflammatory markers: granulocyte macrophage colony-stimulating factor (GM-CSF), interferon gamma (IFN-γ), interleukin-1-beta (IL-1β), interleukin-2 (IL-2), interleukin-4 (IL-4), interleukin-5 (IL-5), interleukin-6 (IL-6), interleukin-8 (IL-8), interleukin-10 (IL-10), tumor necrosis factor-alpha (TNF-α), myeloperoxidase (MPO), high-sensitivity C-reactive protein (hs-CRP), and serum amyloid A (SAA). An additional 3.5 mL of blood was taken on the last day of the four-week
elimination diet to assess changes in the inflammatory markers from the initial blood test. Those who were pregnant, had blood clotting disorders, had previous ALCAT testing, or who were under the age of 18 or over the age of 65 were ineligible to participate in the study.

Participants were informed of the risks and benefits associated with this study and were required to sign written consent (Appendix G) before participating in accordance with the study procedures approved by Northern Illinois University Institutional Review Board (IRB) (Appendix H) and the Institutional Biosafety Committee (IBC) (Appendix I). All subjects were debriefed at the end of the study (Appendix J).

**Experimental Design**

The study was a randomly assigned pre- and post-test double-blind experiment. At the beginning of the study the subjects reported to the nutrition laboratory at NIU to have anthropometrics and blood samples taken. The participants were asked to continue their normal exercise routine as well as dietary intakes before initial testing. The anthropometric measurements and blood samples taken at the beginning were the baseline measurements and were utilized to derive the ALCAT results for 200 food items. When the ALCAT results were determined, the subjects were notified of their individualized diet plans and underwent counseling by nutrition and dietetic graduate students as to what food they needed to eliminate and how to eliminate them. The subjects were placed into two groups by randomly assigning subjects to the treatment group (n=87) and to the placebo group (n=46). The subjects in both groups were required to follow their individualized food elimination diet plans for four consecutive weeks starting after they received their diet plan. At the end of the four weeks,
participants returned to the NIU nutrition laboratory for a second blood draw to measure changes in inflammatory markers and anthropometric measurements.

**Data Collection**

All subjects were required to complete a three-day food record (two weekdays and one weekend day) and a weekly exercise log. They also completed the DSI and MSQ forms and had their weight, height, body fat percentage, and lean body mass recorded at the start of the study and at the completion of the four-week elimination diet.

Anthropometric measurements were taken in the nutrition laboratory at NIU. All subjects were requested to wear lightweight clothing, have bare feet and be well hydrated. Height was measured using a wall-mounted stadiometer (AyrtonS-100, Prior Lake, MN). Lean body mass and percent fat were assessed using bioelectrical impedance (InBody 520, Biospace Inc., Los Angeles, CA). BMI was calculated by the InBody 520, which uses the standard equation (kilogram per meter squared). These measurements were taken at the time of the initial blood draw and again at the time of the final blood draw.

On the first day of the study, subjects had their blood drawn in the nutrition laboratory, (308A Wirtz Hall) by trained phlebotomists wearing medical exam gloves and lab coats. The subject’s arm was prepped with an alcohol swab and blood was drawn using either a 22-gauge needle or a 21- or 23-gauge butterfly placed in a vein in the arm. After the blood was collected, the venipuncture was covered with gauze and light pressure. The site and the gauze were then covered with a Band-Aid. The needles and any gauze soiled by blood were properly disposed of in a clearly labeled sharps container which was readily available in the nutrition laboratory in
Wirtz Hall 308A. Alcohol swabs used to prepare the skin and Band-Aid packaging were disposed of in the garbage.

**Blood Samples**

Four blue-top vials (4.5 mL each) containing 3.8% buffered sodium citrate and one gold-top serum separator tube (SST) were used to collect the blood. The blue-top samples were used to assess food intolerances using ALCAT and the gold-top were used to assess inflammatory markers GM-CSF, IFN-γ, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, TNF-α, MPO, hs-CRP, and SAA. The tubes were labeled with the subject’s name, time, and date of collection. Blue-top vials were immediately inverted several times to ensure proper mixing. The gold-top vials were centrifuged (Compact II Centrifuge made by Clay-Adams, Beckton Dickinson Company) at 3000 RPM for 15 minutes to separate the plasma cells from the serum. All of the vials were then placed into the slots of foam sleeves and placed into a biohazard specimen bag. The requisition form was placed in the front pouch of the specimen bag. The specimen bag was sealed by removing the strip covering the blue adhesive and pressing to close. The specimen bags containing the vials and requisition form were then placed into a silver insulated bag, which was sealed and placed in a box provided by ALCAT. The specimen and its packaging were then placed into a pre-paid UPS Laboratory Pak and sealed tightly. After all blood samples were collected each day, the samples were delivered to the UPS store and shipped overnight to Cell Science Systems (ALCAT headquarters) located at 852 South Military Trail, Deerfield Beech, FL 33442-2985.
Diet Analysis

Diet analysis was performed on the subjects’ three-day food logs (Appendix E). The subjects were trained on how to fill out the three-day food log by approximating serving sizes and documenting exact type of food consumed so that the most accurate analysis could occur. It was be explained that the more accurate the log, the more accurate the diet analysis would be. The goal of this diet analysis was to determine compliance to the recommended elimination diet plan.

Statistical Analysis

Descriptive measures were used to describe the study population. One-way analysis of variance (ANOVA) was used for inferential statistics. Within-and between-group differences in body composition and inflammatory markers at Day1 and at the end of the four-week elimination diet were analyzed using repeated measures ANOVA. Significant main and interaction effects were analyzed using the Bonferonni post hoc method for multiple comparisons. Statistical significance for all data analysis was accepted at the $p<0.05$ level of confidence. All data is expressed as $m \pm SD$. Data was analyzed by using the Statistical Package for Social Sciences (SPSS) for Windows (Version 21.0, 2013, SPSS, Inc, Chicago, IL).
CHAPTER 4

RESULTS

Research Methodology

The purpose of the study was to determine whether the elimination diet plan influenced inflammatory response, body composition and medical symptoms. In this double-blind study, Cell Science Systems randomly assigned every third subject to the placebo group, resulting in 100 subjects being placed in the treatment group (n=100) in which the subjects were given a list of foods to eliminate that they had reactions to based on ALCAT testing and 50 to the placebo group (n=50) in which subjects received a placebo elimination diet. The elimination diets were followed for four weeks.

Characteristics of the Sample

Initially, there were 150 participants who agreed to participate in the study, but due to attrition, the number of samples at post-test was N = 133 (treatment group, n = 87; placebo group, n = 46). Subjects ranged in age from 20-64 years of age, with a mean and standard deviation of $M(+SD) = 37.4(\pm13.18)$, and were predominantly female (n = 114). Participant characteristics are shown in Table 1.
Table 1. Characteristics of Subjects

<table>
<thead>
<tr>
<th>Characteristics Day 1</th>
<th>Treatment (n = 87)</th>
<th>Placebo (n = 46)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>mean (standard error of the mean)</strong></td>
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<tr>
<td>Age (yrs)</td>
<td>37.92 (13.52)</td>
<td>35.78 (12.62)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27.05 (6.17)</td>
<td>26.68 (6.34)</td>
</tr>
<tr>
<td>Body Fat %</td>
<td>31.44 (10.27)</td>
<td>30.27 (10.47)</td>
</tr>
<tr>
<td>Medical Questionnaire (possible range 0-284)</td>
<td>54.67 (31.78)</td>
<td>50.36 (29.51)</td>
</tr>
</tbody>
</table>

Statistical Analysis

The data was transformed from variables with non-normal data distributions. For the inflammation markers hs-CRP, MPO, SAA, IL-6, and IL-8, square root transformations of the data were performed. For lean body mass, BMI, and Medical Symptom Questionnaire scores, log transformations of the data were used. Fat percent data were normally distributed and did not require transformation. All hypotheses were tested using repeated-measures ANOVA.

Hypotheses

Hypothesis 1, subjects in the treatment group would have a lower hs-CRP than those in the placebo group, was not supported. There were no significant main effects of time between pre- and post-test, \( F(2, 130) = 1.83, p = .18 \), or treatment, \( F(2, 130) = .25, p = .62 \), on hs-CRP values. The mean hs-CRP values increased for both the treatment group and for the placebo group over time and this interaction effect, while nearing significance, was not significant, \( F(2, \)
130) = 3.42, \( p = .067 \) (Figure 1). The effects of time, treatment, and the interaction of the two are shown in Table 2.

**Figure 1:** Estimated Marginal Means of hs-CRP
<table>
<thead>
<tr>
<th>Independent variable (n=131)</th>
<th>Effects of time between pre- and post-test $P$ value</th>
<th>Effects of diet intervention (treatment) $P$ value</th>
<th>Interaction effect of time and treatment $P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hs-CRP</td>
<td>0.178</td>
<td>0.619</td>
<td>0.067</td>
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<tr>
<td>MPO</td>
<td>$&lt; 0.001$</td>
<td>0.115</td>
<td>0.596</td>
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<td>SAA</td>
<td>0.384</td>
<td>0.825</td>
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<td>TNF-α</td>
<td>0.067</td>
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<td>0.153</td>
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<td>0.503</td>
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<td>0.954</td>
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<td>0.320</td>
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<td>0.098</td>
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<td>0.340</td>
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<td>IL-5</td>
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<td>0.004</td>
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<tr>
<td>Fat percent</td>
<td>$&lt; 0.001$</td>
<td>0.605</td>
<td>0.233</td>
</tr>
<tr>
<td>BMI</td>
<td>$&lt; 0.001$</td>
<td>0.611</td>
<td>0.003</td>
</tr>
<tr>
<td>MSQ</td>
<td>$&lt; 0.001$</td>
<td>0.628</td>
<td>$&lt; 0.001$</td>
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Hypothesis 2, subjects in the treatment group would have lower MPO than those in the placebo group, was partially supported. Mean values for MPO decreased significantly for both groups over time, $F(2, 131) = 64.44, p < .001$. There was no significant main effect of treatment on MPO, $F(2, 131) = 2.51, p = .12$, and no significant interaction effect of time and treatment, $F(2, 131) = .28, p = .60$ (Figure 2, see Table 2).

**Figure 2**: Estimated Marginal Means of MPO
Hypothesis 3, subjects in the treatment group would have a lower SAA than those in the placebo group, was partially supported. The mean SAA values decreased for the treatment group and increased for the placebo group over time, and this interaction effect was significant, $F(2, 131) = 17.96, p = .035$. There were no significant main effects for time, $F(2, 131) = .76, p = .38$, or for treatment on SAA, $F(2, 131) = .05, p = .83$ (Figure 3, see Table 2).

![Estimated Marginal Means of SAA](image)

**Figure 3:** Estimated Marginal Means of SAA
Hypothesis 4, subjects in the treatment group would have a lower TNF-α than those in the placebo group, was not supported. There were no significant main effects for time, $F(2, 131) = 3.41, p = .067$, or for treatment, $F(2, 131) = 1.41, p = .24$, on TNF-α values. The mean TNF-α values increased for both the treatment group and the placebo group over time and the interaction effect was not significant, $F(2, 131) = 1.62, p = .21$ (Figure 4, see Table 2).

![Figure 4: Estimated Marginal Means of TNF-α](image-url)
Hypothesis 5, subjects in the treatment group would have a lower GM-CSF than those in the placebo group, was not supported. There were no significant main effects for time, $F(2, 131) = 2.07, p = .15$, or for treatment, $F(2, 131) = 1.74, p = .19$, on GM-CSF values. The mean GM-CSF values increased for both the treatment group and the placebo group over time and the interaction effect was not significant, $F(2, 131) = 1.54, p = .22$ (Figure 5, see Table 2).

**Figure 5:** Estimated Marginal Means of GM-CSF
Hypothesis 6, subjects in the treatment group would have a lower IFN-γ than those in the placebo group, was not supported. There were no significant main effects for time, $F(2, 131) = .45, p = .50$, or for treatment, $F(2, 131) = .94, p = .34$, on IFN-γ values. The mean IFN-γ values increased for both the treatment group and the placebo group over time and the interaction effect was not significant, $F(2, 131) = 1.54, p = .91$ (Figure 6, see Table 2).

**Figure 6:** Estimated Marginal Means of IFN-γ
Hypothesis 7, subjects in the treatment group would have a lower IL-1β than those in the placebo group, was not supported. There were no significant main effects of time, $F(2, 131) = .003, p = .95$, or treatment, $F(2, 131) = .13, p = .72$, on IL-1β values. The mean IL-1β values decreased for the treatment group and increased for the placebo group over time, but this interaction effect was not significant, $F(2, 131) = 1.00, p = .32$ (Figure 7, see Table 2).

Figure 7: Estimated Marginal Means of IL-1β
Hypothesis 8, subjects in the treatment group would have a lower IL-2 than those in the placebo group, was not supported. There were no significant main effects of time, $F(2, 130) = .05, p = .83$, or treatment, $F(2, 130) = .03, p = .86$, on IL-2 values. The mean IL-2 values decreased for the treatment group and increased for the placebo group over time, but this interaction effect was not significant, $F(2, 130) = .068, p = .79$ (Figure 8, see Table 2).

**Figure 8:** Estimated Marginal Means of IL-2
Hypothesis 9, subjects in the treatment group would have a lower IL-4 than those in the placebo group, was not supported. There were no significant main effects of time, $F(2, 131) = 2.79, p = .098$, or treatment, $F(2, 131) = .2.69, p = .103$, on IL-4 values. The mean IL-4 values increased for the treatment group and for the placebo group over time and this interaction effect was not significant, $F(2, 131) = .92, p = .34$ (Figure 9, see Table 2).

Figure 9: Estimated Marginal Means of IL-4
Hypothesis 10, subjects in the treatment group would have a lower IL-5 than those in the placebo group, was not supported. There were no significant main effects of time, $F(2, 131) = 2.32, p = .13$, or treatment, $F(2, 131) = 1.90, p = .17$, on IL-5 values. The mean IL-5 values remained nearly the same for the treatment group and for the placebo group over time and this interaction effect was not significant, $F(2, 131) = .54, p = .47$ (Figure 10, see Table 2).

**Figure 10:** Estimated Marginal Means of IL-5
Hypothesis 11, subjects in the treatment group would have lower IL-6 than those in the placebo group was partially supported. Mean values for IL-6 increased significantly for both groups over time, $F(2, 131) = 4.75, p < .03$. There was no significant main effect of treatment IL-6, $F(2, 131) = .04, p = .83$, and no significant interaction effect of time and treatment, $F(2, 131) = 1.88, p = .17$ (Figure 11, see Table 2).

**Figure 11**: Estimated Marginal Means of IL-6
Hypothesis 12, subjects in the treatment group would have lower IL-8 than those in the placebo group, was not supported. There were no significant main effects for time, $F(2, 131) = 2.54, p = .11$, or for treatment on IL-8, $F(2, 131) = .27, p = .61$. There was no significant interaction effect for time and treatment on IL-8, $F(2, 131) = .94, p = .33$ (Figure 12, see Table 2).

![Estimated Marginal Means of IL-8](image)

**Figure 12:** Estimated Marginal Means of IL-8
Hypothesis 13, subjects in the treatment group would have a higher IL-10 than those in the placebo group, was not supported. There were no significant main effects of time, $F(2, 131) = .15, p = .70$, or treatment, $F(2, 131) = .49, p = .49$, on IL-10 values. The mean IL-10 values decreased for the treatment group and increased for the placebo group over time and this interaction effect was not significant, $F(2, 131) = .59, p = .44$ (Figure 13, see Table 2).

**Figure 13**: Estimated Marginal Means of IL-10
Hypothesis 14, subjects in the treatment would have higher LBM than those in the placebo group, was partially supported. Lean body mass decreased for the treatment group and increased for the placebo group over time, and this interaction effect for time and treatment was significant, $F(2, 131) = 8.83, p = .004$. There were no significant main effects for time, $F(2, 131) = .30, p = .58$, or for treatment on lean body mass, $F(2, 131) = .55, p = .46$ (Figure 14, see Table 2).

**Figure 14:** Estimated Marginal Means of Lean Body Mass
Hypothesis 15, subjects in the treatment group would have lower fat percent than those in the placebo group, was partially supported. Mean values for fat percent decreased significantly for both groups over time, $F(2, 131) = 12.17, p < .001$. There was no significant main effect of treatment, $F(2, 131) = .27, p = .61$, and was no significant interaction effect of time and treatment on fat percent, $F(2, 131) = 1.44, p = .23$ (Figure 15, see Table 2).

**Figure 15:** Estimated Marginal Means of Fat Percent
Hypothesis 16, subjects in the treatment group would have lower BMI than those in the placebo group, was partially supported. There was a significant decrease in BMI for both groups over time, \( F(2, 131) = 41.39, p < .001 \), but the treatment group BMI decreased more than did the placebo group BMI. This interaction effect of time and treatment on BMI also was significant, \( F(2, 131) = 9.37, p = .003 \). There was no main effect for treatment on BMI, \( F(2, 131) = .26, p = .61 \) (Figure 16, see Table 2).

**Figure 16:** Estimated Marginal Means of BMI
Hypothesis 17, subjects in the treatment group would have lower MSQ scores than the placebo group, was partially supported. Mean scores for the MSQ decreased significantly for both groups over time, $F(2, 131) = 92.58, p < .001$, but the treatment group’s MSQ scores decreased more than did the placebo group’s scores. This interaction effect of time and treatment on MSQ scores was also significant, $F(2, 131) = 14.59, p < .001$. There was no significant main effect of treatment on MSQ scores, $F(2, 131) = 0.24, p = .63$ (Figure 17, see Table 2).

Figure 17: Estimated Marginal Means of MSQ
CHAPTER 5

DISCUSSION

The results of the present study found that there were significant differences over time between pre- and post-test values for both the treatment and control groups for the following measures: MPO, IL-6, fat percent, BMI, and MSQ values. Significant interaction effects between group assignment and testing time were also found on measures of SAA, LBM, BMI, and MSQ. However, there was not a significant difference between the treatment and placebo groups for the following indices: hs-CRP, MPO, SAA, TNF-α, GM-CSF, IFN-γ, IL-1β, IL-2, IL-4, IL-5, IL-6 IL-8, IL-10, LBM, fat percent, BMI, and MSQ scores.

Subjects were randomly assigned to either the treatment group, which implemented an elimination diet based upon true ALCAT findings, or a placebo group, which implemented an elimination diet based upon food items randomly selected from a list of items to which the subjects did not display an immunological response on their ALCAT results. No significant differences were found between the treatment and placebo groups on any inflammatory marker measurements, body composition measurements, or MSQ scores (see Table 2).

There are multiple factors which may have contributed to the lack of findings in these areas. For example, the list of food items which subjects were required to eliminate from their diets was often extensive, thus making strict dietary compliance challenging. In addition, the strict required dietary adherence may have contributed to the attrition rate, as subjects may have voluntarily withdrawn themselves from the study rather than comply with the prescribed
elimination diet for thirty days. Furthermore, the testing for the inflammatory markers TNF-α, GM-CSF, IFN-γ, IL-1β, IL-2, IL-4, IL-5, and IL-10 did not detect changes for the majority of the participants. For example, 121 of the 133 (83%) participants’ TNF-α values were at or below 0.58 pg/mL both pre- and post-tests and changes below this threshold could not be detected. If future testing can provide greater sensitivity for the aforementioned inflammatory markers, then one may be able to detect significant changes.

Subjects were assessed on measures of inflammatory markers, body composition, and MSQ scores at the start of the study and again thirty days later after implementing their dietary regimen as prescribed. No significant differences were found over the thirty-day span on the following measures: hs-CRP, SAA, TNF-α, GM-CSF, IFN-γ, IL-1β, IL-2, IL-4, IL-5, IL-8, IL-10, and LBM (see Table 2).

Significant differences were found over time for both the treatment and placebo groups on the following inflammatory markers: IL-6 and MPO (see Table 2). Unexpectedly, IL-6 increased in both the treatment and placebo groups while the values for MPO decreased for both the treatment and placebo groups over the duration of the study. These two markers are cytokines, which typically indicate an inflammatory response when elevated. IL-6, when elevated, is characteristic of chronic inflammatory conditions, but unique to IL-6 is its involvement in regulation of metabolic processes.1,37 IL-6, when secreted from adipocytes, interacts with other cytokines that may contribute to the continuation of the inflammatory cycle.69 It works in conjunction with other cytokines, usually driving the reaction, and may increase as it continues to interact with other cytokines. MPO decreased for the treatment group as expected but unexpectedly decreased for the placebo group. It is unclear why these two
particular inflammatory markers demonstrated a significant change while the other inflammatory markers did not.

Significant differences over time were also found on the following body composition markers: fat percent and BMI (see Table 2). Both of these values decreased significantly over the thirty-day period, indicating that both groups lost body fat and lowered their BMI over the course of the dietary elimination period. However, the treatment group had a greater decrease in BMI over time than did the control group, indicating that the avoidance of inflammatory-inducing food items in the experimental group had a greater impact. The loss of fat over time in the experimental group also was greater than in the control group, although not statistically significant. It is possible that these values could have achieved statistical significance with increased dietary compliance; however, a clinically significant loss of body fat was still observed, reflecting a positive response in the experimental group to the elimination of inflammatory foods.

Significant differences were evidenced on pre- and post-test MSQ values as well (see Table 2). These differences were found to be significant over time between the two groups, indicating that both the experimental and control subjects reported experiencing a significant reduction in symptoms of physiological and cognitive distress during the thirty-day period. The treatment group, while not achieving a statistically significant difference in symptom reduction over the placebo group, still reported a greater change than did the control group subjects. It is possible that with greater dietary compliance, these changes could have been statistically significant.

Significant differences between the experimental and control groups on pre and post-test values were discovered on one measure of inflammation: SAA (see Table 2). This inflammatory
marker is elevated in chronic inflammatory disorders.\textsuperscript{12,44} Interestingly, the experimental group demonstrated a significant reduction in this inflammatory marker over the course of the study, while the control group actually demonstrated an increase in this marker during the same period. This suggests that the subjects who avoided foods to which they demonstrated an intolerance on the ALCAT had a decrease in their SAA. Conversely, the subjects who did not avoid the food items to which they had demonstrated intolerance on the ALCAT evidenced an increased SAA, giving credence to a food elimination diet as a means of decreasing inflammatory response as measured by SAA.

A significant interaction effect between the groups over time was also evidenced on lean body mass (see Table 2). The experimental group evidenced a significant decrease in LBM over the course of the study, while the control group actually showed a significant increase in LBM over the same period of time. The loss of LBM is indicative of weight loss, as both fat and muscle are concurrently lost.\textsuperscript{5} Thus, the subjects who eliminated the inflammatory food items from their diets for thirty days lost a significant amount of lean body mass, while the subjects who did not eliminate inflammatory foods actually gained lean body mass. This increase in muscle mass for the control group was unexpected, and the reasons behind this phenomenon may be that over the course of the study the temperatures in the Midwest began to warm and people generally become more active as spring weather appears. Also participants were asked to complete exercise logs throughout the study, but very few participants completed exercise logs beyond the first log, and so it is plausible that this phenomenon can be explained by unreported increased exercise regimens in the control group.

While no significant difference was found between experimental and control groups on any of the measures of inflammatory markers, body composition, or MSQ scores in the current
study, further studies of dietary elimination designed to maximize compliance rate might reveal significant differences between the two groups. The current study demonstrated a significant difference over time between pre- and post-test values for both the treatment and placebo groups for two of the thirteen inflammatory markers (MPO and IL-6), for two of the three body composition markers (fat percent and BMI), and for the measure of health and well-being (MSQ). Significant interaction effects between group assignment and testing time were also found on one measure of inflammation (SAA), two body composition measures (LBM and BMI), and MSQ scores. These results indicate that, although not consistent, the elimination of inflammatory food items had a positive impact on some of the measures studied. Given the significant clinical reduction of symptoms in the experimental group on the aforementioned measures, it is highly possible that statistical significance between groups over the course of the study would have been detected on a greater number of the measures assessed if dietary compliance were increased, attrition decreased, or if testing sensitivity were improved.
CHAPTER 6

LIMITATIONS AND FUTURE RESEARCH

Limitations of the Current Study

The ALCAT was designed to assess the reactivity of each subject to a list of 200 food items. The results were unique for each subject, as it involved reporting the degree of reactivity (mild, moderate, or high) to a specific and individualized food list. Some subjects displayed a reaction to a small list of food items; however, some subjects were instructed to avoid a list of over thirty food items to which they displayed a reaction. This made food elimination challenging and difficult for some of the study participants, as the strict restriction diet involved multiple common and ubiquitous items and ingredients. Furthermore, a great deal of education was required to ensure that subjects understood how to assess the ingredients in their food/beverages in order to avoid the targeted ingredients. The large lists made compliance difficult or even impossible for some participants, which likely contributed to attrition rates. To address concerns regarding compliance and attrition in future studies, it may be helpful to provide a list of food items that are safe to be consumed while on the elimination diet. Menu ideas may also be helpful for subjects in order to help plan their meals over the course of the study.

Attrition was additionally affected by illness and participant obligations. The data collection took place during flu and cold season; several participants were unable to make their
final appointment due to these ailments. Several other participants were unable to find time in their schedules to complete the study due to work and family commitments. It is possible that if the study were to have been conducted at a different time of the year, or if more flexible appointment times were offered, these issues may have been avoided.

**Recommendations for Future Study**

Future studies would likely benefit from increased compliance and decreased attrition rates if subjects were given a list of foods to which they had no reaction along with the list of foods to be avoided at the beginning of the study. Menu plans with ideas for breakfast, lunch, dinner, and snacks given out at the beginning of the study might increase subjects’ understanding of the food elimination diet and therefore increase compliance. As a complete understanding of restricted versus allowable food is necessary for strict adherence to study guidelines, it is critical that all subjects in future studies receive a comprehensive education on food ingredients, food nomenclature, label analysis, grocery store guidelines and restaurant menu food selection criteria. This may eliminate confounding variables in the analysis of study results as well as increase compliance with study guidelines.

Additionally, increased compliance for completing exercise logs would allow changes in exercise to be factored into the data. An online survey that is designed to be quick, easy to use, and accessible from multiple smart devices may increase compliance in future studies.

Finally, in addition to the treatment and placebo groups, future research might include the addition of another control group that would follow their typical diet for four weeks. Compliance for this second control group would most likely be increased, as no dietary avoidance would be required. For this control group, inflammatory markers, body composition and MSQ scores may
show an increased significant difference when compared with the treatment group. This third
group could be a great addition for future research and may increase overall compliance rates as
well as demonstrate greater significant differences in testing results.
REFERENCES


27. Herbig DM. Food elimination based on ALCAT and the effect on overall body inflammation. 2014 (Unpublished thesis) Northern Illinois University, Dekalb, IL.


32. ALCAT: Available for over 25 years. Available at: https://www.alcat.com/.


APPENDIX A
RECRUITMENT FLYER
Do you experience digestive issues after eating certain foods?

Do you crave sweets?

Does your body ache and you can’t figure out why?

If you have answered **YES** to any of these questions, you may be eligible to participate in a Nutrition Research Project in the Spring of 2015

**Why?**
- To determine the effects of food elimination on body inflammation and composition.

**Who can participate?**
- Males and Females 18 to 65 years old
- Individuals with food intolerances, nasal congestion, GERD, or eczema
- People with anxiety, depression, insomnia or chronic exhaustion

**Participant will need to...**
- Be willing to complete a Disease Symptom Inventory and Medical Symptom Questionnaire
- Participate in pre- and post-study blood work & body composition measurements in Wirtz Hall
- Follow an individualized elimination diet for 4 weeks
- Complete 3-day food log and weekly exercise log

**Benefits:**
- **Free** ALCAT allergy test for 200 foods – a $700 value
- **Free** individualized elimination diet based on ALCAT test results
- **Free** analysis of your diet
- **Free** report of body composition measurements using special scale
- **Free** lab work to test the level of inflammation in your body - $1,000 value

If interested in participating
Contact Jodi Hoppensteadt at Elimdietstudy2@gmail.com
APPENDIX B
DISEASE SYMPTOM INVENTORY (DSI)
THE DISEASE SYMPTOM INVENTORY (DSI)
Please rate each of the following disease symptoms for the extent to which they are currently bothering you using the following rating scale:

0 = I do NOT have this symptom  
1 = A Very Mild Effect  
2 = A Mild Effect  
3 = A Somewhat Severe Effect  
4 = A Severe Effect  
5 = An Extremely Severe Effect

1. _____ Migraine Headaches  
2. _____ Irritable Bowel Syndrome  
3. _____ Inflammatory Arthritis  
4. _____ Gastro Esophageal Reflux  
5. _____ Recurrent Sinusitis with Infection  
6. _____ Tension Fatigue Syndrome  
7. _____ Eczema  
8. _____ Recurrent Anxiety  
9. _____ Recurrent Depression  
10. ___ Insomnia  
11. ___ Low Self-Esteem  
12. ___ Chronic Tiredness  
13. ___ Binge Eating  
14. ___ Chronic Tension  
15. ___ Lack of Energy  
16. ___ Food Allergies  
17. ___ Feeling Under Stress  
18. ___ Craving for Sweets  
19. ___ Cravings for Foods other than Sweets  
20. ___ Anorexia  
21. ___ Bulimia  
22. ___ Overeating  
23. ___ Other (write in: _______________________________  
24. ___ Other (write in: ______________________________________)
APPENDIX C
MEDICAL SYMPTOM QUESTIONNAIRE
Medical Symptom Questionnaire

Name ___________________________ Date ________________

Rate each of the following symptoms based upon your typical health profile for:
*Past 30 days*  *Past 48 hours*

**Point Scale**
0 - *Never or almost never* have the symptom  
1 - *Occasionally* have it, effect is *not severe*  
2 - *Occasionally* have it, effect is *severe*  
3 - *Frequently* have it, effect is *not severe*  
4 - *Frequently* have it, effect is *severe*

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<td>Swollen, reddened or sticky eyelids</td>
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<td>Bags or dark circles under eyes</td>
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<td>_____</td>
<td>Blurred or tunnel vision (does not include near or far-sightedness)</td>
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<td>Earaches, ear infections</td>
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<td>_____</td>
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<td>Hay fever</td>
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<tr>
<td>_____</td>
<td>Sneezing attacks</td>
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<tr>
<td>_____</td>
<td>Excessive mucus formation</td>
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<td>_____</td>
<td>Chronic coughing</td>
</tr>
<tr>
<td>_____</td>
<td>Gagging, frequent need to clear throat</td>
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<tr>
<td>_____</td>
<td>Sore throat, hoarseness, loss of voice</td>
</tr>
<tr>
<td>_____</td>
<td>Swollen or discolored tongue, gums, lips</td>
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<tr>
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<td>_____</td>
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<td>_____</td>
<td>Flushing, hot flashes</td>
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<td>Rapid or pounding heartbeat</td>
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<td>_____</td>
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<tr>
<td>LUNGS</td>
<td>Chest congestion, Asthma, bronchitis, Shortness of breath, Difficulty breathing, Total ____</td>
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<tr>
<td>DIGESTIVE TRACT</td>
<td>Nausea, vomiting, Diarrhea, Constipation, Bloated feeling, Belching, passing gas, Heartburn, Intestinal/stomach pain, Total ____</td>
</tr>
<tr>
<td>JOINTS/MUSCLE</td>
<td>Pain or aches in joints, Arthritis, Stiffness or limitation of movement, Pain or aches in muscles, Feeling of weakness or tiredness, Total ____</td>
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<tr>
<td>WEIGHT</td>
<td>Binge eating/drinking, Craving certain foods, Excessive weight, Compulsive eating, Water retention, Underweight, Total ____</td>
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<tr>
<td>ENERGY/ACTIVITY</td>
<td>Fatigue, sluggishness, Apathy, lethargy, Hyperactivity, Restlessness, Total ____</td>
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<tr>
<td>MIND</td>
<td>Poor memory, Confusion, poor comprehension, Poor concentration, Poor physical coordination, Difficulty in making decisions, Stuttering or stammering, Slurred speech, Learning disabilities, Total ____</td>
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<tr>
<td>EMOTIONS</td>
<td>Mood swings, Anxiety, fear, nervousness, Anger, irritability, aggressiveness, Depression, Total ____</td>
</tr>
<tr>
<td>OTHER</td>
<td>Frequent illness, Frequent or urgent urination, Genital itch or discharge, Total ____</td>
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**GRAND TOTAL**: TOTAL ____
APPENDIX D
ALCAT SCREENING & ETHNICITY FORM
ALCAT Screening & Ethnicity Form

NAME_____________________________________

Please list the medications you are currently taking____________________________________
____________________________________________________________________________

Please list any food allergies you have_____________________________________________
____________________________________________________________________________

Please list any food sensitivities or intolerances you have___________________________
________________________________________________________________________________

Ethnicity:
___ American Indian or Alaska Native
___ Asian
___ Black or African American
___ Caucasian
___ Hispanic
___ Native Hawaiian or Other Pacific Islander
___ Other: ______________________
3-Day Food Recall

Name: ________________________

For the following sheet, please do the following:
 ✓ Indicate the time of consumption in the left column
 ✓ Indicate the foods consumed along with estimated measurements (cup, ounce, tablespoon, etc.) in the right column.
 ✓ Include 2 weekdays and 1 weekend day.
 ✓ Please complete each day on a separate sheet.

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<thead>
<tr>
<th>Time:</th>
<th>Food Consumed &amp; Amount</th>
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<tbody>
<tr>
<td>7:45 am</td>
<td>1 whole egg with 2 egg equivalents of egg beaters</td>
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<tr>
<td></td>
<td>2 slices of Brownberry 12-Grain bread</td>
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<tr>
<td></td>
<td>2 Tbsp Skippy peanut butter</td>
</tr>
<tr>
<td></td>
<td>8 oz 1% milk</td>
</tr>
<tr>
<td></td>
<td>8 oz Folgers coffee with 2 Tbsp of hazelnut coffee creamer</td>
</tr>
</tbody>
</table>


APPENDIX F
EXERCISE LOG
Exercise Log

For the following sheet, please do the following:
✓ Indicate the date of exercise in the left column
✓ Indicate the exercise(s) completed along with number of sets and repetitions in each set in the right column.

<table>
<thead>
<tr>
<th>Date:</th>
<th>Exercise(s) Completed:</th>
</tr>
</thead>
<tbody>
<tr>
<td>August 13, 2013</td>
<td>3-mile run at 10:15 pace</td>
</tr>
<tr>
<td></td>
<td>4 sets of abdominal crunches, 8 reps each set</td>
</tr>
<tr>
<td></td>
<td>2-mile walk at moderate pace</td>
</tr>
</tbody>
</table>
APPENDIX G
CONSENT LETTER
Consent to Participate in the Food Elimination Based on ALCAT Testing and the Effect on Overall Body Inflammation Study

You have been invited to participate in a research project sponsored by manufacturer of the ALCAT test and designed to test the effect of food elimination on body inflammation and body composition. The ALCAT test is a food sensitivity test, and is not equivalent to medical allergy testing. You should continue to avoid foods that you know you have allergies or intolerances, regardless, of the ALCAT test results. This study is being conducted by Dr. Judith Lukaszuk, an Associate Professor in Nutrition and Dietetics at Northern Illinois University and Jodi Hoppensteadt, a graduate level nutrition student and Dietetic Intern at NIU.

If you meet the requirements of this study to be determined by completion of disease symptom inventory, you will be asked to follow an individualized elimination diet based on ALCAT testing for four weeks. Your four weeks will begin about one week following your initial screening for this study. You will be provided with a list of foods to avoid for four weeks. You will be randomly assigned to either a control or intervention group. The control group will receive “false” ALCAT reports on their food sensitivities, whereas, the intervention group will receive accurate ALCAT report results. At the end of the study the control group will be provided with accurate ALCAT report results. On day 1 of the study 25 mL (or 5 teaspoons) and at the end of the study, 21.5 mL (or a little over 4 teaspoons) of your blood will be taken to assess food intolerances and to assess thirteen inflammatory markers: granulocyte macrophage colony-stimulating factor (GM-CSF), interferon gamma (IFN-γ), interleukin-1 beta (IL-1β), interleukin-2 (IL-2), interleukin-4 (IL-4), interleukin-5 (IL-5), interleukin-6 (IL-6), interleukin-8 (IL-8), interleukin-10 (IL-10), tumor necrosis factor alpha (TNF-α), myeloperoxidase (MPO), high-sensitivity C-reactive protein (hs-CRP), and serum amyloid A (SAA). You will report to the nutrition laboratory at NIU and have your height, weight, body fat percentage, lean body mass and abdominal waist circumference measured at baseline and at the end of the study. All body measurements will be taken with subjects in lightweight clothing and bare feet. Height will be measured using a wall-mounted stadiometer. Weight, fat mass, percent fat, and fat free mass will be assessed using bioelectrical impedance scale called Biospace. Abdominal circumference will be measured with a cloth tape anteriorly halfway between the lowest lateral portion of the ribcage and the iliac crest. You must be willing to complete a Medical Symptoms questionnaire at the beginning and end of the study as well as 12 weeks after study completion. You can fill in the form via e-mail and send it back to Dr. Judith Lukaszuk at [jmlukaszuk@niu.edu](mailto:jmlukaszuk@niu.edu). You will also be asked to keep a keep a weekly 3-day food record (2 weekdays and 1 weekend day) and an exercise log during the study. We will also be providing you with a questionnaire which asks you your ethnicity, what medications you are taking, and what food allergies or food intolerances you may have.

You understand that participation in this study will involve elimination of specific foods based on ALCAT test results for the duration of the four-week study. The elimination diets will be individualized for you based on the blood drawn on day one and used for the ALCAT test. The blood draw will take approximately 15-20 minutes to complete. The anthropometric measurements will take about 20 to 30 minutes to complete. You are aware that participation is voluntary and may be withdrawn at any time without penalty or prejudice, and that if you have any additional questions concerning this study,
you may contact Dr. Judith Lukaszuk at [redacted] or Jodi Hoppenstead [redacted]. You understand that if you wish further information regarding my rights as a research subject, you may contact the Office of Research Compliance at Northern Illinois University at [redacted].

You understand that the intended benefits of this study include information of the effects of food elimination on body inflammation and body composition. You understand that participation in this study is free and you will not be monetarily compensated although you will receive valuable information regarding what foods you are intolerant to and how much inflammation you have in your body based on the foods you consume.

You understand that there is the potential risk of infection at the site of the blood draw. There is also a chance you may feel dizzy or light headed during the blood draw. Please tell us about these symptoms. We will require you to sit in the chair until your symptoms go away. If your symptoms do not go away in 5 minutes you will be provided with fruit juice to drink. You also may ask to stop the blood draw at any time if you feel uncomfortable with the blood draw procedure. Northern Illinois University policy does not provide for compensation for, nor does the University carry insurance to cover injury or illness incurred as a result of participation in University sponsored research projects. Upon suffering a minor injury, subjects will be referred to their PCP or nearest hospital and in the event of serious injury emergency medical services will be notified immediately.

You understand that all information gathered during this study will be kept confidential by giving all participants a number that is representative of them, and storing the information in a confidential file cabinet, which is locked when not in use. The four-week elimination program results information will only be accessible by the researcher and the advisor.

I understand that my signature below is consent to participate in the Food Elimination Based on ALCAT Testing and the Effects on Body Inflammation and Body Composition Study. I understand that my consent to participate does not constitute a waiver of any legal rights or redress I might have as a result of my participation, and I acknowledge that I have received a copy of this form.

Printed name: ____________________________________________
Signature: ____________________________________________ Date: __________
APPENDIX H
IRB AMENDMENT APPROVAL
Approval Notice

Protocol Amendment

21-Nov-2014

Judith Lukaszuk
Family, Consumer and Nutrition Sciences

RE: Protocol # HS13-0298 “Food elimination based on ALCAT testing and its effect on body inflammation and body composition”

Dear Judith Lukaszuk,

Your Protocol Amendment submission was reviewed and approved under Expedited procedures by Institutional Review Board #1 on 19-Nov-2014.

Please note the following information about your approved research protocol:

If your project will continue beyond that date, or if you intend to make modifications to the study, you will need additional approval and should contact the Office of Research Compliance and Integrity for assistance. Annual review of the project will be necessary until you no longer retain any identifiers that could link the subjects to the data collected. It is important for you to note that as a research investigator involved with human subjects, you are responsible for ensuring that the project has current IRB approval at all times, and for retaining any signed consent forms obtained from your subjects in a secure place for a minimum of three years after the study is concluded. The committee also recommends that the informed
consent include an acknowledgement that the subject, or the subject's representative, that he or she has received a copy of the consent form. In addition, you are required to promptly report to the IRB any injuries or other unanticipated problems involving risks to subjects or others.

Please remember to use your protocol number (HS13-0298) on any documents or correspondence with the IRB concerning your research protocol.

We wish you the best as you conduct your research. If you have any questions or need further help, please contact the Office of Research Compliance and Integrity at (815) 753-8588.
APPENDIX I
IBC APPROVAL
MEMORANDUM

TO: Judith Lukaszuk
School of Family, Consumer & Nutrition Sciences

RE: Continuation of approval of research involving recombinant DNA/pathogenic substances

The following project involving the use of recombinant DNA and/or pathogenic substances was reviewed and continuation of the project, with modifications, was approved by the Institutional Biosafety Committee (IBC) at its meeting on October 16, 2014:

Title:

IBC/ORC #: Biosafety Level: Lab:

*Food elimination based on ALCAT testing and the effects of body inflammation and body composition*

S13-0011
BL-2
DU 161, WZ 308

This approval is effective for one year from the anniversary date of original review, until **October 14, 2015**. If your project will continue beyond that date, you should contact the Office of Research Compliance in the Division of Research & Graduate Studies.
APPENDIX J
DEBRIEFING LETTERS
Debriefing Letter for Control Group Participants
You were randomly assigned to the control group and as such were provided “false” ALCAT report results at the beginning of the study. Attached are your accurate ALCAT report results. Foods included in the red column (indicates a severe intolerance and should be avoided for 6 months), orange column (indicates a moderate intolerance should be avoided for 3-6 months) and yellow column (indicates a mild intolerance should be avoided if possible) should be avoided and foods in the green column indicates acceptable foods so you can eat these foods as long as you are not allergic to them or have from previous experience with these food reacted with a food intolerance. The ALCAT Company will send out a test results guide booklet, a wallet size results card and a meal plan for you to follow based on your ALCAT test results. However, you should consult with your physician before eliminating any foods long term.

Acknowledgement:
I, ________________________, have received my accurate personalized ALCAT test results and understand the foods I need to avoid to potentially reduce inflammation in my body.

Signature: ___________________________ Date: ___________
FOOD ELIMINATION BASED ON ALCAT TESTING AND THE EFFECT ON OVERALL BODY INFLAMMATION

Debriefing Letter for Intervention Group Participants
You were randomly assigned to the intervention group and as such was provided “accurate” ALCAT report results at the beginning of the study. Foods included in the red column (indicates a severe intolerance and should be avoided for 6 months), orange column (indicates a moderate intolerance should be avoided for 3-6 months) and yellow column (indicates a mild intolerance should be avoided if possible) should be avoided and foods in the green column indicates acceptable foods so you can eat these foods as long as you are not allergic to them or have from previous experience with these food reacted with a food intolerance. The ALCAT Company will send out a test results guide booklet, a wallet size results card and a meal plan for you to follow based on your ALCAT test results. However, you should consult with your physician before eliminating any foods long term. At the conclusion of the study, the participants in the control/placebo group will be un-blinded and provided with their ALCAT tests results to determine foods they have sensitivities to and therefore need to avoid.

Acknowledgement:
I, ___________________________, have received my accurate personalized ALCAT test results and understand the foods I need to avoid to potentially reduce inflammation in my body.

Signature: ___________________________ Date: _____________
APPENDIX K
PARTICIPANT’S MEASUREMENT SHEET
Participant #:_____  

### Anthropometric Measurements

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Day One</th>
<th>Day 30</th>
</tr>
</thead>
<tbody>
<tr>
<td>Waist Circumference</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Height</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Body Composition

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Day One</th>
<th>Day 30</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body Fat %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lean body Mass</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>