ABSTRACT

FOOD ELIMINATION BASED ON ALCAT TESTING AND THE EFFECT ON OVERALL BODY INFLAMMATION

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Background: Growing evidence suggests that obesity is associated with systemic inflammation. The level of inflammation can be exacerbated by one or more existing food intolerances. Furthermore, studies have shown that diet quality, not quantity, plays an important role in potential inflammation caused by foods. It is therefore, important to determine if dietary patterns alone or in combination with increased adiposity have a greater effect on systemic inflammation and body composition.

Objective: Hence, the purpose of this study is was to examine the effect of food elimination using the ALCAT food elimination protocol, on body composition and overall body inflammatory markers.

Methods: One hundred thirty-one participants were randomly assigned to either a treatment (n=72) or a control (n=59) group in this pre- and post-test double blind experimental study. All participants followed a 4-week elimination diet based on ALCAT testing protocol, completed a medical symptoms questionnaire at the beginning and end of the study, had their blood drawn for inflammatory markers and provided anthropometric measurements for body composition analysis. In additions Participants kept an exercise log and food log during the study. Within and between group differences in body composition and inflammatory markers were analyzed using
repeated measures ANOVA. Significant main and interaction effects were analyzed using the Bonferonni post-hoc method for multiple comparisons. Univariate analysis of variance was utilized to examine body composition.

**Results:** Both the treatment group and the control groups experienced significant decreases in CRP, MPO, and TNF-α; yet there were no statistically significant differences between the treatment and the control groups. However, the treatment group was found to have a significant (p<0.05) decrease in SAA compared with the control group which showed no change in SAA. Analysis of body composition revealed a significant (p<0.05) decrease in lean body mass, body fat percentage, and waist circumference in both groups; however, the difference was not statistically different between the treatment and the control group even after adjustments for age, gender and baseline outcomes.

**Conclusions:** Overall, there was little evidence to support the ability of elimination diets in decreasing total body inflammation or altering body composition using ALCAT testing protocol.
FOOD ELIMINATION BASED ON ALCAT TESTING
AND THE EFFECT ON OVERALL BODY INFLAMMATION

BY

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CHAPTER 1

INTRODUCTION

Background

Over the last 20 years, there has been a dramatic rise in body weight. Currently, more than one third of adults in the United States are obese (1). Obesity is a major health concern, since it is strongly linked to coronary heart disease, insulin resistance, type 2 diabetes and non-alcoholic fatty liver disease (1,2). Although the rise in obesity has many causes, inflammation is the most common feature which has been implicated in the pathophysiology of all the obesity related disorders.

Inflammation is a protective response by the immune system to harmful stimuli, such as infection, acute illness, trauma, toxins, many chronic diseases and physical stress (2). Chronic inflammation is characterized by the prolonged presence of macrophages in the injured tissue, simultaneously destroying and healing the tissue during the inflammatory process (3-5). Chronic inflammation can lead to systemic inflammation which can impair digestion and absorption which can result in a decreased lean body mass and a decline in nutritional status (2,5). The role of inflammation in the pathology of rheumatoid arthritis (RA), inflammatory bowel diseases (IBD), and asthma are well recognized (5). Yet when obesity is present, the role of inflammation and the contribution to pathology of disease along with other factors, like nutrition, is less clear (5).
Numerous studies have shown that as visceral adipose tissue increases with weight gain so do pro-inflammatory cytokines such as C-reactive protein (CRP), interleukin 1-beta (IL-1-β), interleukin-6 (IL-6), myeloperoxidase (MPO), and tumor necrosis factor-alpha (TNF-α). The cascade of pro-inflammatory cytokines favors insulin resistance which may lead to more weight gain as fat. It becomes a vicious cycle of increased inflammation and more deposition as fat (6-8). Furthermore, the multitude of cytokines released in relation to obesity can change from advantageously protecting the body to damaging by leading to inflammatory diseases (2,4,5).

The nutritional state of an individual can also affect inflammation. For example, undernutrition, which may occur with impaired digestion or absorption, dysfunctional metabolic processing or inadequate intake, can result in malnutrition and protein depletion (2). On the other hand, overnutrition promotes obesity-related health issues, such as diabetes, heart disease, hypertension, metabolic syndrome, and some cancers (2).

Along with nutritional status, dietary intake may play a key role in the inflammatory process. Simple diet and lifestyle changes can positively affect obesity-related inflammation (2,9). Likewise, the level of inflammation can be exacerbated by one or more existing food intolerances. If food intolerance is present, it stimulates the fight or flight response and increases your cravings for that food. This in turn, promotes the greater consumption of that food which will lead to more inflammation. Continued exposure to intolerances provides constant pro-inflammatory stimuli that increase the body’s inflammatory response resulting in tissue damage and loss of barrier function thus leading to systemic inflammation (5,10). What is unknown at this time is if one eliminates food intolerances from the diet will their systemic inflammation, as measured through CRP, IL-1β, IL-6, MPO and TNF-α, decrease, thereby, reducing insulin resistance thus enabling them to lose weight.
Antigen Leukocyte Cellular Antibody Test (ALCAT) is useful in identifying problematic foods in cell-mediated or delayed reactions (2). ALCAT diets require the elimination of intolerant foods and thus should decrease appetite, reduce systemic inflammation, enable weight-loss and improve body composition (11).

Body composition measurements are important in assessing fat mass (FM) and lean body mass (LBM) (12). FM includes essential and non-essential fats. LBM includes protein, water, carbohydrate, and minerals (12). Waist circumference (WC) measurement indicate regional body fat distribution and has been shown to correlate with visceral adipose tissue (12).

**Statement of the Problem**

The purpose of this study was to examine the effect food elimination will have on body composition and on body inflammatory markers using ALCAT testing.

**Independent Variables**

A food elimination diet based on ALCAT test results (treatment group) or a sham list of foods (placebo group) will be provided and followed for four weeks.

**Dependent Variables**

Body composition will be measured in relation to food elimination using FFM, LBM, body fat percent, abdominal WC, and fat mass for both pre- and post-4 week study.

Body inflammatory markers such as CRP, IL-1-β, IL-6, MPO, SAA, and TNF-α will be measured via blood test performed at the NIU Nutrition Lab.
**Hypotheses**

The following hypotheses were investigated during the 4-week elimination program:

1. Subjects randomly assigned to the ALCAT treatment group will have lower CRP at the end of the 4 week study compared to those receiving the placebo.

   *Independent Variable:* Food Elimination Plan

   *Attributes:* Elimination of foods determined by ALCAT based on individual blood tests; varies per person

   *Control:* Participants will receive a placebo food elimination plan

   *Dependent Variable:* Body inflammatory markers

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

2. Subjects randomly assigned to the ALCAT treatment group will have lower IL-1-β at the end of the 4 week study compared to those receiving the placebo.

   *Independent Variable:* Food Elimination Plan

   *Attributes:* Elimination of foods determined by ALCAT based on individual blood tests; varies per person

   *Control:* Participants will receive a placebo food elimination plan

   *Dependent Variable:* Body inflammatory markers

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

3. Subjects randomly assigned to the ALCAT treatment group will have lower IL-6 at the end of the 4 week study compared to those receiving the placebo.

   *Independent Variable:* Food Elimination Plan

   *Attributes:* Elimination of foods determined by ALCAT based on individual blood tests; varies per person
Control: Participants will receive a placebo food elimination plan

*Dependent Variable:* Body inflammatory markers

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

4. Subjects randomly assigned to the ALCAT treatment group will have lower MPO at the end of the 4 week study compared to those receiving the placebo.

*Independent Variable:* Food Elimination Plan

*Attributes:* Elimination of foods determined by ALCAT based on individual blood tests; varies per person

Control: Participants will receive a placebo food elimination plan

*Dependent Variable:* Body inflammatory markers

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

5. Subjects randomly assigned to the ALCAT treatment group will have lower SAA at the end of the 4 week study compared to those receiving the placebo.

*Independent Variable:* Food Elimination Plan

*Attributes:* Elimination of foods determined by ALCAT based on individual blood tests; varies per person

Control: Participants will receive a placebo food elimination plan

*Dependent Variable:* Body inflammatory markers

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

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

*Independent Variable:* Food Elimination Plan
Attributes: Elimination of foods decided by ALCAT based on individual blood tests; varies per person

Control: Participants will receive a placebo food elimination plan

Dependent Variable: Body inflammatory markers

Attribute: Comparison of pre- and post-study blood levels of TNF-α

7. Subjects randomly assigned to the ALCAT treatment group will have lower FM and higher LBM at the end of the study compared to the placebo group.

Independent Variable: Food Elimination Plan

Attributes: Elimination of foods determined by ALCAT based on individual blood tests; varies per person

Control: Participants will receive a placebo food elimination plan

Dependent Variable: FM and LBM

Attributes: FM and LBM assessed using bioelectrical impedance (Biospace)

8. Subjects randomly assigned to the ALCAT treatment group will have lower fat percent at the end of the study compared to the placebo group.

Independent Variable: Food Elimination Plan

Attributes: Elimination of foods determined by ALCAT based on individual blood tests; varies per person

Control: Participants will receive a placebo food elimination plan

Dependent Variable: Fat percent

Attributes: Fat percent assessed using bioelectrical impedance (Biospace)

9. Subjects randomly assigned to the ALCAT treatment group will have a lower abdominal WC at the end of the study compared to the placebo group.
**Independent Variable:** Food Elimination Plan

*Attributes:* Elimination of foods determined by ALCAT based on individual blood tests; varies per person

*Control:* Participants will receive a placebo food elimination plan

**Dependent Variable:** Abdominal WC

*Attributes:* Abdominal WC measured with a cloth tape anteriorly halfway between the lowest lateral portion of the rib cage and the iliac crest.
CHAPTER 2

REVIEW OF LITERATURE

**Introduction**

Obesity rates have more than doubled from 13% to 35.7% in the United States and worldwide over the last two decades (1, 13). It is predicted by World Health Organization (WHO) that 2.5 billion people worldwide will be overweight with a body mass index (BMI) greater than 25 and 700 million people will be obese with a BMI over 30 by the year 2015 (14). Obesity is a major health concern since obesity is strongly linked to inflammation and a number of inflammatory related disorders, such as coronary heart disease, insulin resistance, type 2 diabetes and non-alcoholic fatty liver disease (15,16).

Numerous studies have shown that as visceral adipose tissue increases, pro-inflammatory cytokines such as CRP, IL-1-β, IL-6, MPO, and TNF-α also increase. The cascade of pro-inflammatory cytokines favors insulin resistance leading to more weight gain as fat. It becomes a repetitive cycle of increased inflammation and more deposition as fat (6-8, 15, 16, 18). Furthermore, the multitude of cytokines released in relation to obesity can change from protecting the body to leading to inflammatory diseases (2,4). The presence of low-grade inflammation has been positively associated with adiposity (19). Abdominal weight gain
is a standard indicator of adipose tissue accumulation (6,16) which promotes low-grade chronic inflammation (15-16). Adipose tissue is an active secretory organ that sends out and responds to signals that control appetite, energy expenditure, bone metabolism, endocrine and reproductive systems, insulin sensitivity, as well as immunity and inflammation (6, 16).

Inflammation is a protective response of the immune system to harmful stimuli, such as infection, acute illness, trauma, toxins, many chronic diseases and physical stress (2). Chronic or prolonged inflammation occurs when the body continues to produce inflammatory mediators past the acute response phase (2). Chronic inflammation is characterized by the increased presence of macrophages in the injured tissue, which leads to tissue damage and healing the tissue during the inflammatory process (3). Chronic inflammation can lead to systemic inflammation which can negatively affect many functions of the body, such as digestion and absorption. Inflammatory conditions trigger the immune system to release eicosanoids (n-6 eicosanoids, leukotriene B4, prostaglandin E2) and cytokines (CRP, IL-1-β, IL-6, MPO, TNF-α), which mobilize nutrients needed in the inflammatory process (2,20). As a result, cytokines, especially IL-1-β, IL-6, TNF-α, impact whole body metabolism, body composition and nutritional status (2).

The link between poor nutritional status and impaired immune function has been firmly established (2,20). However, research regarding diet quality, inflammatory markers, and its subsequent effect on immunity is minimal (21,22). A study was conducted to look at the association between healthy eating patterns and immunity and inflammation in overweight or obese menopausal women (21). Boynton et al, found limited evidence that healthy eating habits in postmenopausal women enhanced immune function and decreased inflammation (21). The
study showed that higher diet quality was associated with lower levels of serum CRP or SAA (21). Furthermore, diet quality has been linked to inflammatory markers, specifically CRP and SAA, where the association is largely affected by adiposity. Consumption of a healthier diet potentially leads to a decrease in adipose tissue, which may result in decreased levels of inflammatory markers (21,22). Further research supports the relationship between diet quality and inflammation. In a systematic literature review, foods associated with increased serum levels of CRP and IL-6 included red meats, processed meats, low-fiber foods or refined grains, and alcohol (22). Inversely, consumption of foods such as fruits, vegetables, and whole grains showed decreased serum levels of CRP and IL-6 in the study participants (22). Additionally, the Framingham Heart Study reported that as BMI, visceral adipose tissue, subcutaneous tissue, and WC increased serum levels of CRP and IL-6 also increased (17).

Diet and exercise are known to reduce inflammation (9,19). It is well known that consuming a diet rich in fruits and vegetables, low in refined starches, sugar, saturated and trans-fatty acids reduces the level of inflammatory cytokines. Additionally, a decrease in CRP, IL-6 and TNF-α has been found with diet-induced weight loss (4,20). A study investigated the relationship between inflammation and food digestion and discovered that impairments in intestinal structure, digestive and absorptive function, and barrier function are a result of inflammation originating in the intestine or distant locations (23). Currently, it is unknown what effect the removal of food intolerances from the diet will have on the body. It is suspected that systemic inflammation, as measured through CRP, IL-1β, IL-6, MPO and TNF-α, will decrease, thereby, reducing insulin resistance and promoting weight loss.
The level of inflammation can be exacerbated by one or more existing food intolerances. If food intolerances are present, they will stimulate the fight or flight response and increase food cravings. This in turn, makes one eat a larger serving of that food which will lead to more inflammation. Continued exposure to allergens provides constant pro-inflammatory stimuli which increase the body’s inflammatory response resulting in tissue damage and loss of barrier function thus leading to systemic inflammation (4). Joint pain is a common result of systemic inflammation. TNF-α and IL-1β are believed to promote cartilage damage and CRP has been shown to be elevated in patients with osteoarthritis. Therefore, cartilage breakdown in osteoarthritis may be worsened by inflammation (20). Activated macrophages at the site of inflamed cartilage secrete TNF-α and IL-1, which lead to the loss of the integrity of the cartilage in the joints (4).

**Food Intolerances**

Food allergy by definition is an abnormal immunologic response to a food that occurs in a susceptible individual (24). Allergic reactions usually occur immediately and every time the food is consumed, even in small amounts. Food allergy reactions are facilitated by immunoglobulin E (IgE) antibody (24). Unlike food allergy, food intolerance refers to a variety of non-immunologic reactions, which are mediated by the innate branch of the immune system, occurring after consumption of a particular food (24). These abnormal physiologic responses may be unpredictable due to a gamut of reasons such as intrinsic properties of the food or to biological characteristics of the individual consuming the food (24). These symptoms may not be
reproducible, may be delayed, and often depend on the quantity of the food consumed (24,25). Food intolerances to foods have been reported to cause low-grade inflammation throughout the body (14). For example, consumption of fried, fatty, junk foods and sweets have been associated with increased systemic inflammation and elevated CRP levels (26).

**Prevalence of Food Intolerances in the US**

The prevalence of food allergy appears to be on the rise in Westernized cultures (24). For example, peanut sensitization tripled and reported peanut allergy in children doubled over only a five-year period in both the United States and United Kingdom (24). Although evidence indicates an increasing prevalence, a discrepancy between patient- perceived and physician- confirmed food allergy exists. The prevalence of food allergy, with an immediate immunological response, is highest in infants and toddlers. Cow’s milk allergy is experienced by 2.5% of infants, and up to 8% of children under 3 years of age have allergy to a limited number of foods, mainly cow’s milk, egg, soy, peanut, wheat, fish, shellfish, or tree nuts (24). The prevalence of food allergy decreases slightly with age, affecting almost 4% of the general population (24,25).

In contrast, food intolerance is more common than food allergies but usually go undiagnosed due to delayed symptomology or improper format of testing. Research has shown that upwards of 70-80% of the US population reports having symptoms associated with food intolerances (25). Yet, these individuals are not able to be diagnosed with a food allergy due to the lack of IgE response when tested (25).
Cause of Food Intolerances

Research has found that some primary reasons for food intolerances are an imbalance of fatty acids, chemicals in foods that the liver cannot break down, impaired gut function, and inherited enzyme deficiencies or metabolic disorder (10,24). It is important to note that inherited enzyme deficiencies or metabolic disorders that cause intolerance to food will not change secondary to genetics but if properly addressed, moderate quantities of the problematic food may be tolerated (10,24). However, the remaining aforementioned factors have been linked to unhealthy food consumption where the avoidance of such food results in weight normalization, attenuation of inflammation and reduction of other inflammation-based health issues (10).

Specifically, fatty acid imbalances may result from the overabundance of corn and soy in the diet which contains high omega-6 fatty acids. These omega-6 fatty acids promote high arachidonic acid levels which in turn promote chronic, pro-inflammatory cytokine production (10). Additionally, natural or synthetic chemicals in a variety of foods in today’s diet are difficult for the liver to effectively detoxify and thus activate the immune system (10). For example, salicylates occur naturally in many fruits and vegetables, and can induce a pharmacologically mediated adverse reaction in susceptible individuals. Some examples of food-related factors responsible for non-immunologic reactions include improperly stored fish that became contaminated with histamine, or the presence of tyramine in aged cheese, alcoholic beverages, and cured meats (24). Furthermore, decreased gut function occurs when the gut membrane is compromised. When this natural barrier is damaged, the immune system is called into action as well (10).
Intolerances to foods are mediated by the innate branch of the immune system and thus follow a different pathway than pathogens (10). Food intolerances symptoms are dose-related, chronic and delayed (10). Inflammation, toxic free radicals, and immune chemicals are produced as a result of food intolerances (10). These products may lead to high risk of metabolic, chronic and degenerative diseases (10).

**Symptoms of Food Intolerances**

Cellular reactions to food can cause inflammatory reactions which have been linked to a number of chronic health problems such as obesity, diabetes, migraines, and asthma in addition to skin, heart, joint, and digestive disorders (14,20,25). The extra-intestinal manifestations and other reactions to foods, such as bloating and swelling in the hands, feet, ankles, abdomen, chin and around the eyes, may not display immediate symptoms but can present many hours later. Inflammation associated with food sensitivities can lead to weight gain. Fluid retention caused by inflammation and the release of certain hormones and cytokines, such as IL-6 and TNF-α, are the major causes of the weight gain (14,25)

Poor digestion, malabsorption of nutrients, and thus reduced nutritional status are also symptoms of food intolerances. Research shows that impaired gut function such as digestion, absorption, and barrier function, result from inflammation (12,23). Intestinal inflammation can impair both digestion and absorption within the inflamed area as well as surrounding intestinal areas which makes proper digestion and adequate nutrition challenging (12,23). For example, in inflammatory bowel diseases such as ulcerative colitis and Crohn’s disease, the brush border
activity is reduced, the transit time of food and nutrients is decreased so contents travel through the intestinal tract quicker, and the absorptive mucosa cells may be damaged (12). Celiac disease is a condition where eating gluten triggers an immune response in the small intestine, resulting in inflammation of the mucosa. As a result, the villi become atrophied and flattened, thus impairing digestion and absorption (12). Diarrhea, abdominal pain, malabsorption, and weight loss are symptoms of Celiac disease (12). Some extra-intestinal manifestations of Celiac disease include skin rashes, muscle and joint pain, as well as fertility issues in females (12). Additionally, individuals with food intolerances tend to have poor food intake when not feeling well which exacerbates poor digestion and malabsorption of nutrients (12).

**Inflammatory Markers**

There are a number of inflammatory cytokines in the human body. Each cytokine serves a particular purpose during the inflammation process. In a number of studies, the increased presence of inflammatory cytokines has also been associated with obesity, where these cytokines produced and released by the white adipose tissue (WAT) are responsible for the chronic inflammatory state of obesity (16,27). In healthy individuals, adipocytes produce pro-inflammatory cytokines such as IL-6 and TNF-α (2,6,16,20, 28). IL-6 then stimulates the synthesis of CRP in the liver, all of which is increased with obesity (2,17,28i). Rexrode et al, found a significant and positive association between IL-6 and CRP and all levels of BMI, and WC (29). Another study found that BMI and percent body fat were significant predictors of increased serum levels of IL-6 and CRP, whereas no association between adiposity and TNF-α
was shown (28). Ma et al, from Zhejiang University in Hangzhou, China, reported that several types of obesity were associated with chronic low-grade inflammation (3). Research shows that isolation of one cytokine in the pathophysiology is unlikely and that a compilation of cytokines predict disease (17). Therefore, in the present study multiple inflammatory markers were investigated: CRP, IL-1-β, IL-6, MPO, SAA, and TNF-α.

**C-Reactive Protein**

CRP is a protein that is synthesized in the liver (3,30) and in healthy individuals its level is less than 2mg/L in healthy individuals (15). Therefore, elevated levels of CRP can be used as a strong biomarker of low-grade inflammation (3,15,18,30). IL-6 has been found to be strongly correlated with the synthesis of CRP in response to an increase in visceral adipose tissue. (26,30). Research shows a positive correlation between circulating CRP levels and BMI (15,18,29-31). Moreover, elevated levels of CRP are associated with increased risk of atherosclerotic diseases, stroke, cardiovascular disease and diabetes (15,29). Studies have shown that elderly individuals commonly have elevated serum levels of TNF-α and CRP (20). A study looking at the relationship between adiposity with CRP and IL-6 found that higher CRP and IL-6 levels were observed as BMI levels increased (29). Another study by Khaodhiar et al., concurs with this study and reported almost identical findings (18). Interestingly, Khaodhiar et al., found the association between CRP and BMI to be greater for men as opposed to women due to a higher amount of visceral adipose tissue in males (18). In contrast, Rexrode et al., reported this association stronger in women than men (29). It was also found that weight loss in obese women
resulted in decreased levels of CRP, thus potentially decreasing their risk of cardiovascular disease and diabetes (29). These findings were further supported by research showing that a healthy diet and weight loss were found to reduce CRP levels (30). Additionally, a longitudinal study conducted to examine the effect of BMI on CRP found that as BMI and body mass remained stable, so did CRP levels which coincides with previous research that BMI is positively associated with CRP. (31). Overall, a loss in weight and a decrease in BMI has been shown to decrease levels of CRP in both males and females.

**Interleukin-1-Beta**

IL-1-β is an adipokine that is synthesized in human white adipose tissue, along with TNF-α and IL-6, and exhibits pro-inflammatory properties (20,27). IL-1-β, TNF-α and IL-6 are often used as markers to assess inflammation (32). IL-1-β plays a role in the regulation of SAA during the inflammatory process (33). IL-1-β initiates the production of cyclooxygenase-2 (COX-2), an enzyme that is abundant in activated macrophages and other cells that are seen at the site of inflammation. COX-2 has been shown to be abundant in various carcinomas and to have a central role in tumorigenesis. Thus, IL-1-β is most often associated with pain caused by inflammation.
**Interleukin-6**

IL-6 is a pro-inflammatory cytokine that participates in both trauma-related acute phase response and stress-related inflammatory response (6). A general systemic inflammation can occur when the stress is chronic (6). IL-6 is synthesized in human adipose tissue and plasma concentrations increase with obesity (3,7) where expression of IL-6 is higher in visceral than peripheral adipocytes (7,20). IL-6 obtains pro-inflammatory activity either by itself or by increasing levels of IL-1-β and TNF-α (3). Additionally, IL-6 stimulates the liver production of CRP (3). IL-6 is an adipokine that is synthesized in human white adipose tissue, along with IL-1-β and TNF-α, and exhibits pro-inflammatory properties (20,27). Obesity is related to increased circulating levels of these cytokines (17,20,27,34). In normal weight women with high a high amount of adipose tissue, both IL-6 and TNF-α concentrations were associated with fat mass distribution (27). Higher IL-6 levels were observed with higher BMI levels; lower IL-6 levels were reported with a decrease of BMI (18,29). Elevated IL-6 levels are associated with subsequent heart attacks and diabetes (29).

**Myeloperoxidase**

MPO is a heme protein that constitutes the major component of neutrophils. (32). Therefore, MPO enzyme activity is used to measure neutrophil function (32). Plasma MPO is involved in the releases reactive oxygen species (ROS) and thus may impart damage to proteins, carbohydrates, lipids, and nucleic acids that serve as biological structures (32). This destruction may exacerbate the body’s inflammatory response and potentially result in sepsis (32). During a
state of oxidative stress, increased amounts of neutrophil surround the affected area and release MPO into the bloodstream. Therefore, plasma levels of MPO enzyme activity serves as a biomarker of inflammation, especially when sepsis is present (32).

**Serum Amyloid A**

SAA is an acute phase protein from the apolipoprotein family associated with high density lipoproteins (HDL) in the blood. SAA is produced in the liver and regulated by IL-1, IL-6, and TNF-α in response to inflammatory stimuli, such as stress, injury, infection, or trauma (33). Due to the quick response of SAA, similar to CRP, it is a sensitive biomarker for the acute inflammatory state (21,33). SAA, along with IL-6 and TNF-α, are known to be elevated in obese individuals as these inflammatory markers are expressed in adipose tissue (33). Research shows that elevated systemic SAA concentrations are associated with impaired glucose tolerance and type 2 diabetes mellitus, independent of established type 2 diabetes risk factors, BMI, waist-to-hip ratio, and body fat mass index (33). Research suggests that elevated SAA levels are not the cause of diabetes but a result of the pre-diabetes process (33). Limited research found that healthy eating patterns may contribute to decreased circulating SAA levels such that consumption of a healthier diet potentially leads to a decrease in adipose tissue, which may result in decreased levels of inflammatory markers, specifically CRP and SAA (21).
Tumor Necrosis Factor-alpha

TNF-α is an adipokine that is synthesized in human white adipose tissue, along with IL-1-β and IL-6, and exhibits pro-inflammatory properties (6,27). TNF-α in adipose tissue acts locally but can promote insulin resistance peripherally (7,20,34). Studies have shown that elderly individuals commonly have elevated serum levels of TNF-α and CRP (20). TNF-α affects lipid metabolism and is correlated with high triglyceride levels and low HDL cholesterol levels (20). In a study by DeLany, TNF-α was reported to elicit a chronic inflammatory state thus contributing to the onset of cancer, heart disease, arthritis, and many other health problems (6). This may be due to the fact that TNF-α stimulate the synthesis of IL-6, which in turn stimulates the synthesis of CRP and thus contributes to the maintenance of chronic low-grade systemic inflammation, especially with the presence of obesity (15). Another study found that as BMI increases up to a BMI of 40, TNF-α also increases; above 40 there were no further increases of TNF-α (18).

Downside of Systemic Inflammation

Inflammation can cause alterations in gastrointestinal functions that last long after the acute inflammation has been resolved (20). There are nutritional consequences of cytokine-modulated actions as a result of chronic inflammation. The brain, endocrine system, liver, muscles, blood and gastrointestinal tract are target areas of cytokine-modulated behavior. The brain will experience a sickness syndrome that includes fatigue, apathy, cognitive dysfunction, anorexia, and sleepiness resulting in weight loss due to decreased food intake (2). The endocrine
system displays a euthyroid sickness, anorexia and an increase in the metabolic rate which results in muscle wasting due to decreased food intake (2). Increased synthesis of positive acute phase proteins, decreased synthesis of negative acute phase proteins, increased fatty acid synthesis, increased lipolysis and decreased lipoprotein lipase are all seen in the liver which results in increased edema and hypertriglyceridemia. Increased insulin resistance is observed in the muscles and results in hyperglycemia (2). In the blood, decreased red blood cells production occurs as well as a redistribution of albumin, pre-albumin and iron which results in anemia and increased edema (2). However, the increase in white blood cells, primarily leukocytes, results in activated inflammatory mediators which can interfere with lipolysis and thus inhibit weight loss (10). Lastly, in the gastrointestinal tract, the rate of protein breakdown increases, the gastric secretions decrease, gastrointestinal mobility slow down, and emptying time increases, this combination results in decreased protein reserves (2).

**Body Composition**

Body composition is utilized in tangent with other assessment tools to provide a precise depiction of an individual’s overall health (2). Anthropometric methods used to assess body composition are based on the theory that the body is made up of two chemically distinct sections; fat and fat-free mass (35). Areas of focus in this study are fat mass, fat percent, lean body mass, and WC. BMI was found to be independently associated with IL-6 and CRP and unfit-overweight participants had significantly higher IL-6 and CRP (19). Studies show that diet-induced weight loss reduces serum concentrations of CRP, IL-6, and TNF-α (15,20).
Fat-Free Mass

FFM consists of muscle (skeletal and non-skeletal), soft lean tissues, and the skeleton and is a mixture of water, protein, and minerals with muscle acting as the major protein source (35). FFM is a measurement used to assess body fatness or overweightness and obesity. In fact, FFM is often preferred over BMI since it is a more accurate assessment tool of LBM (36). FFM is expressed in kilograms (kg) (36).

Percent Body Fat

Body fat (BF) is the primary way the body stores energy and is sensitive to severe malnutrition (35). Percent BF is measurement used to assess body fatness in regards to overweightness and obesity (36). Often times, percent BF is preferred over BMI since it is a more accurate assessment tool of adiposity (36). Percent BF is expresses as a percentage (%) (36).

In a study by Wong et al., the levels of IL-6 and CRP were measured with weight loss and again with a decrease in percentage of body fat; the results were similar (17). The results showed that as percent BF and weight decreased both IL-6 and CRP declined (17).
Lean Body Mass

LBM is a component of body composition, calculated by subtracting body fat weight from the total body weight. This is calculated to distinguish between body fat mass and LBM (36). LBM has typically been used for prescribing proper levels of medications and for assessing metabolic disorders. (36). Research shows that LBM is superior to total body weight as a clinical measure as body fat is less relevant for metabolism (36). LBM is expressed in kg (36).

Waist Circumference

WC measurement is an established assessment of abdominal visceral adipose tissue (6,12). WC is found by measuring the distance around the smallest area below the rib cage and above the umbilicus with the use of a non-stretchable tape measure (2,12). In obese individuals, the waistline may be more difficult to locate, therefore, finding the point halfway between the inferior surface of the ribs and the top of the iliac crest may be the best location for WC measurement on obese individuals (6). Measurements over 35 inches for women and over 40 inches for men are considered independent risk factors for disease (2,6). However, these parameters may not be as useful for individuals with a BMI of 35 or more (2).

Bioelectrical Impedance Analysis

Bioelectrical Impedance Analysis (BIA) is a technique to analyze body composition and is based on the principle that, relative to water, lean tissue has a greater electrical conductivity
and lower impedance than fatty tissue due to its electrolyte content (2). BIA is a safe, non-invasive, portable and rapid method to measure body composition, particularly LBM (2,12). BIA is a reliable method for large populations (12). For the best results, the individual should be well-hydrated, abstain from alcohol, caffeine, or diuretics in the last 24 hours and abstain from exercise in the last 4 to 6 hours (2). However, fever, electrolyte imbalance, and extreme obesity may affect the reliability of the measurements (2).

**ALCAT**

**History and Purpose**

The ALCAT test utilizes a food sensitivity blood test to identify potential cellular allergic reactions for over 350 foods, chemicals and herbs (2,11,25). ALCAT is useful in identifying food intolerances in cell-mediated or delayed inflammatory reactions. These inflammatory reactions are linked to chronic health problems like obesity and diabetes, as well as skin, heart, joint, and digestive disorders (25). It is strongly recommended that the ALCAT test be followed up with a subject-specific elimination diet and clinical observation (2). ALCAT diets require the elimination of intolerant foods and thus should decrease appetite, reduce systemic inflammation, enable weight-loss and improve body composition (11,14).
ALCAT Testing

The ALCAT test measures the effect of the food on the cells in the innate immune system to determine if a food intolerance is present (10). Specifically, ALCAT is an indirect measurement of the presence of prostaglandins, cytokines, and leukotrienes released from the degranulation of leukocytes in the presence of an allergen and measures the change of leukocyte via an automated computer analysis, called the ROBOCat II (2,14). The degree of food intolerance is determined by comparing the size and volume of the leukocytes post reaction to the baseline leukocyte sample given by the individual (14). Based on the level of leukocyte reactivity when tested, the foods will be labeled as acceptable foods or as a mild, moderate, or severe intolerance (25). According to Kaats, et al., the ALCAT test was found to be an effective test that offers individualization to the patient being tested (11). Furthermore, blind studies have revealed that ALCAT is the most accurate test to support a strong correlation between clinical symptoms and food (10).

Justification for Food Elimination

The purpose of a food elimination diet is to remove the suspected food(s) from the diet for specific time period, usually 4-12 weeks, and followed by a reintroduction phase where the food is brought back into the diet. Food elimination diets are designed on an individual basis (2) and in the case of this study the elimination diets for the ALCAT group were based on the ALCAT test results. Since food intolerance results from activation of the innate immune system,
the avoidance of the problematic food(s) will result in improved gut integrity and healed gut barrier, increased liver detoxification, and better overall nutritional status (10).

Successful weight loss and effective healthy eating patterns rely heavily on proper identification of factors related to food intolerance (10). In a double blind study by Akmal et al., an 83.4% correlation was determined between ALCAT test results and a statistically significant number of patients exhibiting food sensitivity related extra-intestinal symptoms, such as migraines, irritable bowel syndrome, eczema and other conditions that are often observed as co-morbidities in obese patients (14). Kaats et al., found that the ALCAT testing and corresponding diet plan, used by the ALCAT group and compared to the control group showed positive results, such as an improved body composition and a decrease in self-reported disease symptoms (11). According to the Baylor University study, evidence supports the effectiveness of ALCAT testing in improving BMI and/or weight when the participants followed the recommended elimination diet based on ALCAT testing (14). For example, in the Baylor University study, it was shown that 98% of the participants, following the elimination diets based on ALCAT testing, lost weight, which was primarily from fat loss, and/or saw improvements in overall body composition (14). Additionally, these participants reported improvements in physical performance, sense of well-being, abdominal bloating and digestive issues (14).

**Conclusion**

Research has shown that obesity is a major cause of systemic inflammation (30). Furthermore, studies have shown that diet quality, not solely quantity of intake, plays an
important role in potential inflammation caused by foods (11,14,30). Additionally, it is important to determine if dietary patterns alone or in combination with increased adiposity have the greatest effect on systemic inflammation and body composition. Food intolerance is more common than food allergies. Research has shown that upwards of 70-80% of the US population reports having food intolerance experiences (25). Yet, intolerances to foods usually go undiagnosed due to delayed symptomology or improper format of testing. Hence, the purpose of this study was to examine what effect food elimination had on body composition and on overall body inflammatory markers using ALCAT testing.
CHAPTER 3

METHODS

Participants

One hundred forty-six participants were selected to take part in this study. Participants were recruited by posting an advertisement in the local paper, by posting flyers on campus or in fitness centers within a 50 mile radius of Northern Illinois University (NIU) DeKalb campus (Appendix A), and via social media such as Facebook and LinkedIn. Those individuals meeting the Disease Symptom Inventory (DSI) criteria of having at least two or more of the symptoms listed and rating them as “somewhat severe effect” were selected for the study. Participants completed a disease symptom inventory (Appendix B), a medical survey questionnaire (Appendix C), and an ALCAT screening form (Appendix D). Participants kept a three-day food record including two weekdays and one weekend day (Appendix E), kept an exercise log for one week (Appendix F), and had 25 mL of blood taken to assess food intolerances at baseline and levels of CRP, IL-1-β, IL-6, MPO, SAA, and TNF-α, at baseline. Participants had an additional 3.5 mL of blood taken on the last day of the 4-week long study to retest levels of CRP, IL-1-β, IL-6, MPO, SAA, and TNF-α. Those individuals who were pregnant, had hemophilia, or were under the age of 18 or over the age of 65 were ineligible to participate in the study.

All participants were informed of the risks and benefits associated with this study and
were required to give written consent to participate (Appendix G) in accordance with the study procedures approved by Northern Illinois University Institutional Review Board (Appendix H) and the Institutional Biosafety Committee (Appendix I), prior to enrollment in this study. All participants were debriefed at the end of the study (Appendix J).

**Experimental Design**

This was a randomly assigned pre- and post-test double blind experimental study. Participants were assigned to groups using a random number chart. On day one and the last day of the four-week long study, subjects reported to the nutrition laboratory at NIU to have anthropometrics and blood samples taken. The blood samples determined ALCAT results for 200 foods (only on day one), 50 functional foods and medicinal herbs, and 50 chemicals and molds and inflammatory marker levels (both pre and post study).

All participants were assigned and counseled on an individualized elimination diet plan based on ALCAT testing (n=73) or sham list of foods (n=73). This diet plan was followed for four consecutive weeks. An exercise log was completed to control for extraneous body composition changes.

**Data Collection**

The participants completed a three-day food record (two weekdays and one weekend day), a weekly exercise log, complete the disease symptom inventory and medical symptom inventory, had their height, weight, body fat percentage, lean body mass and abdominal WC measured at baseline and at the end of the month long study (Appendix K).
Anthropometry

Participants reported to the nutrition laboratory at NIU. All body measurements were taken with participants in light weight clothing and bare feet. Height was measured using a wall-mounted stadiometer (Ayrton S-100 Prior Lake, MN). Weight, FM, percent fat, and FFM were assessed using bioelectrical impedance (InBody 520, Biospace Inc. Los Angeles, CA).

BMI, using the standard equation (kilogram per meter squared), was calculated by the InBody 520.

Abdominal WC was measured with a cloth tape anteriorly halfway between the lowest lateral portion of the ribcage and the iliac crest.

Blood Samples

In addition to anthropometric data collection, participants had their blood drawn in the Nutrition Laboratory (308A Wirtz Hall) by trained phlebotomists while wearing gloves and a lab coat. After the subjects arm site was prepared by cleaning with an alcohol swab, either a 22 gauge needle or a 21 or 23 gauge butterfly was placed in an arm vein in order to obtain blood samples. After the blood was taken, the venipuncture site was covered with gauze and slight pressure was applied to the site. The site was then covered with a band-Aid. The needles and gauze products covered with blood were disposed of in clearly labeled sharp containers which were available in the Nutrition Laboratory in 308A Wirtz Hall.

Four blue top vials (4.5 mL each) containing 3.8% buffered sodium citrate and one gold top serum separator (SST) was obtained. The Blue top blood sample assessed food sensitivities using ALCAT and the gold top was used to assess inflammatory markers CRP, IL-6, IL-1β, SAA, MPO and TNF-α. The tubes were labeled with the participant's name, time and date of
collection. Blue top vials were immediately inverted several times to ensure proper mixing. The gold top vial was 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 vials were placed into the slots of the foam sleeves which were then be placed into a biohazard specimen bag. The requisition form was placed in the front pouch of the specimen bag. The strip covering the blue adhesive was removed and the bio-hazard specimen bag was sealed. The specimen bag with vials and requisition were placed into a silver insulated bag and the bag was sealed. The insulated bag was placed in the box provided by ALCAT. The specimen were placed into the pre-paid UPS Laboratory Pak and sealed tightly. The specimens were transported to the local UPS store and sent overnight to Cell Science Systems 852 South Military Trail (ALCAT headquarters) in Deerfield Beach, FL.

**Diet Analysis**

Diet analysis was performed using a three-day food log that the participants in this study completed. Participants were instructed on how to approximate serving sizes and how to identify ingredients in their food so that the most accurate analysis can occur. It was explained that the more accurate the log, the more accurate the diet analysis will be. The purpose of this was to determine if the participants were following the recommended elimination diet plan. Diets were to be analyzed to the nearest total calorie and nearest ½ gram of protein. NutritionCalc Plus (McGraw-Hill Companies, Columbus, OH, 2009) was utilized for the data entry and analysis of the three-day food log. A graduate nutrition student who had training with the system entered the data.
Statistical Analysis

Descriptive measures were analyzed using a one-way analysis of variance (ANOVA). Within and between group differences in body composition and inflammatory markers at day one 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 Bonferroni post-hoc method for multiple comparisons. This data was expressed as m ± SD. Univariate analysis of variance was utilized to examine body composition. Three response variables (lean body mass, percentage BF, and WC) were analyzed with univariate regression adjusted by age, gender, and baseline outcomes. This data was expressed as F value and significance (p) level. Statistical significance for all data analysis was accepted at the p<0.05 level of confidence. 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 this study was to determine whether the elimination of foods determined to cause an inflammatory response would lead to a decrease of inflammation and an improvement of body composition. In this double blind study, subjects were randomly assigned by Cell Science Systems to either the control group (n=59) in which the participants received a placebo elimination diet or the treatment group (n=72) in which the participants were given an accurate list of foods they had severe and moderate reactions to, based on ALCAT testing. Those foods were eliminated for 4 weeks.

Participants

One hundred forty-six participants were enrolled in the study and completed the initial data collection process. The participants were randomly assigned to the treatment or control group. Fifteen participants were withdrawn from the study due to lack of response to a scheduling email for the follow-up appointment. One hundred thirty-one participants, control group (n=72) and treatment group (n=59), completed all phases of the study.

There were no significant differences in age, BMI, percentage BF, height, WC, weight, and medical symptom questionnaire scores between participants in the
treatment or control group at the start of the study. Descriptive data is shown in Table 1 and Figure 1.

Table 1: Physical Characteristics of Subjects

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group</th>
<th>Data</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group Totals</td>
<td>Control</td>
<td>59</td>
</tr>
<tr>
<td></td>
<td>Treatment</td>
<td>72</td>
</tr>
<tr>
<td>Age (years)</td>
<td>Control</td>
<td>34.85 ± 11.52</td>
</tr>
<tr>
<td></td>
<td>Treatment</td>
<td>34.79 ± 12.91</td>
</tr>
<tr>
<td>BMI</td>
<td>Control</td>
<td>27.15 ± 6.56</td>
</tr>
<tr>
<td></td>
<td>Treatment</td>
<td>27.45 ± 5.93</td>
</tr>
<tr>
<td>Body Fat %</td>
<td>Control</td>
<td>31.35 ± 10.71</td>
</tr>
<tr>
<td></td>
<td>Treatment</td>
<td>31.59 ± 9.51</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>Control</td>
<td>168.33 ± 8.28</td>
</tr>
<tr>
<td></td>
<td>Treatment</td>
<td>167.51 ± 11.69</td>
</tr>
<tr>
<td>Waist Circumference (in)</td>
<td>Control</td>
<td>88.22 ± 13.20</td>
</tr>
<tr>
<td></td>
<td>Treatment</td>
<td>90.69 ± 17.16</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>Control</td>
<td>77.48 ± 18.66</td>
</tr>
<tr>
<td></td>
<td>Treatment</td>
<td>78.76 ± 20.58</td>
</tr>
<tr>
<td>Medical Questionnaire Day 1</td>
<td>Control</td>
<td>56.20 ± 31.22</td>
</tr>
<tr>
<td></td>
<td>Treatment</td>
<td>58.46 ± 32.49</td>
</tr>
</tbody>
</table>
Inflammatory Markers

CRP, MPO, SAA, TNF-α

Four inflammatory markers, CRP, MPO, SAA and TNF-α, showed a significant decrease at the end of the four-week study. There was a significant decrease in CRP levels for both the treatment group \((p=0.0024)\) as well as for the control group \((p=0.0259)\) during the study. \((2.44 \pm 3.83\) to \(1.09 \pm 1.35\) and \(2.54 \pm 4.43\) to \(1.18 \pm 1.24\), respectively). Similarly, there was a significant decrease in MPO levels for both the treatment group \((p<0.0001)\) as well as for the control group \((p<0.0001)\) during the study \((302.78 \pm 245.85\) to \(181.06 \pm 134.39\) and \(352.00 \pm 288.25\) to \(167.81 \pm 112.00\), respectively). Likewise, there was a significant decrease in TNF-α levels for both the treatment group \((p<0.0001)\) as well as for the control group \((p<0.0009)\) during the study. \((0.81 \pm 0.50\) to \(0.67 \pm 0.32\) and \(0.74 \pm 0.14\) to \(0.65 \pm 0.14\), respectively). However, there was a significant decrease in SAA levels for the treatment group \((p=0.0017)\) but not for the
control group (p=0.0761) during the study. (51.08 ± 46.96 to 30.92 ± 37.91 and 42.60 ± 40.68 to 31.55 ± 35.10, respectively). Full results can be found in Table 2 and Figures 2-4.

**IL-1-β and IL-6**

There were no significant decreases in IL-1-β or IL-6 levels for either the treatment or the control group during the 4 week study.

**Comparison of Changes in Inflammatory Markers**

When analyzing the levels of inflammatory markers at day one and day thirty for control and treatment groups, mixed results were found. There was a significant decrease in CRP, MPO, TNF-a in both the control (p=0.0259, p<0.001, p=0.009, respectively) and treatment groups (p=0.0024, p<0.001, p<0.001, respectively). There was a significant decrease in SAA for the treatment group (p=0.0017) but not the control group. There was no significant decrease in IL-1-β or IL-6 noted for either the control or the treatment group. See Figure 4.
Table 2: Inflammatory Marker Changes over the Course of the 4-Week Study

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group</th>
<th>Day 1</th>
<th>Day 30</th>
<th>Comparison - Day1 &amp; Day 30</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRP</td>
<td>Control</td>
<td>2.54 ± 4.43</td>
<td>1.18 ± 1.24</td>
<td>1.36 ± 4.57*</td>
</tr>
<tr>
<td></td>
<td>Treatment</td>
<td>2.44 ± 3.83</td>
<td>1.09 ± 1.35</td>
<td>1.35 ± 3.63*</td>
</tr>
<tr>
<td>IL-1-β</td>
<td>Control</td>
<td>0.46 ± 0.36</td>
<td>0.40 ± 0.47</td>
<td>0.06 ± 0.60</td>
</tr>
<tr>
<td></td>
<td>Treatment</td>
<td>0.63 ± 1.76</td>
<td>0.45 ± 0.98</td>
<td>0.19 ± 0.81</td>
</tr>
<tr>
<td>IL-6</td>
<td>Control</td>
<td>3.34 ± 1.03</td>
<td>3.25 ± 0.68</td>
<td>0.08 ± 0.62</td>
</tr>
<tr>
<td></td>
<td>Treatment</td>
<td>3.62 ± 2.59</td>
<td>3.60 ± 1.51</td>
<td>0.02 ± 1.87</td>
</tr>
<tr>
<td>MPO</td>
<td>Control</td>
<td>352.00 ± 288.25</td>
<td>167.81 ± 112.00</td>
<td>184.2 ± 278.4*</td>
</tr>
<tr>
<td></td>
<td>Treatment</td>
<td>302.78 ± 245.85</td>
<td>181.06 ± 134.39</td>
<td>121.7 ± 221.3*</td>
</tr>
<tr>
<td>SAA</td>
<td>Control</td>
<td>42.60 ± 40.68</td>
<td>31.55 ± 35.10</td>
<td>11.05 ± 47.02</td>
</tr>
<tr>
<td></td>
<td>Treatment</td>
<td>51.08 ± 46.96</td>
<td>30.92 ± 37.91</td>
<td>20.15 ± 52.37*</td>
</tr>
<tr>
<td>TNF-α</td>
<td>Control</td>
<td>0.74 ± 0.14</td>
<td>0.65 ± 0.14</td>
<td>0.09 ± 0.19*</td>
</tr>
<tr>
<td></td>
<td>Treatment</td>
<td>0.81 ± 0.50</td>
<td>0.67 ± 0.32</td>
<td>0.14 ± 0.22*</td>
</tr>
</tbody>
</table>

*indicates p<0.05

Figure 2: Inflammatory Markers at Day One of the 4-Week Study
**Figure 3:** Inflammatory Markers at Day Thirty of the 4-Week Study

**Figure 4:** Comparison of Change in Inflammatory Markers at the End of the 4-Week Study
Body Composition

Univariate Analysis of Variance was utilized to examine body composition. Three response variables (lean body mass, body fat percentage, and WC) were analyzed with univariate regression adjusted by age, gender, and baseline outcomes.

Lean Body Mass

There was a significant increase in LBM from day one to day thirty (F=5.793, p=0.018). According to the estimated marginal means of LMB at day thirty, the treatment group had a higher LBM mean than the placebo group. However, when factoring in age and gender and when comparing the treatment and control groups in relation to LBM, no significant correlations were found. Full results can be found in Table 3. See Figure 5.

Body Fat Percentage

Overall, a significant decrease in body fat percent was found (F=527.628, p<0.001). There was a significant decrease in body fat percent from day one to day thirty (F=2099.220, p<0.001). According to the estimated marginal means of body fat percent at day thirty, the treatment group had a higher body fat percentage mean than the placebo group. When factoring in age and gender and when comparing the treatment and control groups in relation to body fat percentage, no significant correlations were found. See Figure 6.
Waist Circumference

Overall, a significant decrease in WC was found (F=145.605, p<0.001). There was a significant decrease in overall WC from day one to day thirty (F=562.339, p<0.001). According to the estimated marginal means of WC at day thirty, the treatment group had a lower WC mean than the placebo group. However, when factoring in age and gender and when comparing the treatment and control groups in relation to body fat percentage, no significant correlations were found. See Figure 7.

Table 3: Changes in Body Composition over the Course of the 4-Week Study

<table>
<thead>
<tr>
<th>Variable</th>
<th>Comparison Factor</th>
<th>F Value</th>
<th>Significance Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>LBM D30</td>
<td>Overall change</td>
<td>1.990</td>
<td>0.085</td>
</tr>
<tr>
<td></td>
<td>Age</td>
<td>0.968</td>
<td>0.327</td>
</tr>
<tr>
<td></td>
<td>LBM D1</td>
<td>5.793</td>
<td>0.018*</td>
</tr>
<tr>
<td></td>
<td>Group</td>
<td>0.295</td>
<td>0.588</td>
</tr>
<tr>
<td></td>
<td>Gender</td>
<td>0.213</td>
<td>0.645</td>
</tr>
<tr>
<td></td>
<td>Group &amp; Gender</td>
<td>0.133</td>
<td>0.716</td>
</tr>
<tr>
<td>Body Fat % D30</td>
<td>Overall change</td>
<td>527.628</td>
<td>0.000*</td>
</tr>
<tr>
<td></td>
<td>Age</td>
<td>0.017</td>
<td>0.896</td>
</tr>
<tr>
<td></td>
<td>Body Fat % D1</td>
<td>2099.220</td>
<td>0.000*</td>
</tr>
<tr>
<td></td>
<td>Group</td>
<td>0.957</td>
<td>0.330</td>
</tr>
<tr>
<td></td>
<td>Gender</td>
<td>3.341</td>
<td>0.070</td>
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<tr>
<td></td>
<td>Group &amp; Gender</td>
<td>0.637</td>
<td>0.426</td>
</tr>
<tr>
<td>WC D30</td>
<td>Overall change</td>
<td>145.605</td>
<td>0.000*</td>
</tr>
<tr>
<td></td>
<td>Age</td>
<td>0.601</td>
<td>0.440</td>
</tr>
<tr>
<td></td>
<td>WC D1</td>
<td>562.339</td>
<td>0.000*</td>
</tr>
<tr>
<td></td>
<td>Group</td>
<td>0.014</td>
<td>0.906</td>
</tr>
<tr>
<td></td>
<td>Gender</td>
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<td>0.292</td>
</tr>
<tr>
<td></td>
<td>Group &amp; Gender</td>
<td>0.475</td>
<td>0.492</td>
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</tbody>
</table>

*= statistically significant with p<0.05.
Covariates appearing in the model are evaluated at the following values: Age = 34.82, LBM D1 = 52.3975

**Figure 5:** Estimated Marginal Means of Lean Body Mass at Day 30

Covariates appearing in the model are evaluated at the following values: Age = 34.82, Fat %D1 = 31.4865

**Figure 6:** Estimated Marginal of Means of Body Fat Percentage at Day 30
Figure 7: Estimated Marginal of Means of Waist Circumference at Day 30

MQ Day 1 and MQ Day 30

A Pearson’s correlation was run to determine the relationship between 131 participants’ MQ Day one and MQ day thirty scores. Pearson correlation for the data revealed a strong, positive correlation between MQ Day One and MQ Day 30 (r=0.690, n=131, p<0.01, two-tails).

Dietary Factors

Diet Compliance

Both treatment and control groups were provided an elimination diet to follow for four consecutive weeks. The treatment group was found to have 88.9% dietary compliance (n=64), whereas the placebo group was found to have 98.3% compliance (n=58). Only a total of 6.9%
non-compliance (n=9) existed for both groups combined. Full results can be found in Table 4. See Figure 8.

**Table 4: Dietary Factors over the Course of the 4-Week Elimination Diet**

<table>
<thead>
<tr>
<th>Factor</th>
<th>Group</th>
<th>Total in Group</th>
<th>Number Participants</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dietary Compliance</td>
<td>Control</td>
<td>59</td>
<td>58</td>
</tr>
<tr>
<td></td>
<td>Treatment</td>
<td>72</td>
<td>64</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>131</td>
<td>122</td>
</tr>
<tr>
<td>Gluten Intolerance</td>
<td>Control</td>
<td>59</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td>Treatment</td>
<td>72</td>
<td>48</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>131</td>
<td>75</td>
</tr>
<tr>
<td>Fluoride Intolerance</td>
<td>Control</td>
<td>59</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>Treatment</td>
<td>72</td>
<td>33</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>131</td>
<td>53</td>
</tr>
<tr>
<td>Glyphosate Intolerance</td>
<td>Control</td>
<td>59</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>Treatment</td>
<td>72</td>
<td>39</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>131</td>
<td>47</td>
</tr>
</tbody>
</table>

**Figure 8: Dietary Compliance over the Course of the 4-Week Study**
Gluten Intolerance

Both treatment and control groups were provided an elimination diet to follow for four consecutive weeks. The treatment group was found to have 66.7% of participants with gluten intolerance (n=48), whereas the placebo group was found to have 45.8% of participants with an intolerance to gluten (n=27). No gluten intolerance was found for 42.7% of both groups combined (n=56). See Figure 9.

![Gluten Intolerance (n=131)](image)

**Figure 9:** Gluten Intolerance Discovered During the 4-Week Study

Fluoride Intolerance

From the treatment and control groups, fifty-three participants were found to have fluoride intolerance. The treatment group was found to have 45.8% fluoride intolerance (n=33), whereas the placebo group was found to have 34.5% fluoride intolerance (n=20). No Fluoride intolerance was found for 59.2% of both groups combined (n=77). See Figure 10.
Figure 10: Fluoride Intolerance Discovered During the 4-Week Study

Glyphosate Intolerance

From the treatment and control groups, 47 participants were found to have glyphosate intolerance. The treatment group was found to have 54.2% glyphosate intolerance (n=39), whereas the placebo group was found to have 13.8% glyphosate intolerance (n=8). See Figure 11.

Figure 11: Glyphosate Intolerance Discovered During the 4-Week Study
CHAPTER 5

DISCUSSION

This was the first study to test whether food elimination based on ALCAT testing would alter body composition and decrease body inflammation. Unfortunately, none of the nine hypotheses tested were supported as evidenced by the results. The findings of this study suggest both groups had significant decreases for three of the six inflammatory markers, CRP, MPO, TNF-α. In addition, SAA was found to significantly decrease in the treatment group only. However, when comparing the treatment and control groups for each inflammatory marker, there were no significant differences between them. Similarly, both treatment and control groups had significant decreases for LBM, percentage BF, and WC from day one to day thirty. The findings of this study concur with other studies in that a decrease in WC is strongly correlated with decreased levels of CRP (29,30). Previous studies also have found that diet quality was inversely associated with CRP and SAA levels such that as diet quality improved, levels of CRP and SAA decreased (21). The findings of this study differ from previous studies that found a significant treatment effect with reduction in IL-6 and TNF-α based on ALCAT testing and associated elimination diets (10). Additional studies have shown elimination diets based on ALCAT testing to significantly improve body composition, specifically decreased body fat percent, and increased lean body mass (11).
**Inflammatory Markers**

Both the treatment group and the control groups experienced significant decreases in CRP, MPO, and TNF-α; yet, the treatment group was not found to be statistically different than the control group. Studies have shown that diet induced weight loss decreases CRP, IL-6, TNF-α, which may provide some rationale for the observed decreases in CRP & TNF-α along with improved body composition (20,30). According to Rexrode, et al., CRP is more strongly correlated with WC than IL-6, which may explain a decrease in CRP and not IL-6 (29,30).

Additionally, the treatment group was found to have a significant decreased in SAA, whereas the control group showed no significant change in SAA levels. SAA is inversely associated with diet quality and obesity, where a high quality diet and decrease in adipose tissue was associated with lower levels of SAA or CRP (21). No significant findings were associated with IL-β-1 and IL-6 for either group.

Overall, there was little evidence to support a treatment effect of elimination diets based on ALCAT testing on the decreased levels of overall body inflammation. This is most likely due to the inclusion of chemicals, molds, medicinal herbs, and functional foods, in addition to food for the elimination diets. It is suspected that lack of knowledge of foods containing these items decreased dietary compliance. Although not part of the initial focus of this study, an unexpected and interesting finding was that dietary compliance, and specific intolerances to gluten, fluoride and glyphosate, may have skewed the final results. Dietary compliance is importance to consider, especially when analyzing the aforementioned intolerances because the control group was more compliant (98.3%) than the treatment group (88.9%). In regards to gluten intolerance, about half of control group were found to have gluten intolerance. Therefore, if told to avoid similar items (wheat, rye, barley, malt) from green list that are similar or contain gluten, then the avoidance of
gluten all together will decrease inflammation. This potentially skewed the treatment effect.

Conversely, about half of treatment group was fluoride and glyphosate intolerant. If the treatment participants were not avoiding tap water (drinking, cooking or adding to foods) or foods containing glyphosate, this could have also skewed results and decreased the treatment effect. The skewed results would also occur if the control group, which had a better compliance rate, was avoiding glyphosate.

Furthermore, because the control group eliminated ingredients found in numerous other products, total body inflammation decreased; thus creating skewed result of the treatment effect. For example, sunflower oil is an ingredient found in many commercial food items. In order to effectively eliminate sunflower oil, fast foods and commercial snack foods also must be eliminated. Although the individual intolerant to the sunflower oil, eating a healthier, higher quality diet leads to a reduction of adiposity which can further lead to decreased levels of body inflammation (2,9,21). Had this not occurred, it is suspected that a significant treatment effect would have been evident.

**Body Composition**

Analysis of body composition revealed a significant decrease in lean body mass, body fat percentage, and WC from day one to day thirty; however, the treatment group was not found to be statistically different from the control group even after adjustment for age, gender and baseline outcomes. The findings of this study differ from previous studies that found a significant positive correlation between elimination diets based on ALCAT testing and improved body composition (11,14). One reason may be in how the elimination diets for the treatment group were designed. For example, the treatment group only received a list of foods to avoid...
from the most severe and moderate categories and not the mild category. Had the treatment elimination diets included the mild category, it is suspected that a significant treatment effect in regards to decreased inflammation and improved body composition would have been found. Additionally, the control group received a placebo list of foods to avoid but some lists included food items that closely resembled or were associated with foods on the participant’s accurate list. This created mixed results and thus no significant difference between the treatment and control groups.

**Medical Symptom Questionnaire**

Comparison of the day one with the day thirty medical symptom questionnaire revealed a strong, positive correlation between day one and day thirty. Collectively, medical symptomology improved over the course of the four-week study for a large percentage of the 131 participants. No analyses were run to determine which group showed the greatest improvement. Similar studies have examined the relationship of medical symptomology and food sensitivities with a Disease Symptom Inventory (DSI), however, this study utilized the self-reported Medical Symptom Questionnaire (MQ) to assess such a relationship (11).

**Dietary Factors**

In regards to dietary compliance, the control group was found to have a higher percentage of dietary compliance than the treatment group. This may partly be due to the complexity of the elimination diets as the diets included foods, functional foods, medicinal herbs, chemicals and molds. Nutritional education was provided to participants along with the elimination diets to ascertain foods associated with functional foods, medicinal herbs, chemicals and molds just as
was done in previous studies (11). Other intolerances to gluten, fluoride, and glyphosate were present in both groups. Despite offering nutrition education, lack of knowledge or understanding regarding foods containing gluten and how these chemicals affect certain foods or beverages may have decreased dietary compliance unknowingly on behalf of the participants.

Approximately 65% of the participants that finished the study completed a 3-day food log. Due to the lack of return rate, the results were not analyzed as these numbers may not be entirely representative of the group as a whole.

**Conclusion**

Overall, there was little evidence to support a treatment effect of elimination diets based on ALCAT testing on the decrease of overall body inflammation and body composition. This is most likely due to how the elimination diets were administered, as the items to avoid were vague and overwhelming, along with poor dietary compliance of the treatment group. Furthermore, it is suspected that a significant treatment effect in regards to decreased inflammation and improved body composition would have been found, if the following conditions were met: 1) only food/beverage items were included in the elimination diets (exclude chemicals, molds, medicinal herbs, and functional foods, 2) the treatment elimination diets included the mild category and 3) the placebo lists of foods to avoid were clearly different from the accurate list of foods with no overlapping food items.
CHAPTER 6

LIMITATIONS AND FUTURE RESEARCH

Limitations

The ALCAT testing consisted of 200 foods, 50 functional foods and medicinal herbs, and 50 chemicals and molds. As a result, the elimination diets were complex; requiring a lot of education to make sure participants knew exactly foods/beverages were associated with the functional foods, medicinal herbs, chemicals and molds and thus what to avoid. Future studies might consider ALCAT testing for foods only to increase participant diet compliance and an improved treatment effect. It is also recommended that elimination diets specifically list food items to be avoided to increase diet compliance.

Additionally, mixed treatment results were observed due to conflicting placebo elimination diets. Many of the placebo elimination diets given to participants in the control group included items closely related to, or contained in food/beverage items with which the participant was intolerant. For example, baker’s yeast was on multiple placebo lists where the participants had gluten as a reactant food on the true results; by eliminating food/beverage items with baker’s yeast, gluten was also eliminated. This resulted in the participants experiencing a slight treatment effect as evidenced by decreased MQ day 30 scores. It is recommended that future studies use
extreme caution when assigning placebo elimination diets so that foods to be avoided during the course of the study are not associated with true reactant food items.

Conversely, the elimination diets provided to the treatment group were not inclusive enough. The treatment group received reactant items to avoid from the most severe (red) and the moderately severe (orange) intolerance lists, but not from the mildly severe (yellow) list. As a result, more items from the mildly severe list have the chance to be consumed more frequently, have the potential to become a moderate or severe intolerance and thus create more inflammation in the body while simultaneously preventing improved body composition. It was also noted that many participants had extensive mild intolerance lists, yet relatively minimal severe and moderate intolerance lists. Due to the continued consumption of the mildly intolerant items, inflammation in the body increased while simultaneously preventing improved body composition. Both of these circumstances allowed for mixed results of treatment effect.

Participants were asked to maintain their exercise regimen throughout the study based on the self-reported activity level on the day one exercise log. The pre- and post-study exercise log was used to ensure this consistency, however, many participants reported changing the exercise regimen during the study. Additionally, not all participants returned the day thirty exercise log. Studies using a more accurate protocol to monitor exercise regimen may yield different results.

**Future Research**

A future study could use the protocol for a four-week elimination diet but simplify the content of the elimination diet to food/beverage items only. A simpler, more specific list of foods to eliminate would better assist the participants with dietary compliance. A more inclusive elimination diet for the treatment group which included severe, moderate and mild reactant foods
would better encompass the factors suspected to increase inflammation and allow for a more accurate treatment effect when comparing the control and treatment groups. Future studies could use the same protocol for Three-Day Food Logs but employ a more stringent plan to increase the return rate. A future study could use a more accurate protocol to monitor exercise which may yield different results.
REFERENCES


25. ALCAT: Available for over 25 years. Available at: [https://www.alcat.com/](https://www.alcat.com/)


35. Gibson RS. *Principles of Nutritional Assessment.* Oxford University Press. 1990:182

APPENDIX A
RECRUITMENT FLYER
ATTENTION: Adults 18 to 75 years of age

Do you crave sweets?

Do you experience digestive issues after eating certain foods?

Does your body ache and you cannot figure out why?

If you have answered YES to any of these questions, you might be eligible to participate in a Nutrition Research Project in Spring 2014

Who’s Requested?
- Males and Females aged 18 years to 65 years of age
- People with food intolerances, nasal stuffiness, chronic tiredness, GERD or eczema.
- People with recurrent anxiety, recurrent depression, insomnia or stressed out
- Exclusion criteria includes pregnant women and people with bleeding disorders

What is Required of You?
- Complete a Disease Symptom Inventory and Medical Symptom Questionnaire
- Complete pre- and post- study blood work & body composition measurements
- Follow an individualized elimination diet for 4 weeks
- Complete 3-day food log and weekly exercise log

Why?
- To determine effect of food elimination on body inflammation and body composition

What are the Benefits to you?
- FREE ALCAT allergy test for 200 foods - $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

Who to Contact?
- Dawn Herbig at elimdietstudy@yahoo.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
2 = A Mild Effect
4 = A Severe Effect
1 = A Very Mild Effect
3 = A Somewhat 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: ________________________________)
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

**HEAD**
- _______ Headaches
- _______ Faintness
- _______ Dizziness
- _______ Insomnia
  Total _______

**EYES**
- _______ Watery or itchy eyes
- _______ Swollen, reddened or sticky eyelids
- _______ Bags or dark circles under eyes
- _______ Blurred or tunnel vision
  (does not include near or far-sightedness)
  Total _______

**EARS**
- _______ Itchy ears
- _______ Earaches, ear infections
- _______ Drainage from ear
- _______ Ringing in ears, hearing loss
  Total _______

**NOSE**
- _______ Stuffy nose
- _______ Sinus problems
- _______ Hay fever
- _______ Sneezing attacks
- _______ Excessive mucus formation
  Total _______

**MOUTH/THROAT**
- _______ Chronic coughing
- _______ Gagging, frequent need to clear throat
- _______ Sore throat, hoarseness, loss of voice
- _______ Swollen or discolored tongue, gums, lips
- _______ Canker sores
  Total _______

**SKIN**
- _______ Acne
- _______ Hives, rashes, dry skin
- _______ Hair loss
- _______ Flushing, hot flashes
- _______ Excessive sweating
  Total _______

**HEART**
- _______ Irregular or skipped heartbeat
- _______ Rapid or pounding heartbeat
- _______ Chest pain
  Total _______
<table>
<thead>
<tr>
<th>Category</th>
<th>Symptoms</th>
<th>Total</th>
</tr>
</thead>
<tbody>
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<td><strong>LUNGS</strong></td>
<td>Chest congestion</td>
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</tr>
<tr>
<td></td>
<td>Asthma, bronchitis</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Shortness of breath</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Difficulty breathing</td>
<td></td>
</tr>
<tr>
<td><strong>DIGESTIVE TRACT</strong></td>
<td>Nausea, vomiting</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Diarrhea</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Constipation</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Bloating feeling</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Belching, passing gas</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Heartburn</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Intestinal/stomach pain</td>
<td></td>
</tr>
<tr>
<td><strong>JOINTS/MUSCLE</strong></td>
<td>Pain or aches in joints</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Arthritis</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Stiffness or limitation of movement</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pain or aches in muscles</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Feeling of weakness or tiredness</td>
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</tr>
<tr>
<td><strong>WEIGHT</strong></td>
<td>Binge eating/drinking</td>
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</tr>
<tr>
<td></td>
<td>Craving certain foods</td>
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</tr>
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<td></td>
<td>Excessive weight</td>
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<td>Compulsive eating</td>
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<td></td>
<td>Water retention</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Underweight</td>
<td></td>
</tr>
<tr>
<td><strong>ENERGY/ACTIVITY</strong></td>
<td>Fatigue, sluggishness</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Apathy, lethargy</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Hyperactivity</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Restlessness</td>
<td></td>
</tr>
<tr>
<td><strong>MIND</strong></td>
<td>Poor memory</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Confusion, poor comprehension</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Poor concentration</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Poor physical coordination</td>
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</tr>
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<td></td>
<td>Difficulty in making decisions</td>
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</tr>
<tr>
<td></td>
<td>Stuttering or stammering</td>
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</tr>
<tr>
<td></td>
<td>Slurred speech</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Learning disabilities</td>
<td></td>
</tr>
<tr>
<td><strong>EMOTIONS</strong></td>
<td>Mood swings</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Anxiety, fear, nervousness</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Anger, irritability, aggressiveness</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Depression</td>
<td></td>
</tr>
<tr>
<td><strong>OTHER</strong></td>
<td>Frequent illness</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Frequent or urgent urination</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Genital itch or discharge</td>
<td></td>
</tr>
</tbody>
</table>

**GRAND TOTAL**

**TOTAL**
APPENDIX D
ALCAT SCREENING AND 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: ______________________
APPENDIX E
FOOD LOG SHEET
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.**

<table>
<thead>
<tr>
<th>Time:</th>
<th>Food Consumed &amp; Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>7:45 am</td>
<td>1 whole egg with 2 egg equivalents of egg beaters</td>
</tr>
<tr>
<td></td>
<td>2 slices of Brownberry 12-Grain bread</td>
</tr>
<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>
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 Dawn Herbig, 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 nine inflammatory markers: C-Reactive Protein (CRP), Fibrinogen, Interleukin-1 beta (IL-1 beta), Interleukin-6 (IL-6), Myeloperoxidase (MPO), Receptor Activator of Nuclear Factor Kappa-B Ligand (RANKL), Serum Amyloid A (SAA), Tumor Necrosis Factor alpha (TNF-alpha), and Zonulin. 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 light weight 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. 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 (815) 753-6352 or Dawn Herbig (847) 927-7808. 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 (815) 753-8588.

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
COMPLETE IRB APPLICATION
Application for Institutional Review of Research
IN Volving H uman S ubjects

Note: Please complete this form thoroughly keeping in mind that the primary concern is the potential risk (economic, ethical, legal, physical, political, psychological/emotional, social, breach of confidentiality, or other) to the participants. Provide copies of all materials to be used in the investigation. The Institutional Review Board (IRB) must have enough information about the transactions with the participants to evaluate the risks of participation.

Name(s) and employee ID for faculty, Z-ID for students
Dr. Judith Lukaszuk (A111938), Dawn M. Herbig (Z916080), Dr. Josephine Umoren(A101184), Dr. Masih Shoikani (A135885)

Status: ☐ Faculty ☐ Graduate Student ☐ Undergraduate Student

Department:
Family Consumer and Nutrition Sciences and Medical Lab Sciences Program

Mailing Address (if not department):
FCNS

Phone: 815-753-6352 E-mail jnlukaszuk@niu.edu

Project Title:
FOOD ELIMINATION BASED ON ALCAT TESTING AND ITS EFFECT ON BODY INFLAMMATION AND BODY COMPOSITION

Proposed Data Collection Start Date: September, 2013

Note: Unless the authorized departmental reviewer (e.g., chair or designee) has deemed on the screening form that IRB review is not needed, all projects must receive formal written clearance from the IRB Chair (or an IRB member designated by the Chair) prior to the start of data collection.

Type of Project (Check one)
☐ Departmental Research (faculty/student projects not externally funded and not indicated below)

☐ Graduate Thesis/Dissertation (IRB application should be submitted AFTER proposal defense)
Advisor/Committee Chair (& e-mail):

☐ Undergraduate Project (Senior thesis/capstone, research rookies, independent study)
Advisor/Committee Chair (& e-mail):

☐ Externally Sponsored Research
A complete copy of the grant proposal or contract must accompany this application form for IRB review to take place.

• Source of Funding:
American Medical Testing Laboratories with Cell Science Systems, Corp.

• Title of grant proposal (if different from IRB protocol):
Food Elimination Based on ALCAT Testing and its Effects on Body Inflammation and Body Composition

• Name of principal investigator on grant proposal:
Dr. Judith Lukaszuk

• Office of Sponsored Projects file number (Note: this is not the grant number):
CSP:

☐ Other
Specify:
Part I: Purpose and Procedures:

1) Describe the purpose of your study and the reason(s) this study is needed. Include any necessary background information and a description of your hypothesis or your research question.

The purpose of this study is to determine what effect food elimination based on Antigen Leukocyte Cellular Antibody Test (ALCAT) testing versus food elimination based on a placebo (PL) will have on body composition and on weight body inflammatory markers. The objectives of this study are to measure IL-6, TNF-alpha, IL-1 beta, myeloperoxidase (MPO), hs-CRP, SAA, RANKL, Fibrinogen and Zonulin; body composition measurements FFM, LBM, body fat % and fat mass; Disease Symptom Inventory (DSI) scores and medical symptom questionnaire scores within and between subjects pre versus post food elimination based on ALCAT or placebo. There are limited studies available regarding this topic, which is why this study is necessary.

Hypothesis 1 (H1): Subjects randomly assigned to the ALCAT treatment group will have lower IL-6 at the end of the study versus those receiving the PL.
Hypothesis 2 (H2): Subjects randomly assigned to the ALCAT treatment group will have lower TNF-alpha at the end of the study versus those receiving the PL.
Hypothesis 3 (H3): Subjects randomly assigned to the ALCAT treatment group will have lower IL-1 beta at the end of the study versus those receiving the PL.
Hypothesis 4 (H4): Subjects randomly assigned to the ALCAT treatment group will have lower Myeloperoxidase (MPO) at the end of the study versus those receiving the PL.
Hypothesis 5 (H5): Subjects randomly assigned to the ALCAT treatment group will have lower hs-CRP at the end of the study versus those receiving the PL.
Hypothesis 6 (H6): Subjects randomly assigned to the ALCAT treatment group will have a lower SAA levels at the end of the study versus those receiving the PL.
Hypothesis 7 (H7): Subjects randomly assigned to the ALCAT treatment group will have lower Fibrinogen levels at the end of the study versus those receiving the PL.
Hypothesis 8 (H8): Subjects randomly assigned to the ALCAT treatment group will have lower RANKL levels at the end of the study versus those receiving the PL.
Hypothesis 9 (H9): Subjects randomly assigned to the ALCAT treatment group Zonulin will change at the end of the study versus those receiving the placebo.
Hypothesis 10 (H10): Subjects randomly assigned to the ALCAT treatment group will have a lower fat mass, and fat percent at the end of the study than those receiving the placebo.
Hypothesis 11 (H11): Subjects randomly assigned to the ALCAT treatment group will have a lower lean body mass at the end of the study than those receiving the placebo.
Hypothesis 12 (H12): Subjects randomly assigned to the ALCAT treatment group will have a lower abdominal waist circumference at the end of the study than those receiving the placebo.

2) The following items will help the IRB reviewers understand the step-by-step procedures of your study:

2A) Explain the participant eligibility and exclusion criteria that will be used.

Those individuals meeting the Disease Symptom Inventory (DSI) criteria of having at least two or more of the symptoms listed and rating them as "somewhat severe effect" will be selected for the study. Subjects must be willing to fill in a disease symptom inventory, keep a 3 day food record (2 weekdays and 1 weekend day) an exercise log, and fill in an ethnicity questionnaire. They must be willing to complete a Medical Symptoms Questionnaire on days 1, at the end of the study and 12 weeks after study completion. They must be willing to have 25 mL (Day 1) 21.5 mL (at the end of the study) of blood taken to assess food intolerances), hs-CRP, Receptor activator of nuclear factor kappa-B ligand (RANKL), serum amyloid A (SAA), Fibrinogen, Zonulin, MPO, IL-6, IL-1 beta and TNF-alpha on day 1 and at the end of the study. Subjects four week time period will begin once they receive the results of their ALCAT test (approximately one week after the blood samples are sent off to be evaluated). The participants will be sent an e-mail of foods they need to avoid for the next four weeks. Those individuals who are pregnant or who have bleeding disorders will be ineligible to participate in the study.

2B) Explain the recruitment procedures (how will participants learn about the study?). If using the snowballing technique, please explain who contacts potential participants (other participants or the researcher).

*Please attach recruitment scripts, flyers, or postings [Appendix A]*

One hundred forty subjects will be selected to take part in this study. Subjects will be recruited by posting an advertisement in the local paper and by posting flyers on campus or in fitness centers within a 50 mile radius of Northern Illinois University (NIU) DeKalb campus. Recruitment flyers will also be posted via social media, such as Facebook, and sent via e-mail.
2C) Explain the consent process (verbal and/or written procedures for informing participants of the nature of the study and what they will do).

[Please attach all documents (assent, consent, parent permission – Appendix B) that are appropriate for each group of subjects participating in the study. Consent forms should be prepared for adult participants (age 18 or over). Assent forms should be prepared for minor subjects appropriate to their ages, and permission form(s) for parents or legally authorized representatives should also be prepared. For children too young to comprehend a simple explanation of participation, parental permission is sufficient only if the research will provide direct benefit to the subject, a member of the subject's family, or other children with the same condition as the subject.]

All participants will be informed of the risks and benefits associated with this study and will be required to give written consent to participate in accordance with the Institutional Review Board at Northern Illinois University. See Appendix B for the written consent form to be used.

2D) Describe the data collection procedures including what data will be collected, how it will be collected (include a description of any interventions to be used), the duration of participation in the study session(s), and how the session(s) will end.
Voluntary participants including students, faculty and staff from Northern Illinois University and residents within a 50 mile radius of the DeKalb campus will be recruited for this pre and post-test double blind randomized experimental study. Subjects will be assigned to groups using a random number chart. Subjects will report to the nutrition laboratory (Wirtz hall room 308A) at NIU. All body measurements will be taken with subjects in light weight clothing and bare feet. Height will be measured using a wall-mounted stadiometer. Weight, fat mass, percent fat, and FFM will be assessed using bioelectrical impedance (Biospace 520). Abdominal circumference will be measured with a cloth tape anteriorly halfway between the lowest lateral portion of the ribcage and the iliac crest. For anthropometry measurements, the subjects will complete a 3-day food record including 2 weekdays and 1 weekend day(Appendix C), an exercise log(Appendix C), ethnicity form (Appendix C) complete the disease symptom inventory (Appendix C) and medical symptom inventory(Appendix C), have their height, weight, body fat percentage, lean body mass and abdominal waist circumference measured at baseline and at the end of the month long study(Appendix C). Two exceptions to above is that the Medical Symptom Questionnaire will be sent to the participants via e-mail 12 weeks after study completion to fill in again and subjects will have a medication and food allergy and intolerance screening form (Appendix C) they will need to complete on only Day 1 of the study. The medications will be screened to determine if an individual is taking blood thinners. If so the laboratory personnel who will be taking blood from that subject will be notified of this information prior to the blood draw. On Days 1 and at the end of the study subjects will report to the Nutrition Lab at NIU to have their body composition and blood taken to determine food intolerances using the ALCAT test and to determine their inflammatory state by checking IL-6, TNF-Alpha, RANKL, SAA, IL-1 Beta, hs-CRP, Zonulin and MPO. Blood samples will be drawn by Dr. Shokrani (certified medical technologist), Jeanne Isabel (Director of the Medical Lab Sciences program), Ellen Olsen (Student Lab Coordinator and phlebotomist) and Laura Lemons (phlebotomist) while wearing gloves. After the subjects arm site is prepared by cleaning with an alcohol swab either a 22 gauge needle or a 21 or 23 gauge butterfly will be placed in an arm vein in order to obtain blood samples. The site of the venipuncture after the blood is taken will be covered with gauze and slight pressure will be applied to the site. The sight will then be covered with a Bandaid. The needles and gauze products covered with blood will be disposed of in clearly labeled biohazard containers which are available in the Nutrition Lab Wirtz hall 308A..

Four blue vials (4.5 mL each) will be filled after access is obtained via an arm vein in order to determine food sensitivities. The labeled tubes will have the participant's name and study number. All tubes used for the ALCAT test will be placed in plastic Ziploc biohazard bags and then placed in a box that has been provided by the ALCAT. All pre-labeled vials of blood will be FED EXED in the provided box overnight to ALCAT to assess for food intolerances.

In the same venipuncture site, blood samples will be collected in (1-2) 3.5 ml goldtop tubes (SST tubes). On Day one of the study 2(3.5 mL) gold top vials will be filled. The extra tube is needed to validate the blood sample values. At the end of the study only one gold top vial will be needed. The labeled tubes will have the participant's name and study number. The blood in the SST tubes will be used to assess inflammatory markers (IL-6, IL-1β, TNF-Alpha, MPO, hs-CRP, SAA, RANKL, Zonulin and Fibrinogen). On the first day of the study the blood for one of the gold tubes will be spun in a centrifuge to separate the serum from the whole blood, the other gold top tube will be sent back as whole blood. Either one (end of study) or two gold tubes (Day one of study) will be placed in the biohazard bag with the other blood samples, sealed in the ALCAT test kit box and FED EXED overnight to ALCAT for further analyses.

Blood samples will be transported to FED EX either by the PI (Dr. Łukaszuk) or her graduate assistant Dawn Herbig. Additionally, the subjects will complete a weekly 3-day food record (including 2 weekdays and 1 weekend day) and an exercise log for the duration of the study. Those individuals who are pregnant, have a bleeding disorder which prevents them from clotting or if they are under the age of 18 or over the age of 85 will be ineligible to participate.

All participants will be informed of the risks and benefits associated with this study and will be required to give written consent to participate in accordance with the Institutional Review Board at Northern Illinois University.
Please note: It is the researcher’s responsibility to seek out permission to use copyrighted materials. Please indicate whether you have permission to use any copyrighted materials for your project:

☐ Yes, I have permission to use any copyrighted materials for this project
☐ No, I do not yet have permission to use any copyrighted materials for this project
☐ This is not relevant for the materials being used in this project

26) If applicable, explain the procedures for providing compensation.

2F) If applicable, explain the procedures for debriefing participants. Please attach a debriefing script or sheet [Appendix D]

At the conclusion of the study, those subjects that were in the PI group will be debriefed and told provided with the ALCAT results with a listing of correct food to which they tested intolerant.

Reminder: As appendices to this application, attach copies of all: A) Recruitment information [script/flyer/etc.], B) Informed consent documents [assent/parent permission/scripts/etc.], C) Materials [questionnaires/surveys/interview questions/listing of all information/data to be collected/etc.], D) Debriefing information [documents/scripts], E) Referral list [if appropriate]. It is the responsibility of the researcher to obtain any relevant permission for copyrighted materials. If the research involves an oral interview or focus group discussion that could evolve as it progresses, include a list of discussion topics and any “starter” questions for each topic that can reasonably be expected to be covered. If a draft of a written questionnaire or survey is attached, it should be clearly labeled as such and a final version must be submitted before data collection begins. PLEASE NOTE THAT ANY ITEMS CAN BE ATTACHED AS SEPARATE DOCUMENTS IF NEEDED.

Part II: Research Participants

3) Participant demographics:

- Gender:  ☐ M  ☐ F  ☑ Both
- Estimated age(s):
  18 - 65 years of age.
- Are any subjects under age 18?  ☐ Yes  ☑ No
- Potentially vulnerable populations (please indicate if any of the following groups are the target population of the study)
  ☐ Pregnant women & fetuses
  ☐ Prisoners
  ☐ Decisionally impaired/mentally disabled
  ☐ Specific ethnic group(s) (list in box):
    None of the above
  If any potentially “vulnerable populations” have been indicated above, please explain the necessity for using this particular group, or if specific groups are excluded from the study, please indicate the exclusion criteria used.

- Target number of participants in the entire study (including controls) from start to finish (keep in mind that this is just an estimate of the total):
  140 participants

4) Please explain any outside institutional (i.e., schools, hospitals) approval you will need to obtain and how approval will be sought. Provide scripts, letters, or emails providing any information that will be used to obtain needed approvals/permission. It is the responsibility of the researcher to follow all applicable policies of any outside institution(s).

Part III: Risk/Benefit assessment

5) What knowledge/benefit(s) to the field will be gained from the study?
The participants may feel better when they avoid foods they are intolerant to and it may also decrease their total body inflammation and improve their body composition such as decreased fat % and increased muscle mass.

6) What direct benefit(s) are there to the participant(s) (if any) from the proposed research? [For example, learning a new skill, psychological insight, teaching experience] [Please note that compensation is NOT considered a direct benefit.]

Participants will receive the following benefits from this study: ALCAT test and personalized elimination diet, and a report of levels of inflammatory markers.

7) Describe any potential risks (breach of confidentiality, economic, ethical, legal, physical, political, psychological/emotional, social, or other) to the subjects posed by the proposed research. (Note: Some studies may have “no reasonably foreseeable risks.”) Investigators are required to report all unexpected and/or adverse events to the IRB. Therefore, it is important that you list all reasonably anticipated risks because unanticipated adverse events may need to be reported by NIU to NIHPR.

Minimal risk of infection at the site that blood was drawn.

8) Federal regulations require that researchers use procedures that minimize any risks to participants. What procedures will be used to minimize each risk and/or deal with the challenge(s) stated in “7” above?

Blood will be drawn in the NIU Nutrition Lab by Dr. Shokrani, Jeanne Isabel or a phlebotomist properly trained in blood draws.

9) If support services are required to minimize risk of harm to participants, explain what will be provided (list of services available – Appendix E). [A resource list for the DeKalb area is available on the ORC website – if using this, please provide a copy with your application.]

If the subject feels light headed or faint when getting their blood drawn, they will be instructed to remain seated in a chair until their symptoms abate. They will be provided fruit juice if their symptoms do not abate in 5 minutes. If subjects feel uncomfortable, he or she can discontinue his/her blood being drawn.

10) How do the potential benefits of the study justify the potential risks to the participants?

The potential of infection is minimal as blood will be drawn by trained individuals using a sterile procedure. The information provided by ALCAT test, inflammatory marker blood test, and diet analysis has great potential to improve the quality of life of the participants, if knowledge is properly applied.

Part IV: Consent Document Variations

11) Will audio, video, or film recording be used? Yes □ No ☒

If yes, specify the recording format to be used.

Please keep in mind that specific consent must be sought in the informed consent document(s) by including a separate signature/date line giving consent for recording. This is in addition to the signature/date line giving consent to participate in the research project.

12) Will this project require the use of consent/assent documents written in a language other than English? Yes □ No ☒

Reminder: If non-English documents will be used, please have the document translator provide documentation (email or written) that the translation is equivalent to the English version. [This can be done after the protocol is approved in order to minimize the number of changes needed.]

13) Are you requesting a waiver of a signed informed consent document? Yes □ No ☒

Please indicate the justification for requesting this waiver:

☐ The only record linking the subject to the research would be the signed consent document and the principal risk of the research would be breach of confidentiality.

☐ The research involves minimal risk to the subjects and involves no procedures for which written consent is normally required outside of the research context (e.g., online surveys).

14) Are you requesting a waiver/alteration of some other aspect of the informed consent document?
[This section is relevant for studies involving deception.]

Yes ☒ No ☐

14a) Please explain which aspects of informed consent will be missing or altered along with a justification for the change.

After participants sign the informed consent form (Appendix B), they will be randomly assigned to the treatment or the control group. The treatment group will receive an accurate list of individualized foods to be eliminated from the diet for 4 weeks. The control group will receive a placebo list of foods to eliminate that should produce no results as the list of foods will be foods that can be eaten without intolerance issues per ALCAT test. The consent form will make it very clear to subjects that they will be participating in an intervention or control group with the latter group getting a "false" list of foods to eliminate for four weeks. At the end of the study both groups will be un-blinded and the control group will receive an accurate list of foods to avoid based on the ALCAT results.

14b) Please explain how the project meets all of the following criteria:

1) The research presents no more than minimal risk of harm to the participants.

The control group will receive an placebo list of foods to eliminate that should produce no results as the list of foods will be foods that can be eaten without issue per ALCAT test. This placebo list should have no potential harm to the participants.

2) The waiver/alteration will not adversely affect the rights or welfare of the participants.

The participants in the control group will be provided with their ALCAT test results to determine foods they have sensitivities to at the end of the study.

3) The research could not practically be carried out without the waiver or alteration.

This study is designed to be a double blind study so that the participants or primary researcher will be bias free during the study. Also if people knew they were in the PL group they would likely not participate in the study.

4) Whenever appropriate, the participants will be provided with additional pertinent information after participation.

Yes, participants in the control group will receive the accurate individualized ALCAT test results containing foods that ALCAT test results determined they are sensitive to.

15) Will any HIPAA protected health information be collected as part of the data? Yes ☐ No ☒

If yes, describe the procedures for protecting the information.

The participant health information will be protected as the paper records will be stored in a locked filing cabinet. Electronic records will be accessible only by the primary researcher.

[Please provide a copy of your HIPAA disclosure form to be given to participants.]

16) Will any protected school records be collected as part of the data? Yes ☐ No ☒

If yes, describe the procedures for protecting the information.

Part V: Confidentiality and Anonymity

17) Will identifying information be connected to the data (even through an identification key linking identities to a pseudonym or code that is kept separate from the data)? Yes ☒ (confidential data) No ☐ (anonymous data)

18) If you answered yes to the above question, describe precautions to insure the privacy of the subjects, and the confidentiality of the data, both in your possession and in reports and publications.

Participants will be assigned to groups using a random number chart separate from the data. During the study, participants will be referred to as the number he/she was assigned. The participant health information will be protected as the paper records will be stored in a locked filing cabinet. Electronic records will be accessible only by the primary researcher. Three years following the publication of the study, the records will be destroyed via shredding paper documents and erasing computer files.
19) How will the records (data, recordings, and consent forms) be stored? Also indicate how long records will be kept and how and when they will be disposed of.

The participant health information will be protected as the paper records will be stored in a locked filing cabinet. Electronic records will be accessible only by the primary researcher.

Three years following the publication of the study, the records will be destroyed via shredding paper documents and erasing computer files.

**Part VI: Does this project involving deception?**

Yes ☒ No □

20) Describe the deception being used. Be sure to clarify whether this is deception by omission (an important aspect of the study is withheld from the participants) or commission (the participant is misled about some aspect of the study) or both. [Complete Item 14 if aspects of consent are missing.]

The deception will be commission as the treatment group will knowingly receive accurate ALCAT test results and personalized elimination diets, whereas, the control group will receive a list of foods to avoid that they are not sensitive or intolerant to.

21) Why is deception a necessary and unavoidable component of the experimental design?

Without deception, bias may interfere with the participants’ willingness to complete the 4-week elimination diet and thus skew the results. The PL group at the end of the study will receive an accurate list of the foods that they are sensitive to.

22) Debriefing of participants will be:

☒ Immediate (directly following the research session)
☐ Delayed

☒ Full (all aspects of deception will be revealed)
☐ Partial (some aspects of deception will remain unexplained)

a) If debriefing is delayed, why is the delay necessary, and when will it occur?

b) If debriefing is partial, why is the partial debriefing necessary? Would the participant be harmed in any way by full debriefing?

c) If debriefing is partial, will full debriefing occur later?

d) Does the presence of deception increase risk of harm to the participants?

No ☒ Yes □

e) Is the respondent free to withdraw his/her data after being fully debriefed?

Yes ☒ No □

23) Who will provide the debriefing?

Dawn Herbig or Judith Lukassnik

**Reminder:** Please include a copy of your debriefing script/sheet with this application [Appendix D].

**Part VII: Credit and Compensation**

24) If participants will receive course credit for participation, please describe it below.
25) If participants will receive some other form of compensation for participation, please describe it below.

26) Describe any alternative tasks that will be available for participants to earn the credit or compensation.

Part VIII: Conflict of interest
27) Do any of the researchers conducting this study have any potential conflicts of interest?
   
   [Conflicts of interest may include financial or personal interest, or any condition in which the investigator’s judgment regarding a primary interest may be biased by a secondary interest.] Yes □ No □

28) If yes to the above question, please describe the nature of the conflict of interest.

Please use the following link to access the NIU research conflict of interest policy:

Part IX: Researcher Qualifications
29) In addition to listing the investigators’ names, indicate their qualifications to conduct procedures to be used in this study (specifically describe past experience conducting research with humans or how training will occur).

The individuals that will have direct contact with the participants are Dr. Judith Lukaszuk, Dawn Herbig, Dr. Shokrani, Jeanne Isabel, Ellen Olson and Laura Lemons.

Dr. Lukaszuk has a Ph.D. in Exercise Physiology, a MS in Nutrition and Dietetics, and is a registered and licensed dietitian. Dr. Judith Lukaszuk has experience obtaining muscle samples from subjects and maintaining a sterile field while doing so. She also has experience obtaining blood samples and using gloves and disposing of unused blood samples using the biohazard container. Dr. Lukaszuk gained this experience in 1997-1999 while conducting her doctoral study on the Effect of creatine monohydrate on anaerobic exercise performance. The study was approved by the IRB and the University of Pittsburgh and the General Clinical Research Center at the University of Pittsburgh Medical Center as this study was funded by the NIH. Dr. Lukaszuk has more recent experience taking blood samples from subjects using a finger stick and obtaining urine samples from subjects from 2010-2012.

Dawn Herbig has a BA in Sociology, BS in Nutrition and Dietetics and is under the supervision of Dr. Lukaszuk.

Dr. Shokrani is a certified Medical laboratory scientist and has extensive experience in handling human specimen such as urine and blood. Dr. Shokrani has published his work which was based on using and handling human specimen.

Jeanne Isabel has more than 40 years of work experience working at NIU and has 10 years of work experience working exclusively as a phlebotomist.

Ellen Olson, BS, MLS and Laura Lemons, Phlebotomy Certificate are both phlebotomists working in the Medical Lab Sciences Department at NIU. Ellen is the student lab coordinator and has over 20 years of experience and Laura has 19 years of experience.

Dawn Herbig is a graduate student who has learned and assisted with the anthropometrics lab last year for Dr. Lukaszuk’s FCNS 415 and FCNS 416 Nutrition in Clinical Care courses last year. Dawn completed the CITI training course in Nov 2012.
30) State the date of completion of CITI Human Subjects Protection training program(s) for the individuals listed in the above question. [Note: NIU Policy requires that research investigators must complete appropriate training before conducting human subjects research.] If you have comparable training, please attach certification indicating this.

CITI (Collaborative Institutional Training Initiative) training is thorough and well recognized:
https://www.citiprogram.org/Default.aspx

Dr. Lukasznik attends the IRB inservice given at FCNS yearly.

Dr. Shokran has attended numerous workshops and has a lot of experience with obtaining blood samples safely.

Jeanne Isabel, Ellen Olson and Laura Lemoons are all phlebotomists with the proper training to handle blood products safely.

To be completed by investigator and confirmed by advisor (if student project) and departmental reviewer. Initials indicate all required parties ratify that application is complete:

Checklist of items required to accompany completed application form:
1. ____ Complete grant proposal/contract (for externally funded projects)
2. ____ All surveys, questionnaires, interview questions, or other instruments to be used
3. ____ Subject recruitment/introductory materials
4. ____ Informed consent documents (must select at least one):
   - Consent form for adults (if participants are age 18 or over)
   - Assent form for minors (if participants are under age 18)
   - Parental permission form (if participants are under age 18)

Initial indicating all listed materials are attached and application is complete; INCOMPLETE APPLICATIONS WILL NOT BE PROCESSED. The investigator will be notified of deficiencies in the application via e-mail from the Office of Research Compliance (ORC); if no response is received by the ORC within five (5) working days the application will be considered void.

Investigator _______ Advisor (if student project) _______ Department Chair/Designee _______

REQUIRED SIGNATURES: ALL PROJECTS

CERTIFICATION

I certify that I have read and understand the policies and procedures for research projects that involve human subjects and that I intend to comply with Northern Illinois University Policy. Any changes in the approved protocol will be submitted to the IRB for written approval prior to those changes being put into practice unless it involves an immediate safety issue for the subject during a procedure. (In such instances, the researcher is required to promptly notify the IRB after the fact.) I also understand that all non-exempt projects require review at least annually.

Investigator(s) Signature(s) Date

Signature of Faculty Advisor (Student Project Only) Date

Authorized Departmental Review:

☐ Project qualifies for Administrative Review.
  Cite the appropriate exempt category: ______________________

☐ Project qualifies for Subcommittee Review.
  Cite the appropriate expedited category: ______________________

☐ Project is referred for review by the convened IRB.

Signature of Authorized Departmental Reviewer Printed name Date

Return this form, together with necessary documentation, to the Office of Research Compliance, Lowden Hall, 301. For information or additional assistance with the approval process, please call the office at (815) 753-8598 or access the ORC web page at www.orc.niu.edu.
APPENDIX I
DEBRIEFING LETTERS
FOOD ELIMINATION BASED ON ALCAT TESTING AND THE EFFECT ON OVERALL BODY INFLAMMATION

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 J
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>