Diet and Adipose Tissue Inflammation

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Abstract

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Background: Adipose tissue inflammation is a possible link between obesity and type 2 diabetes mellitus. In obesity adipose tissue macrophages (ATMs) are thought to undergo a phenotypic switch to a pro-inflammatory state resulting in chronic low-grade inflammation that is correlated with insulin resistance. The mechanism producing this phenotypic switch is unclear. This pilot study seeks to explore the hypothesis that the pro-inflammatory state of ATMs in obesity occurs due to an environment of metabolic dysfunction and nutrient excess characterized by elevated levels of free fatty acids, glucose, and insulin.

Methods: Sixteen overweight or obese individuals with evidence of insulin resistance were randomized to follow either a control diet based on the United States Department of Agriculture's 2010 Dietary Guidelines for Americans or the Anti-Inflammatory Milieu (AIM) diet designed to reduce the exposure of ATMs to the hypothesized pro-inflammatory stimuli. The AIM diet had a low glycemic load, low insulinemic index, and a low content of long-chain fatty acids. Subjects followed the diet for twelve weeks while attending bi-weekly group meetings. At baseline and at the intervention conclusion subjects were tested for markers of metabolic activation on ATMs, and adipose tissue expression of pro-

inflammatory and anti-inflammatory markers. An exit survey was used to conduct an exploratory analysis of the feasibility for free-living individuals to adopt each study diet.

Results: No significant differences between the AIM and USDA diet groups were observed in the changes in levels of metabolic activation markers or number of ATMs, or in adipose tissue expression of inflammatory markers. Weight loss occurred in all but one of the sixteen subjects with an average weight change in the AIM group of -5.8 ± 3.9 kg and an average weight change in the USDA group of -5.0 ± 3.2 kg. A non-significant trend towards a moderate increase in the average numbers of ATM and downstream inflammatory markers was observed in each diet group. No significant differences were seen in subject perceptions of the study diet experience between diet groups. Subjects in each diet group tended to regard the diets as feasible and satisfying.

Discussion: This study suggests that a diet aimed at minimizing daily exposure of adipose tissue to glucose, insulin, and long-chain fatty acids provides no unique benefit to decreasing adipose tissue inflammation compared with the generally recommended 2010 USDA Dietary Guidelines. Weight loss among subjects in each diet group may have contributed to potential increases in inflammatory markers. Beyond the presumed benefit of weight loss, it remains uncertain if either study diet is beneficial or harmful in the long term with regard to adipose tissue inflammation and associated co-morbidities. Overall the study participants were a motivated group of individuals who felt that they benefited from both study diets, and who are likely to continue the study diets in the future.

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Background

Introduction

The obesity epidemic

Overweight and obesity are health challenges worldwide. Beginning in the 1970s and continuing through today developed countries have experienced a rising prevalence of obesity and since then developing countries have joined the trend.[1] A body mass index (BMI) of 25 to 29.9 kg/m^2 is classified as overweight and a BMI of ≥ 30 kg/m^2 as obese. Between 1980 and 2013 the global prevalence of BMI ≥ 25 kg/m^2 increased from 28.8% to 36.9% in men, and from 29.8% to 38% in women.[2] In the past 8 years the rise in obesity rates slowed in developed countries but rates continued to increase in developing countries.[2] In the United States the 2011-2012 National Health and Nutrition Examination Survey (NHANES) found that 16.9% of youth and 34.9% of adults were obese.[3] In the period between 2000 and 2012 the rise in obesity prevalence in the United States appeared to taper or level, however no declines were detected.[3,4] Obesity therefore remains a high priority condition in clinical practice and public health.

Consequences of the obesity epidemic

There is an association between obesity and increased mortality risk.[5-9] For example, Adams et al. found that risk of death was strongly associated with obesity in both men and women of all ages, races, and ethnicities.[5] There is less consensus about the relationship between mortality risk and overweight. Whitlock et al. found excess mortality increased progressively as BMI rose above the optimal BMI range of 22.5 – 25.0 kg/m^2.[8] However, Flegal et al. found that overweight (BMI of 25 - <30 kg/m^2) was associated with slightly but significantly lower all-cause mortality risk than normal weight.[9]

A major concern for overweight and obese individuals is the risk of comorbid conditions, notably a range of chronic diseases. Obesity is a significant risk factor for type 2 diabetes, cardiovascular disease (CVD), the components of metabolic syndrome, and various cancers such as those of the esophagus, liver, pancreas, colon and rectum, prostate, breast and endometrium.[10-13] Analyses of NHANES data from 1976-2010 found that BMI was one of the most important predictors of diabetes prevalence and demonstrated that a representative sample of US adults with diabetes was more likely to be obese than adults who did not have diabetes.[14,15] In Canada more than half the cases of type 2 diabetes have been said to be attributable to obesity.[16] Weight loss, among other lifestyle interventions, has been shown to decrease risk of type 2 diabetes.[17] Obesity is importantly associated with CVD, which includes coronary heart disease, myocardial infarction, angina pectoris, congestive heart failure, stroke, hypertension, and atrial fibrillation.[10] Weight loss may also reduce risk factors for CVD complications.[18] Additionally, obesity is associated with increased risk of osteoarthritis, gallbladder disease, acute pancreatitis, nonalcoholic fatty liver disease, obstructive sleep apnea, and depressive disorder.[10]

Because obesity is related to morbidity, it often carries with it the burden of increased healthcare spending and reduced quality of life.[11] In 2005 the US may have spent as much as $190 billion in obesity-related healthcare expenses.[19] Expenses are incurred from direct costs, such as inpatient and outpatient services. Indirect costs related to ill health also contribute to the economic burden.[20] These

can include missed work days, reduced productivity at work, disability claims, higher insurance payments for employers, workplace accidents and injuries, and fewer working years due to premature mortality.[20] Additionally, the economic cost of diabetes is interesting given its association with obesity. In 2012 the American Diabetes Association estimated that in the US the cost of diagnosed diabetes was $245 billion, of which $176 billion was spent in direct medical cost and $69 billion was due to reduced productivity.[21]

Treatment options for obesity

Treatments for obesity include management and intervention strategies, but unfortunately have varying long term success. Lifestyle modification of diet, exercise, and behavior is a common first approach to obesity management. This can produce modest immediate effects. For example, Wadden et al. found that intervention groups lost an average of 0.3-6.6 kg after 6 months of lifestyle intervention.[22] Pharmacological intervention, including products such as orlistat or metformin, in addition to lifestyle modification could produce a greater weight loss effect at 12 months compared to lifestyle modification alone.[23,24] However, maintaining weight loss of 5% or more for longer than a year may be limited to only 20% of those intending to lose weight.[25] Surgical interventions, such as gastric bypass surgery, are treatment alternatives for obesity. Surgery commonly results in substantially greater weight loss and comorbidity reduction but it is generally reserved for morbidly obese individuals with a BMI > 40 kg/m2, is a higher risk intervention, and can have negative side effects such as increased substance abuse and mental disorders.[26,27] The difficulty of effectively treating obesity and maintaining long term weight loss could help explain the persistence of the obesity epidemic. Obesity treatment may benefit from shifting the focus from weight loss to alternative mechanisms to improve comorbid complications and quality of life.

One potential intervention is to decrease the severity of adipose tissue inflammation in an attempt to break a vicious metabolic cycle that contributes to co-morbidities associated with obesity.[28] Low grade chronic, systemic inflammation is associated with obesity and may contribute to insulin resistance and type 2 diabetes as well as other complications.[28] Systemic inflammation often occurs in conjunction with adipose tissue inflammation.[28]

Adipose tissue inflammation

Chronic low-grade inflammatory processes in adipose tissue

Adipose tissue is an endocrine organ that plays an important role in metabolism, health, and obesity. It expresses a variety of factors called adipokines that can influence a large range of processes such as food intake, insulin sensitivity, inflammation, and immunity.[29] Two key adipokines are adiponectin and leptin.[29] Adiponectin increases insulin sensitivity in the liver and muscles, increases free fatty acid (FFA) oxidation, and decreases serum concentration of FFA, glucose, and triacylglycerol.[30] Leptin regulates appetite and helps maintain energy homeostasis, but also has numerous other roles in the body, including in the immune system.[29,30]

Adipocytes enlarge as an individual becomes obese resulting in systemic metabolism alterations.[30] In obese individuals plasma adiponectin concentration decreases, which is correlated with

insulin resistance and hyperinsulinemia.[30] Conversely, leptin concentration increases but target cells become resistant to its action.[30] The enlarged adipocyte undergoes altered perilipin expression contributing to increased FFA and glycerol release from the adipocyte, which contributes to insulin resistance.[30] With increasing obesity adipose tissue produces greater quantities of pro-inflammatory factors such as tumor necrosis factor alpha (TNF-α), interleukin 6 (IL-6), interleukin 1 beta (IL-1β), interleukin 8 (IL-8), monocyte chemotactic protein 1 (MCP-1), resistin, visfatin, inducible nitric oxide synthase (iNOS), transforming growth factor beta-1, plasminogen activator inhibitor type 1 (PAI-1), tissue factor, and factor VII.[30-32] Obese individuals often experience chronic low-grade inflammation that is characterized by increased plasma levels of C-reactive protein (CRP) and inflammatory cytokines such as those listed above.[33] TNF-α, IL-6, and resistin, among other cytokines, are critical to the induction and maintenance of the obesity-associated inflammatory response.[32] Chemokines such as MCP-1 and IL-8 attract monocytes to adipose tissue and once they infiltrate they differentiate into macrophages and become key producers of inflammatory mediators, creating a vicious inflammatory cycle.[29,33]

Obesity-linked adipose tissue inflammation is associated with insulin resistance and glucose intolerance

An association between adipose tissue inflammation and insulin resistance in conjunction with obesity has been observed in numerous mouse models. For example, Hotamisligil et al. found that adipose tissue in obese mice expresses five- to tenfold more TNF-α messenger RNA per unit of total RNA than adipose tissue from lean control mice.[34] Since TNF-α is a key element of the inflammatory process, this indicated a link between obesity and adipose tissue inflammation. By neutralizing TNF-α in vivo the authors demonstrated that increased TNF-α mediated insulin resistance in the obese mice. Uysal et al. conducted further investigations into the effects of TNF-α on insulin resistance in obese mice.[35] Using obese wild type and obese TNF-α gene knock-out mice they discovered that the obese TNF-α knock-out mice were less insulin resistant. Likewise, using obese wild type and obese p55 and p75 TNF-α receptor knock-out mice they saw a similar pattern of reduced insulin resistance in the obese TNF-α receptor knock-out mice. This suggested that TNF-α action in obesity contributes to insulin resistance. Additionally they found that the circulating FFA in the obese wild type mice was elevated compared to lean mice, while the obese TNF-α knock-out mice had lower levels of FFA similar to those in the lean mice. It is thought that elevated FFA associated with obesity contributes to insulin resistance.[35] These experiments demonstrate that inflammation in adipose tissue contributes to systemic insulin resistance, at least in part by increasing FFA levels. Perreault and Marette conducted a similar study exploring the effects of iNOS gene knock-out in obese mice.[36] They saw that obese iNOS knock-out mice were insulin sensitive similar to that of lean mice while obese wild type mice had elevated insulin resistance. This demonstrated that an elevated production of iNOS in obese adipose tissue may be another link between inflammation and insulin resistance.

Additionally, inflammatory markers decrease with weight loss. Vieira et al. showed that high fat diet-induced obese mice had reduced adipose tissue inflammation when weight loss was induced by a low fat diet and/or exercise.[37] The authors saw decreased TNF-α and MCP-1 gene expression with simultaneously improved insulin sensitivity. Kosteli et al. found high fat diet-fed obese mice that underwent an extended period of caloric restriction-induced weight loss had a decreased number of adipose tissue macrophages (ATM) after an initial acute increase in ATMs at the instigation of weight

loss.[38] Because ATMs produce factors such as TNF-α and MCP-1, their reduction in the adipose tissue over time with weight loss resulted in a reduced chronic inflammatory state.

The same pattern can be observed in human studies leading to the hypothesis that adipose tissue inflammation in humans is a pathophysiological event and a primary cause of insulin resistance. Hotamisligil et al. moved forward from rodent models to investigate the relationship between obesity and adipose tissue expression of TNF-α and insulin resistance in humans.[39] They found that the adipose tissue in obese premenopausal women expressed significantly more TNF-α and these subjects had greater insulin resistance than lean controls. A weight reduction program in the obese subjects resulted in improved insulin sensitivity and decreased adipose tissue production of TNF-α. Le et al. observed macrophage infiltration in the adipose tissue of obese minority young adults.[40] Obese subjects with evidence of adipose tissue inflammation had greater fasting insulin, lower pancreatic beta cell function, higher TNF-α concentration, and greater liver fat and visceral adipose tissue mass than their obese peers that did not show signs of adipose tissue inflammation. These findings were independent of ethnicity and sex. Pietilainen et al. conducted a study in monozygotic twin pairs discordant for obesity to evaluate the effect of acquired obesity on inflammatory and macrophage markers.[41] They found that independent of genetic influences, obesity increased adipose tissue expression of the inflammatory marker TNF-α and the macrophage marker CD68, and decreased adipose tissue expression of adiponectin. This was correlated with insulin resistance. This twin study also confirms the findings of Weisberg et al. who determined that the macrophage content of adipose tissue in mice and humans was positively correlated with BMI and with adipocyte size.[42] Their study concluded that adipose tissue macrophages were the primary source of TNF-α and other proinflammatory molecules in adipose tissue. The chronic inflammation observed in conjunction with obesity is not only associated with insulin resistance but also with a higher risk for other chronic diseases such as type 2 diabetes, cardiovascular diseases, various cancers, autoimmune diseases, and inflammatory diseases.[29]

Macrophage contribution to adipose tissue inflammation

Macrophage accumulation is associated with both BMI and adipocyte size in mice and humans

ATM have taken center stage in the concept of adipose tissue inflammation and its associated co-morbidities. Macrophages derive from monocytes that move into the tissues from blood and differentiate in response to damaged tissues, damaged cells, pathogens, or cytokines.[28] Macrophages residing in specific tissues are thought to influence tissue homeostasis by clearing dead cells and debris.[28] Macrophages are not homogenous and can consist of different phenotypes that influence their function as well as the quality, duration, magnitude, and specificity of inflammatory responses.[28] For example, in the classic inflammatory process toll like receptors on macrophages recognize specific classes of attacking microbes and in response initiate the release of cytokines.[43] This is a first step in the development of the classic immune system response to invading pathogenic bacteria.[43]

Macrophage accumulation in adipose tissue is positively associated with an increase in body weight.[44] As adipocytes enlarge with overnutrition they secrete MCP-1 and other chemokines.[44] This creates a chemotactic gradient that draws monocytes into the adipose tissue where they become ATMs.[44] Once in the adipose tissue the ATMs can undergo a phenotypic switch toward a more

inflammatory cell type, which secretes a number of pro-inflammatory factors such as TNF-α, IL-6 and MCP-1 that can contribute to insulin resistance.[42,45] The chemokines produced by pro-inflammatory ATMs continue to attract more macrophages creating a feed forward inflammatory process.[44, 46]

Hypothesis of M1 vs M2 activation states in adipose tissue macrophages

Activation of macrophages by environmental stimuli has classically been described as existing in one of two possible states, which have been defined in vitro.[47] The "classically activated" (M1) state occurs in response to a pro-inflammatory stimulus such as lipopolysaccharide (LPS) present on gram-negative bacteria or interferon gamma (INFγ) produced by lymphocytes in response to antigen.[47] These signals are indicative of an attack by a foreign pathogen that necessitates immune system mobilization. The M1 activated macrophage produces pro-inflammatory factors such as TNF-α, IL-6, and iNOS.[47] The second activation state is termed "alternatively activated" (M2) and occurs in response to a number of stimuli such as interleukin 4 (IL-4) and interleukin 13 (IL-13).[47] The M2 macrophage produces anti-inflammatory factors such as IL-10 and arginase, and are also thought to be involved in tissue repair.[47]

Lumeng et al. used a mouse model to demonstrate that ATMs undergo a phenotypic switch from an anti- to a pro-inflammatory state under conditions of high-fat feeding and increased body weight.[47] First they saw the number of ATMs increase with the onset of obesity and the formation of crownlike structures, a cardinal sign of macrophage accumulation in adipose tissue. These ATMs had an elevated expression of the pro-inflammatory cytokines IL-6 and iNOS . Alternatively, lean mice ATMs showed a higher expression of IL-10 and arginase, and lower expression of the pro-inflammatory factors. The authors found that IL-10 protected adipocytes from effects of TNF-α, such as reduction in insulin sensitivity. The authors hypothesized that these anti- and pro-inflammatory ATM could be similar to M2 and M1 macrophages, respectively. They proposed that either elevated factors such as MCP-1, TNF-α and FFA in obesity induced M1 activated macrophages to enter adipose tissue or that existing M2 ATMs were phenotypically switched to the M1 state.

Since the proposal of the M1/M2 ATM paradigm there has been much exploration of the differences between what were hypothesized to be M1 and M2 ATMs. In addition to stimulation by T-helper 1 (Th1) type cytokines such as LPS and INFγ, ATMs with a pro-inflammatory phenotype may be activated by interferon regulatory factor (IRF), signal transducers and activators of transcription (STAT), suppressor of cytokine signalling (SOCS), and exposure to excess lipids, notably saturated fatty acids.[46,48] ATMs with an anti-inflammatory phenotype are thought to play a role in adipose tissue homeostasis through actions such as tissue remodeling and inflammation resolution.[46] These ATMs may be activated by transcription factor PPAR-gamma, adiponectin, Th2 type cytokines such as IL-4 and IL-13, and unsaturated fatty acids, notably the omega-3 polyunsaturated fatty acids DHA and EPA.[46] In obese adipose tissue ATMs tend to group in crown like structures and express pro-inflammatory markers while in lean adipose tissue ATMs are interstitially spaced and express anti-inflammatory markers.[46] Despite these observed differences between macrophages with distinct inflammatory phenotypes, much of the initial research in the field did not determine if ATMs were in fact M1 or M2 macrophages. Macrophages that displayed pro- or anti-inflammatory characteristics were simply labeled as M1 or M2 without further verification of cell identifiers. Although the M1/M2 model is often used to explain adipose tissue inflammatory processes, an accumulating body of evidence has found that this simple dichotomy may not accurately capture the entire picture of elements at play.

Hypothesized alternatives to M1 and M2 paradigm

It is likely that the concert of events contributing to adipose tissue inflammation in obesity is more complicated than the simple M1/M2 ATM hypothesis suggests. The distinction between M1 and M2 ATMs in humans is not as clearly defined as it is in vitro or in rodent adipose tissue. First, it had never been well defined which cell surface markers differentiate M1 from M2 macrophages in humans. Also, in humans M2 ATMs may secrete both pro- and anti-inflammatory cytokines or ATMs may be positive for both M1 and M2 cell surface markers.[46] Macrophages can modify their phenotype in response to their environment, but a clear understanding of how this switching may occur in obesity remains elusive.[49] It may be more likely that macrophage phenotypes exist on a spectrum that includes complex expressions of both M1 and M2 characteristics.[49] Furthermore these gene expressions possibly exist on a multipolar spectrum that is affected by the many cells and ligands present in each different tissue and varying inflammatory states.[50]

Despite the focus on adipose tissue inflammation as a major etiologic factor in insulin resistance, some studies demonstrate a positive role for adipose tissue inflammation. Asterholm et al. developed a mouse model to explore the role of adipose tissue inflammation in high-fat-diet induced metabolic disturbances.[51] In mice bred to reduce the adipocyte host immune response by inhibiting a number of adipose-specific pro-inflammatory signaling pathways, they were able to observe the metabolic effects of a high fat diet without the induction of the inflammatory response in adipose tissue. They found that adipogenesis rates decreased, adipose tissue expansion and remodeling were impaired, glucose tolerance decreased, high fat diet-induced hepatic steatosis increased, and intestinal permeability increased contributing to chronic systemic inflammation. The authors concluded that acute adipose tissue inflammation was necessary for healthy adipose tissue growth and maintenance, and enabling safe storage of excess nutrients.

ATMs also play a positive role in protecting adipocyte function during periods of increased lipolysis and free fatty acid concentrations.[38] Macrophages are heavily recruited to adipose tissue with initiation of weight loss in order to phagocytose excess lipid and possibly reduce adipocyte stress.[38] After a prolonged period of weight loss ATM quantity decreases.[38] Additionally Xu et al. found that in obesity M1 polarization was not observed at all, but rather a greater quantity of ATMs increased the inflammatory process in adipose tissue.[52] They observed a key role of ATM lysosome function that effectively allowed ATMs to buffer the increased accumulation of lipids in adipose tissue that accompanied obesity.

A new emerging model of adipose tissue inflammation

The picture of ATM mechanisms that influence chronic low grade inflammation in obesity has become more complex since the initial proposal of the M1/M2 model. Kratz et al. conducted a study to explore an alternative mechanism promoting the pro-inflammatory activation of ATMs.[53] The authors identified three M1 macrophage cell surface marker proteins, CD274, CD38, and CD319, which are found on in vitro-differentiated and classically activated M1 macrophages in both humans and mice. These markers were strongly expressed in macrophages from the airway of cystic fibrosis patients with confirmed lung infections with gram-negative bacteria, indicating that the M1 phenotype was activated in the traditional setting of bacterial stimuli. Interestingly, these markers were not significantly

expressed on ATMs from obese human subjects or high-fat diet-induced obese mice, despite the fact that these ATMs showed elevated cytokine expression characteristic of inflammation compared to the ATMs obtained from lean human and mice counterparts. However, when the mouse ATMs were treated with the traditional bacterial stimuli LPS and IFNγ, they began to express the classic M1 surface marker proteins, demonstrating that the phenotypic switch of ATMs to M1 activation was possible under appropriate conditions. In line with the findings by Xu et al., these data clearly demonstrated that pro-inflammatory ATMs in mice or humans in the setting of obesity are distinct from classically activated M1 macrophages.

Kratz et al. proposed a new mechanism responsible for activating the inflammatory process in the ATMs that they termed "metabolic activation".[53] They hypothesized that metabolic activation occurs as a result of exposure to an environment with high levels of glucose, insulin, and palmitate, a setting that is characteristic in the obese individual. Palmitate is thought to act as the strongest driver of the phenotypic switch. ATMs isolated from obese humans and mice specifically overexpressed the surface proteins ABCA1, CD36, and PLIN2. In addition, other surface markers associated with M2 alternatively activated macrophages were suppressed. The authors tested the hypothesis of metabolic activation by treating monocyte derived macrophages with media conditioned by visceral adipose tissue from obese humans or mice. They found elevated expression of the pro-inflammatory cytokines TNF-α and IL-1β, as well as the surface markers ABCA1 and CD36. When the monocyte derived macrophages were treated with sputum from a cystic fibrosis patient they found enhanced production of TNF-α, IL-1β, and the M1 surface markers CD274 and CD38. This demonstrated that disease specific biological fluids could produce distinct surface marker expressions in naïve macrophages. The authors quantified the ATM surface marker expression of ABCA1 and CD36 in adipose tissue obtained from obese subjects and found that these markers were elevated in comparison to those expressed by macrophages from adipose tissue samples from lean subjects and in comparison to those expressed by airway macrophages from cystic fibrosis patients. This indicated that macrophage metabolic activation correlated with adiposity. This correlative relationship was absent for M1 surface markers. The authors saw a similar positive correlation between adiposity and expression of pro-inflammatory cytokines and metabolic activation markers in ATM obtained from obese mice. The study concluded that pro-inflammatory activated ATMs in obese adipose tissue are not the same classically activated M1 phenotype as previously suggested. The metabolically activated macrophage is likely a product of an environment of metabolic dysfunction and nutrient excess characterized by elevated levels of palmitate, glucose, and insulin.

The Anti-Inflammatory Milieu (AIM) diet study

The modern Western diet often consists of foods that promote a high glycemic load and high insulinemic index while concurrently being high in long chain fatty acids. This can create an environment with elevated levels of palmitate and other fatty acids, glucose, and insulin, which together produce conditions amenable to metabolic activation of ATMs as Kratz and colleagues have shown in vitro. Additionally, excess caloric intake may contribute to obesity. Together these conditions may

trigger or contribute to adipose tissue inflammation, which in turn may independently contribute to insulin resistance and ultimately glucose intolerance. The hypothesis of the connection between the modern Western diet and conditions of metabolic activation of ATMs is the basis for the aims of this study. For the purposes of the study a diet plan was created called the Anti-Inflammatory Milieu (AIM) diet, which sought to minimize daily exposure of adipose tissue to glucose, insulin, and long-chain fatty acids. The control diet was modeled after the USDA 2010 Dietary Guidelines for Americans.

Primary aim: To investigate whether consumption of the AIM diet reduces the metabolic activation of ATMs compared to the control diet, as assessed by quantifying the ATM cell surface expression (relative mean fluorescence intensity, rMFI) of the metabolic activation markers CD36 and ABCA1.

Secondary aim: To compare the impact of the AIM versus control diet on endpoints downstream of metabolic activation of ATMs, specifically a) adipose tissue expression of TNF-α, IL-1β and IL-6, and b) adipose tissue expression of adiponectin

Exploratory aim: To assess the participant experience during the dietary intervention as measured by a survey designed to understand how acceptable and feasible it is for free-living participants to adopt each of the two study diets.

We hypothesized that the rMFI of metabolic activation markers on ATMs and the downstream endpoints of metabolic activation of ATMs would decrease from baseline in each diet group with a greater decrease occurring in the AIM diet group.

The exploratory aim above seeks to investigate how health behavior change is affected during the study. Health behavior change is difficult to maintain after an active intervention period.[54] As an example of dietary behavior change, a number of studies have explored why heart failure patients struggle to follow a sodium restricted diet.[55-57] A common barrier identified was lack of knowledge including non-awareness of recommended guidelines, inability to read the nutrition facts panel, inability to distinguish packaging between high and low sodium foods, lack of nutritional and cooking knowledge, and unfamiliarity with the rationale for following a low sodium diet.[55-57] Additional barriers included interference with socialization, inability to eat out, conflict with family, limited food choices, lack of palatability, and lack of motivation to cook healthy food when eating alone.[56,57]

Strategies that may facilitate dietary behavior change are environmental and social changes that accompany an intervention, self-monitoring of dietary intake, and receiving feedback from clinicians and biochemical markers.[54,58] Highly motivated individuals are more likely to maintain demanding dietary changes over a period of several years.[58] Motivation to sustain change may be affected by individual preferences, family variables, demographics, lifestyle factors, and sociocultural variables that affect the food environment such as food marketing.[58] If participants in the AIM study receive adequate education and social support, are highly motivated, and regard the dietary intervention as acceptable and feasible then behavior change via adoption of the study diet may be expected to follow.

The exit survey participants completed at the end of the dietary intervention was used to evaluate four concepts relating to adoption of the study diet and maintaining dietary behavior change

into the future. They were asked to comment on acceptability, feasibility, barriers, and benefits and drawbacks of the study diet. These were used to understand participant motivation to sustain the dietary changes experienced in the study and to inform future intervention research on relatively restrictive diets.

Methods

Study design

A randomized parallel design study was conducted which included a 12-week dietary intervention. Once enrolled in the study, participants were block randomized into either the USDA diet ("Diet A") group or the AIM diet ("Diet B") group. At baseline participants completed an initial clinical assessment and met with the study coordinator to discuss their diet assignment and receive detailed oral and written instructions as well as recipes. They were not told which diet they were randomized to, the name of the diet other than Diet A or Diet B, the nature of the two intervention diets, or the study hypothesis being tested. After starting the diet, subjects participated in six group meetings for dietary counseling every other week. At the end of the 12-week intervention period participants completed a second clinical assessment including the same procedures performed at the baseline clinical assessment.

Subjects

A total of 16 women and men with a BMI ≥ 28 kg/m^2 and a baseline homeostasis model assessment insulin resistance index (HOMA-IR) > 2.0 were recruited using flyers, local newspapers, campus publications, and online websites. Interested candidates called a study recruiting telephone line and completed a phone screening interview that asked questions about anthropometrics, medical history, medications, and dietary and lifestyle habits. If candidates passed the phone screening they were provided additional information about the study through the consent forms and asked to come to an in-person screening visit at Fred Hutchinson Cancer Research Center (FHCRC). Interested candidates came to the visit after a 10-hour overnight fast. The consent forms were reviewed and candidates could ask questions. After providing informed consent, interested candidates then underwent in-person screening procedures. Women of childbearing age completed a urine pregnancy test and were immediately excluded if pregnant. Screening procedures included measurement of height, weight, and vital signs, completion of a detailed medical and medication history form, and a 20 mL fasted blood draw to analyze for CRP, glucose, and insulin concentrations, and thyroid, liver, and kidney disease. The following inclusion and exclusion criteria were used to screen candidates:

Inclusion criteria:	**Exclusion criteria:**
· Age 18 – 75 years · Body Mass Index (BMI) ≥ 28 kg/m^2 · Homeostasis model assessment insulin resistance (HOMA-IR) index > 2.0 · Body weight within 10% of current weight within the 3 months before starting the study · Able to come to the FHCRC Prevention Center for one 1-hour pre-study visit and two clinic visits of ~ 5-hour duration each · Able and willing to attend bi-weekly dietary group counseling sessions at the FHCRC during the 12-week intervention period	· Any previous or current use of antidiabetic medications or insulin · Presence or history of major chronic inflammatory or autoimmune disease (e.g., lupus, rheumatoid arthritis, Hashimoto's thyroiditis, inflammatory bowel disease, celiac disease, multiple sclerosis), malabsorption syndromes, or diseases of the liver, thyroid, or kidneys (stage IV or later chronic kidney disease) · Food allergies or intolerances against major study foods · Intake of drugs likely to interfere with study endpoints, including corticosteroids and anabolic steroids,

· Willingness and ability to follow the dietary regimen · Able to complete repeated 4-day food records before and during the dietary intervention. · Willingness to maintain usual lifestyle habits (other than diet) throughout the study (e.g., physical activity habits) · Ability to understand, speak, and write in English · Ability to provide informed written consent	hormone replacement therapy, NSAIDS (more than 3 times per week and more than 600 mg per day), warfarin (within 3 months of starting the study), antibiotics or probiotics (within 2 weeks of starting the study) · Presence or recent history of anemia (within 3 months of starting the study) · Participation in another study that includes an intervention of any kind or a blood draw >300 mL over 3 months · Alcohol intake > 2 drinks per day · Use of tobacco products, eCigarettes, or recreational drugs on more than 2 days per month · Current or recent (within 12 months of starting the study) pregnancy or breastfeeding

Individuals who were eligible and interested in participating in the study attended a pre-study clinic visit where they completed consenting procedures for the main phase of the trial. They were educated on how to collect a stool sample at home and how to keep a 4-day dietary record at home. They were asked to complete these procedures prior to the first clinic visit.

Study diets

The intervention diet was the Anti-Inflammatory Milieu (AIM) diet. The goal of this diet was to minimize daily exposure of adipose tissue to glucose, insulin, and fatty acids. This diet included a low glycemic index, low glycemic load, low insulinemic index, and low long-chain fatty acid content. The diet included protein-rich foods such as legumes, eggs, fish, and lean meats. Foods with a high glycemic index were eliminated or minimized such as refined grains, starchy tubers, added sugars, and baked grain products including breads, crackers, cookies, pastries, cake, cold cereals, and pizza. These were replaced with low glycemic index foods such as legumes, vegetables, fruit, and cooked whole grains. Dairy foods were excluded because of their high insulinemic index. Sources of long-chain fatty acids were limited such as fatty meats and vegetable oils, and were replaced by coconut oil, medium chain triglyceride (MCT) oil, and limited amounts of butter fat (ghee). Participants in this diet group were provided extra-virgin coconut oil and medium-chain triglyceride (MCT) oil to assist with adherence.

The control diet was modeled after the USDA 2010 Dietary Guidelines for Americans. The diet included grains at least 50% of which were supposed to be consumed as whole grains, as well as low-fat dairy, legumes, fruit, vegetables, fish, vegetable oils and margarines, and limited quantities of meat, eggs, nuts and seeds. Participants were encouraged to lower intake of sodium to less than 2300 mg (1500 mg over 51 years old) per day, consume less than 10% of calories from saturated fat, limit consumption of trans and solid fats, added sugars, refined grains, and alcohol. The dietary instructions used specific language or examples from the USDA 2010 Dietary Guidelines for Americans as much as possible. Participants in this group were provided extra-virgin olive oil, canola oil, and sunflower oil as their principal sources of added fats and oils.

Clinical procedures

Participants began the intervention with the baseline clinical assessment. They were admitted to clinic after a 10-hour fast and returned their stool sample and 4-day diet record. They completed a questionnaire assessing their health, medication, supplement use, and physical activity habits over the previous 12 weeks. They also completed a food frequency questionnaire (FFQ). Anthropometrics measured included height, weight, waist and hip circumference, and vital signs measured included blood pressure, temperature, and pulse. They underwent an adipose tissue biopsy and a DEXA-scan to assess body composition. At the end of the 12-week intervention the participants concluded the intervention with the follow-up clinical assessment in which they completed the same procedures as in the first clinic visit. They completed another FFQ and took an exit survey (see **Appendix 1**) to evaluate the participant experience in the study.

Laboratory analysis

The biopsied adipose tissue underwent collagenase digestion using Collagenase I (Worthington Biochemical Corp., Lakewood, NJ; final concentration 1 mg/mL) in PBS with 50 U/mL DNAse I for 1 h at 37°C. Following digestion, the tissue slurry was passed through a 180 µm mesh filter, which was rinsed twice with 5 mL PBS + 1% BSA before centrifugation for 5 min at 1200 rpm. After removal of the adipocyte layer, the cell pellet containing the stromavascular cells (SVC) was then resuspended in 1X red cell lysis solution and incubated for 2 min at room temperature. The SVC were then washed twice with cold staining buffer (0.2%BSA/0.09%NaN_3/PBS), passed through another filter (35 µm) and finally resuspended in 1 mL of staining buffer for counting.

Multi-parameter flow cytometry was performed on SVC freshly isolated from adipose tissue. Individual cell populations were labeled using a combination of up to ten directly conjugated primary antibodies all purchased from either BD Pharmagen (San Jose, CA), BioLegend (San Diego, CA), or Beckman-Coulter (Brea, CA). They included AlexaFlour (AF700)-conjugated CD11c; Allophycocyanin (APC)-conjugated CD206; APC-Cy7 conjugated CD14; Fluorescein Isothiocyanate (FITC)-conjugated ABCA1; Krome Orange conjugated CD45; Peridinin Chlorophyll Protein Complex (PerCP)-conjugated CD15, CD20; Phycoerythrin (PE)-conjugated CD36; PE-Texas Red (TxR) conjugated CD16; and PE-Cy7 conjugated 1c. Samples were analyzed immediately following labeling using a LSRII flow cytometer (Beckton Dickinson, Franklin Lakes, NJ) to collect at least 30,000 events in a broadly defined lymphocyte-monocyte-granulocyte gate, defined by forward- and side-scatter attributes. Analysis was carried out with FlowJo software, version 9.4.1 (TreeStar, Ashland, OR) using histogram and dot plot analyses on the live cell gate. Live cells were defined by the absence of fluorescence associated with the uptake of 4'.6-Diamidino-2-phenylindole, 2HCl (DAPI; EMD Chemicals, Gibbstown, NJ and/or Molecular Probes, Invitrogen, Carlsbad, CA), a reactive dye that binds to cellular amines of membrane-compromised (i.e., dead) cells. Measurements of cell surface CD36 and ABCA1 expression were assessed by measuring rMFI of these markers in gated adipose tissue macrophages, relative to appropriate isotype controls. The number of ATM cells were counted per gram of tissue.

Adipose tissue gene expression was measured by qPCR. Total RNA was extracted from whole adipose tissue using RNeasy Lipid Tissue kit (Qiagen, Hilden, Germany) and quantified using RiboGreen (Invitrogen Corp., Carlsbad, CA). cDNA synthesis was carried out on ~1 mg of total RNA using the RETROscript® Kit (Ambion/Applied Biosystems, Austin, TX) and PCR performed using pre-designed

TaqMan® Gene Expression Assays (Applied Biosystems, Austin, TX) on an ABI Prism® 7900HT Sequence Detection System. β-glucuronidase and 18s rRNA were measured as housekeeping genes. Genorm was used to calculate a normalization factor that was applied to all target genes, which include adiponectin, IL-1β, IL-6, and TNF-α.

Statistical analysis

Statistical analyses were performed using SPSS software (version 24, SPSS Inc., Chicago IL). Normality of distributions for all variables was assessed by Shapiro-Wilk tests and by checking histograms and normal plots. Results are expressed as means ± standard deviations (SD) for normally distributed variables or as median (25^{th} percentile, 75^{th} percentile) for non-normally distributed variables. Changes over time were compared by repeated-measures analysis of variance (RM-ANOVA), with 'time' (baseline vs. completed 12-week diet) as the within-subjects factor, and intervention group (USDA vs. AIM) as the between-subjects factor. For this analysis variables were log 10-transformed if the residuals were not normally distributed, which was the case for the mRNA concentrations of genes encoding inflammatory mediators TNF-α, IL1β, and IL6; and ATM number and the rMFI of the pro-inflammatory marker CD36. No adjustments were made for multiple testing because all measures of adipose tissue inflammation were interpreted together, i.e. a change in a single biomarker was not interpreted as indicative of a change in 'inflammation'. Independent-samples Mann-Whitney tests were used to compare exit survey data among the two diet arms. The survey data was coded with a numerical identifier for each possible answer choice. Additionally, survey data was tabulated and graphed using Microsoft Excel software (version 16, Microsoft, Redmond WA). An alpha-error of $P < 0.05$ was considered significant for all analyses.

Results

Baseline characteristics of the study population are presented in **Table 1**. None of the characteristics were significantly different between the AIM and USDA diet groups. Weight change during the 12-week intervention period is shown in **Figure 1**. All but one subject lost weight regardless of study group. One subject in the USDA group remained weight stable and no subjects gained weight. The average weight change in the USDA group was -5.0 ± 3.2 kg and the average weight change in the AIM group was -5.8 ± 3.9 kg.

The analysis of number of ATM and relative mean fluorescence intensity (rMFI) of markers of metabolic activation is presented in **Table 2**. No significant time x diet group interaction was found, indicating that no endpoint changed differently in one intervention group compared to the other. When analyzed for the main effect of time only, ABCA1 rMFI was significant ($p = 0.004$). On further analysis of this significant change in ABCA1 rMFI, the significance of the effect of time was lost when adjusted for weight change throughout the 12-week intervention period (data not shown). Similar results were seen in the adipose tissue expression of genes encoding mediators of inflammation presented in **Table 3**. Again, no significant time x diet group interactions were detected for any endpoint. Adiponectin showed a possible trend only when analyzed for the main effect of time ($p = 0.085$). Again, when adjusted for weight change any possible trend in adiponectin disappeared (data not shown).

There were no significant differences between the two diet intervention groups for any of the survey questions. Only one question evaluating the ease of incorporating the diet into everyday life showed a trend toward significance ($p = 0.065$) between the diet groups. The mean responses for this question are seen in **Figure 2**. On a scale of 1 = most difficult and 4 = easiest, the USDA group averaged an ease of diet incorporation of 3.125 compared to 2.25 for the AIM group. Difficulty following the AIM diet was most commonly reported at the beginning of the intervention (3 subjects) while difficulty following the USDA diet was most commonly reported in the middle of the intervention (4 subjects), however one AIM subject did not answer this question (**Figure 3**).

There was little variation between the groups regarding how closely subjects reported following the diet (**Figure 4**). On a scale of 1 = least closely and 4 = most closely, the USDA group averaged a closeness of 3.5 compared to 3.375 for the AIM group. In both intervention groups the greatest number of subjects reported deviating from the study diet 2-3 times per month (USDA = 4 subjects, AIM = 3 subjects) (**Figure 5**). However, 2 USDA subjects reported deviating from the diet once per week and 1 USDA subject reported deviating from the diet 2-3 times per week. Likewise, 1 AIM subject reported deviating from the diet once per week, 1 AIM subject reported deviating from the diet 2-3 times per week, and 2 AIM subjects reported deviating from the diet 4-6 times per week.

Time constraints were a barrier to diet adherence for all 16 subjects regardless of intervention group (**Figure 6**). Additionally, every type of barrier presented in the survey was reported by at least 2 subjects in each group. A total of 7 subjects in the AIM group noted eating out and craving non-diet foods as barriers and a total of 6 subjects in the USDA group noted eating out and the work place as barriers. Occurrence of drawbacks and benefits that subjects reported are shown in **Figure 7**. There was an overall emphasis on benefits with a total of 5 drawbacks reported, 68 benefits reported, and 39 non-responses counted for all 16 subjects regardless of diet group.

All subjects reported that the study had a neutral effect (AIM = 1 subject), somewhat positive effect (USDA = 4 subjects; AIM = 2 subjects), or very positive effect (USDA = 4 subjects; AIM = 5 subjects) on their quality of life (**Figure 8**). No subjects in either group reported a negative effect of the diet on their quality of life. The majority of all subjects reported that they liked the study diet a little (USDA = 3 subjects, AIM = 2 subjects) or a lot (USDA = 5 subjects, AIM = 6 subjects) and no subjects reported that they disliked the diet (**Figure 9**). Finally, **Figure 10** shows subject satisfaction with each diet was high. All 8 subjects in the AIM group and 7 subjects in the USDA group reported the benefits of the diets were worth the sacrifices, while 1 subject in the USDA group reported they were unsure. In both the USDA and AIM groups 7 subjects reported they would continue the diet into the future, while 1 subject in each group was unsure. All 8 subjects in the USDA group and 7 subjects in the AIM group reported they would recommend the diet to others, while one person in the AIM group said they would not.

Discussion

No significant changes were observed in the number or metabolic activation markers of ATMs or downstream inflammatory markers between the AIM and USDA diet groups. Weight loss occurred in all but one subject with a slightly greater average weight loss in the AIM group. No significant differences were seen in subject perceptions of the study diet experience between diet groups. However, responses showed generally positive feedback regarding the feasibility, barriers, benefits, drawbacks, and satisfaction with the diets.

Although not statistically significant, the average levels of ABCA1 rMFI, TNFα, IL-1β, and adiponectin slightly increased in each diet group. Perhaps the study was underpowered to show a true change in these values that would become more significant with more participants. If indeed these adipokine levels modestly increased it would be contrary to expectation given that this generally signals an increase in pro-inflammatory markers with the exception of adiponectin.

As described earlier, studies conducted in humans and rodents have found that obesity and an environment with elevated levels of palmitate and other fatty acids, glucose, and insulin is associated with increased expression of adipose tissue inflammatory markers. This study anticipated a decrease in metabolic inflammatory markers with a greater decrease in the AIM diet that was specifically designed to minimize suspected pro-inflammatory factors in adipose tissue, compared to the USDA diet. However, we saw some possible evidence of increased inflammatory markers or no change, and no significant differences between study groups. An important outcome of this study that may help explain these results is the weight loss that occurred in both study groups. Since the design of this study there has been an expansion in the literature that explores the relationship of weight loss and ATM inflammatory markers.

As mentioned previously, Kosteli et al. showed that in obese mice macrophages were recruited to adipose tissue at the initiation of weight loss on a moderate caloric restriction diet.[38] ATMs appeared to be recruited by the release of FFA due to lipolysis and the ATMs showed a rapid increase in intracellular lipid drops. The authors hypothesize that this may moderate extracellular increases in FFA and preserve adipocyte function. As triglyceride stores in the mice decreased and lipolysis fell with prolonged weight loss the ATM quantity was reduced.[38] Belza et al. found that negative energy balance in obese humans did not reduce inflammatory markers such as hs-CRP and IL-6.[59] However, in a following weight maintenance period these markers decreased possibly suggesting that reduction in body fat stores and weight stabilization at a new lower level drives down adipose tissue inflammation. Interestingly, adiponectin did not increase in response to fat loss.[59] In one of the previously mentioned studies by Hotamisligil et al. a small sample of obese subjects showed a decrease in TNFα expression in adipose tissue only once weight stable for 3 weeks following a 12-week weight reduction program.[39] Salas-Salvado et al. conducted a weight loss intervention in obese subjects and found that in negative energy balance conditions subcutaneous adipose tissue expression of IL-1, TNFα, MCP-1, IL-6, and adiponectin increased.[60] The authors hypothesized that in a calorie restricted environment the enhanced expression of these cytokines provokes the mobilization of stored lipids. Likewise Magkos et al. saw either no change or increased adipose tissue gene expression of IL-6, TNFα, MCP-1 and macrophage markers CD68 and EMR1 in obese subjects after achieving a 5% weight loss.[61] However the

authors observed that after 11%-16% weight loss, subcutaneous adipose tissue expression of these inflammatory genes was downregulated. Other studies in overweight and obese subjects who followed diet and exercise-induced weight loss interventions did not find associated changes in gene expression of inflammatory markers such as IL-6, TNFα, and MCP-1.[62,63] However, the study by Tam et al. did observe increases in adiponectin in each weight loss intervention arm.[62] Various other studies have shown improved adipose tissue inflammatory profiles after either modest or dramatic weight loss, characterized by decreased expression of pro-inflammatory factors such as IL-6 and MCP-1.[64-66]

Thus, there is no clear or consistent explanation for how weight loss affects adipose tissue inflammation but it appears that initial weight loss is unlikely to produce immediate improvements in ATM quantity or gene expression of inflammatory markers in adipose tissue. It is possible that the subjects in this study were experiencing ongoing weight loss at the time of follow-up and therefore adipose tissue inflammatory markers may have been more likely to be elevated. A potential concern is that the weight loss process in the study subjects may have masked any potential inflammatory benefits or differences between the diets. If the study had encompassed a longer intervention period, one can suspect subjects could reach a new weight stabilization that could present the opportunity to see a decrease in pro-inflammatory markers or a difference in the diet group outcomes. Interestingly, there was no caloric restriction enforced in either of the study diets. Participants could eat as much as they liked as long as they chose foods that fit within the study diet pattern. However, being that both diets were restrictive by nature and that energy density of the study foods was almost certainly lower than that of foods that make up the habitual diet of our participants, weight loss happened almost universally in the subjects regardless of diet group.

The exit survey conducted at the follow-up visit showed an overall positive response to each of the study diets. Unsurprisingly participants in the AIM group reported that their study diet was more challenging to incorporate into everyday life than those participants in the USDA group, which was expected given that the AIM diet was more restrictive. Likewise participants in the AIM group reported deviating from the study diet more frequently than those in the USDA group. If there is no distinct anti-inflammatory benefit or other general benefits to following the AIM diet and participants lost weight in both study groups, a free-living individual may chose to follow the USDA diet since it appears to be somewhat easier to follow and is less restrictive. Interestingly AIM diet participants reported more difficulty following the diet at the beginning of the intervention and USDA diet participants reported more difficulty following the diet in the middle of the intervention. This may be because the more restrictive AIM diet was more difficult to learn and adapt to at first. For future study design purposes it may be helpful to consider providing more intensive or more frequent diet support at the beginning of an intervention for participants on restrictive diets in order to optimize diet adherence.

Every participant in the study experienced barriers to diet adherence, with the universal obstacle being time. What takes extra time for each individual can be different as seen in these two participant's comments:

> "The main obstacle is time to prepare and shop for new foods, and to modify recipes if having other people over."

> "Lack of time to prepare meals after work. I missed going out to dinner with friends. Doing dishes (by hand) all the time was a pain..."

The time required to follow the diet can take many forms such as shopping, preparing food, modifying recipes for others, and doing more dishes than usual. Study coordinators should pay particular attention to addressing the time barrier throughout future dietary intervention studies. Preparing participants to expect increased demands on their time prior to a study start would establish participant understanding of their commitment and open the door for continued communication about this challenge during the study. Other frequently reported barriers were eating out and the workplace for each diet group, and craving non-diet foods for the AIM diet group. This suggests that following a diet within the context of varied social environments is challenging and participants need to be coached on how to adhere to their diet in social settings. It is unsurprising that more AIM diet participants reported craving non-diet foods given the more restrictive nature of their diet. In future diet intervention studies, pointedly discussing barriers before and throughout the intervention would be helpful since each participant is likely to experience their own unique combination of obstacles.

Of the options presented on the exit survey few participants noted drawbacks of their study diet while more participants overall reported benefits gained from their study diet. This could be related to participant motivation resulting in an inherently optimistic outlook on effects of the diet, or the question simply may not have included appropriate types of benefit and drawback examples for participants to choose from. Some of the most positive responses were weight loss and increased energy level for participants from each group, and decrease in everyday physical pain for participants in the AIM group. Some participants reported the following benefits of the diet:

> "Less constipation… and decreased acid reflux even if I miss a dose of pantoprazole."

> "... including more vegetables and fruit (in my diet) became easier over time… and increased awareness (about food and nutrition) was a good and hopefully long lasting benefit."

> "Consistent dry cough I had had went away, joint pain and swollen feeling I had in my ankles and knees is gone, and chronic non-healing skin lesions on right shin area are finally healing."

Despite this positive outlook many participants did not respond to the survey options and it is far from an exhaustive list of possible outcomes experienced while following either of the study diets. Notably, an option addressing gastroesophageal reflux disease (GERD) was not included and many participants mentioned GERD resolution anecdotally either in person or in the write-in section provided. Additionally, there is no certainty that any benefits or drawbacks experienced during the intervention period are directly associated with the study diet. These could be products of other environmental factors in these free-living individuals.

Finally, satisfaction in both diet groups was largely positive. No subjects reported a negative effect of their diet on quality of life, or disliking their diet. Likewise almost all subjects reported the diet was worth the sacrifices, that they are likely to continue the diet into the future, and all but one person (AIM subject) will recommend this diet to others. There was little difference among the diet groups in

all these evaluations of satisfaction. This may be due to weight loss experienced by almost all the subjects, anecdotal improvements in various health conditions, increased knowledge of food and nutrition gained from group meetings during the study, or any other factor not captured by the exit survey. Regardless of the source of satisfaction, this is a positive starting point for these subjects to continue this health behavior change into the future.

As mentioned previously, highly motivated individuals are more likely to maintain a dietary change for a longer period of time.[58,67] The participants in this study were motivated from the beginning considering they self selected to pursue inclusion in the study. Likewise, their satisfaction with each of the study diets at the conclusion of the intervention is motivation for them to continue. Successful dietary behavior change depends on nutrition education so participants can understand how to follow a particular diet and what is the reasoning for following the diet.[58] Throughout this intervention participants attended bi-weekly group meetings that included an education piece on nutrition, information on how to follow their specific diet, and a check in with other members of their diet group. Another behavioral strategy used to promote dietary change is to foster self-efficacy and provide feedback on progress.[58,67] Again, participants in this study received such validation of their efforts, encouragement, and feedback at the group meetings. However, regular feedback will be difficult to maintain after the study concludes because the bi-weekly meeting structure organized by the study team will be unavailable to participants. Social relationships between people, communities and environments influence behavior change.[67] A person's complex environment, including elements such as family variables, demographics, and lifestyle factors, influences their desire and ability to adhere to a diet long term.[58] Moving forward participants will have to reconcile their desire to continue the diet with their social environment and what they may or may not be able to modify within it. For example, family members may grow tired of accepting the participant's diet or a participant may become less willing to put forth extra time and effort to adhere to the diet in the face of other lifestyle priorities.

This study has some limitations. The sample size was small and there was an uneven distribution of female to male participants. The P-values showed no trend toward differential effects with regard to adipose tissue inflammation therefore it is unlikely that these results will motivate a larger trial. Another limitation is that this study was conducted in free-living individuals who likely naturally deviated from the diet. It is impossible to know how accurately the participants followed their study diet without providing all foods throughout the intervention. This may have a larger effect on follow-up measurements than we can know. Nonetheless, occasional deviation from the diet is a likely occurrence for an individual attempting to follow a diet on their own time and therefore may provide a useful picture of realistic outcomes of the study diets in practice. Finally, there was a lack of a validated tool available to understand the participant experience during a dietary intervention and assess the likelihood they will continue the behavior change into the future.

Conclusion

Results from this study suggest that a diet aimed at minimizing daily exposure of adipose tissue to glucose, insulin, and long-chain fatty acids provides no unique benefit to decreasing adipose tissue inflammation compared with the generally recommended 2010 USDA dietary guidelines. Contrary to our hypothesis, neither of these diets resulted in decreased adipose tissue inflammatory markers after 12 weeks of dietary intervention and instead may have encouraged a moderate rise in some of these

markers. Participants in each group experienced weight loss while following the diets, which may initially lead to an increase in adipose tissue inflammatory markers. Despite no significant differences detected in participant feedback among the two study diets there was a positive response to following each diet. Overall the participants were a motivated group of individuals who are well positioned to continue the study diets into the future. Whether the general public would have success following either of the study diets is unclear. People likely have a wide variety of motivation levels, access to diet information and instruction, feedback options, group support, and social environmental contexts. This makes it difficult to presume how successfully an individual would be able to follow either the AIM or USDA diet, though having access to a guided intervention is clearly an advantage. It is uncertain if beyond the presumed benefit of weight loss the outcomes of either study diet are beneficial in resolving adipose tissue inflammation. We do not know if levels of adipose tissue inflammation markers would return to baseline or decrease once weight stabilization was achieved on either diet. Future studies can be designed to include a longer intervention period in order to reach weight stabilization or focus on other possible nutrition mechanisms that may affect adipose tissue inflammation in obesity and ultimately decrease the risk of associated co-morbidities such as diabetes.

References

1. Swinburn BA, Sacks G, Hall KD, et al. The global obesity pandemic: shaped by global drivers and local environments. *Lancet.* 2011;378(9793):804-814.
2. Ng M, Fleming T, Robinson M, et al. Global, regional, and national prevalence of overweight and obesity in children and adults during 1980-2013: a systematic analysis for the Global Burden of Disease Study 2013. *Lancet.* 2014;384(9945):766-781.
3. Ogden CL, Carroll MD, Kit BK, Flegal KM. Prevalence of childhood and adult obesity in the United States, 2011-2012. *Jama.* 2014;311(8):806-814.
4. Flegal KM, Carroll MD, Kit BK, Ogden CL. Prevalence of obesity and trends in the distribution of body mass index among US adults, 1999-2010. *Jama.* 2012;307(5):491-497.
5. Adams KF, Schatzkin A, Harris TB, et al. Overweight, obesity, and mortality in a large prospective cohort of persons 50 to 71 years old. *N Engl J Med.* 2006;355(8):763-778.
6. Katzmarzyk PT, Janssen I, Ardern CI. Physical inactivity, excess adiposity and premature mortality. *Obes Rev.* 2003;4(4):257-290.
7. Freedman DM, Ron E, Ballard-Barbash R, Doody MM, Linet MS. Body mass index and all-cause mortality in a nationwide US cohort. *Int J Obes (Lond).* 2006;30(5):822-829.
8. Whitlock G, Lewington S, Sherliker P, et al. Body-mass index and cause-specific mortality in 900 000 adults: collaborative analyses of 57 prospective studies. *Lancet.* 2009;373(9669):1083-1096.
9. Flegal KM, Kit BK, Orpana H, Graubard BI. Association of all-cause mortality with overweight and obesity using standard body mass index categories: a systematic review and meta-analysis. *Jama.* 2013;309(1):71-82.
10. Pi-Sunyer X. The medical risks of obesity. *Postgrad Med.* 2009;121(6):21-33.
11. Visscher TL, Seidell JC. The public health impact of obesity. *Annu Rev Public Health.* 2001;22:355-375.
12. Calle EE, Rodriguez C, Walker-Thurmond K, Thun MJ. Overweight, obesity, and mortality from cancer in a prospectively studied cohort of U.S. adults. *N Engl J Med.* 2003;348(17):1625-1638.
13. Renehan AG, Tyson M, Egger M, Heller RF, Zwahlen M. Body-mass index and incidence of cancer: a systematic review and meta-analysis of prospective observational studies. *Lancet.* 2008;371(9612):569-578.
14. Nguyen NT, Nguyen XM, Lane J, Wang P. Relationship between obesity and diabetes in a US adult population: findings from the National Health and Nutrition Examination Survey, 1999-2006. *Obes Surg.* 2011;21(3):351-355.
15. Menke A, Rust KF, Fradkin J, Cheng YJ, Cowie CC. Associations between trends in race/ethnicity, aging, and body mass index with diabetes prevalence in the United States: a series of cross-sectional studies. *Ann Intern Med.* 2014;161(5):328-335.
16. Janssen I. The public health burden of obesity in Canada. *Can J Diabetes.* 2013;37(2):90-96.
17. Knowler WC, Barrett-Connor E, Fowler SE, et al. Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. *N Engl J Med.* 2002;346(6):393-403.
18. Poirier P, Giles TD, Bray GA, et al. Obesity and cardiovascular disease: pathophysiology, evaluation, and effect of weight loss: an update of the 1997 American Heart Association Scientific Statement on Obesity and Heart Disease from the Obesity Committee of the Council on Nutrition, Physical Activity, and Metabolism. *Circulation.* 2006;113(6):898-918.
19. Cawley J, Meyerhoefer C. The medical care costs of obesity: an instrumental variables approach. *J Health Econ.* 2012;31(1):219-230.
20. Trogdon JG, Finkelstein EA, Hylands T, Dellea PS, Kamal-Bahl SJ. Indirect costs of obesity: a review of the current literature. *Obes Rev.* 2008;9(5):489-500.

21. Association AD. Economic costs of diabetes in the U.S. in 2012. *Diabetes Care.* 2013;36(4):1033-1046.
22. Wadden TA, Butryn ML, Hong PS, Tsai AG. Behavioral treatment of obesity in patients encountered in primary care settings: a systematic review. *Jama.* 2014;312(17):1779-1791.
23. Dombrowski SU, Knittle K, Avenell A, Araujo-Soares V, Sniehotta FF. Long term maintenance of weight loss with non-surgical interventions in obese adults: systematic review and meta-analyses of randomised controlled trials. *Bmj.* 2014;348:g2646.
24. Peirson L, Douketis J, Ciliska D, Fitzpatrick-Lewis D, Ali MU, Raina P. Treatment for overweight and obesity in adult populations: a systematic review and meta-analysis. *CMAJ Open.* 2014;2(4):E306-317.
25. Wing RR, Phelan S. Long-term weight loss maintenance. *Am J Clin Nutr.* 2005;82(1 Suppl):222s-225s.
26. Gloy VL, Briel M, Bhatt DL, et al. Bariatric surgery versus non-surgical treatment for obesity: a systematic review and meta-analysis of randomised controlled trials. *Bmj.* 2013;347:f5934.
27. Kovacs Z, Valentin JB, Nielsen RE. Risk of psychiatric disorders, self-harm behaviour and service use associated with bariatric surgery. *Acta Psychiatr Scand.* 2016.
28. Sun S, Ji Y, Kersten S, Qi L. Mechanisms of inflammatory responses in obese adipose tissue. *Annu Rev Nutr.* 2012;32:261-286.
29. Conde J, Scotece M, Gomez R, et al. Adipokines: biofactors from white adipose tissue. A complex hub among inflammation, metabolism, and immunity. *Biofactors.* 2011;37(6):413-420.
30. Greenberg AS, Obin MS. Obesity and the role of adipose tissue in inflammation and metabolism. *Am J Clin Nutr.* 2006;83(2):461s-465s.
31. Trayhurn P, Bing C, Wood IS. Adipose tissue and adipokines--energy regulation from the human perspective. *J Nutr.* 2006;136(7 Suppl):1935s-1939s.
32. Goran MI, Alderete TL. Targeting adipose tissue inflammation to treat the underlying basis of the metabolic complications of obesity. *Nestle Nutr Inst Workshop Ser.* 2012;73:49-60; discussion p61-46.
33. Zeyda M, Stulnig TM. Obesity, inflammation, and insulin resistance--a mini-review. *Gerontology.* 2009;55(4):379-386.
34. Hotamisligil GS, Shargill NS, Spiegelman BM. Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. *Science.* 1993;259(5091):87-91.
35. Uysal KT, Wiesbrock SM, Marino MW, Hotamisligil GS. Protection from obesity-induced insulin resistance in mice lacking TNF-alpha function. *Nature.* 1997;389(6651):610-614.
36. Perreault M, Marette A. Targeted disruption of inducible nitric oxide synthase protects against obesity-linked insulin resistance in muscle. *Nat Med.* 2001;7(10):1138-1143.
37. Vieira VJ, Valentine RJ, Wilund KR, Antao N, Baynard T, Woods JA. Effects of exercise and low-fat diet on adipose tissue inflammation and metabolic complications in obese mice. *Am J Physiol Endocrinol Metab.* 2009;296(5):E1164-1171.
38. Kosteli A, Sugaru E, Haemmerle G, et al. Weight loss and lipolysis promote a dynamic immune response in murine adipose tissue. *J Clin Invest.* 2010;120(10):3466-3479.
39. Hotamisligil GS, Arner P, Caro JF, Atkinson RL, Spiegelman BM. Increased adipose tissue expression of tumor necrosis factor-alpha in human obesity and insulin resistance. *J Clin Invest.* 1995;95(5):2409-2415.
40. Le KA, Mahurkar S, Alderete TL, et al. Subcutaneous adipose tissue macrophage infiltration is associated with hepatic and visceral fat deposition, hyperinsulinemia, and stimulation of NF-kappaB stress pathway. *Diabetes.* 2011;60(11):2802-2809.

41. Pietilainen KH, Kannisto K, Korsheninnikova E, et al. Acquired obesity increases CD68 and tumor necrosis factor-alpha and decreases adiponectin gene expression in adipose tissue: a study in monozygotic twins. *J Clin Endocrinol Metab.* 2006;91(7):2776-2781.
42. Weisberg SP, McCann D, Desai M, Rosenbaum M, Leibel RL, Ferrante AW, Jr. Obesity is associated with macrophage accumulation in adipose tissue. *J Clin Invest.* 2003;112(12):1796-1808.
43. Marieb EN, Hoehn K. *Anatomy and Physiology.* 3rd ed. San Francisco, CA: Pearson Benjamin Cummings; 2008.
44. Osborn O, Olefsky JM. The cellular and signaling networks linking the immune system and metabolism in disease. *Nat Med.* 2012;18(3):363-374.
45. Xu H, Barnes GT, Yang Q, et al. Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. *J Clin Invest.* 2003;112(12):1821-1830.
46. Hill AA, Reid Bolus W, Hasty AH. A decade of progress in adipose tissue macrophage biology. *Immunol Rev.* 2014;262(1):134-152.
47. Lumeng CN, Bodzin JL, Saltiel AR. Obesity induces a phenotypic switch in adipose tissue macrophage polarization. *J Clin Invest.* 2007;117(1):175-184.
48. Castoldi A, Naffah de Souza C, Camara NO, Moraes-Vieira PM. The Macrophage Switch in Obesity Development. *Front Immunol.* 2015;6:637.
49. Kraakman MJ, Murphy AJ, Jandeleit-Dahm K, Kammoun HL. Macrophage polarization in obesity and type 2 diabetes: weighing down our understanding of macrophage function? *Front Immunol.* 2014;5:470.
50. Gordon S, Pluddemann A, Martinez Estrada F. Macrophage heterogeneity in tissues: phenotypic diversity and functions. *Immunol Rev.* 2014;262(1):36-55.
51. Wernstedt Asterholm I, Tao C, Morley TS, et al. Adipocyte inflammation is essential for healthy adipose tissue expansion and remodeling. *Cell Metab.* 2014;20(1):103-118.
52. Xu X, Grijalva A, Skowronski A, van Eijk M, Serlie MJ, Ferrante AW, Jr. Obesity activates a program of lysosomal-dependent lipid metabolism in adipose tissue macrophages independently of classic activation. *Cell Metab.* 2013;18(6):816-830.
53. Kratz M, Coats BR, Hisert KB, et al. Metabolic dysfunction drives a mechanistically distinct proinflammatory phenotype in adipose tissue macrophages. *Cell Metab.* 2014;20(4):614-625.
54. Ory MG, Lee Smith M, Mier N, Wernicke MM. The science of sustaining health behavior change: the health maintenance consortium. *Am J Health Behav.* 2010;34(6):647-659.
55. Neily JB, Toto KH, Gardner EB, et al. Potential contributing factors to noncompliance with dietary sodium restriction in patients with heart failure. *Am Heart J.* 2002;143(1):29-33.
56. Sheahan SL, Fields B. Sodium dietary restriction, knowledge, beliefs, and decision-making behavior of older females. *J Am Acad Nurse Pract.* 2008;20(4):217-224.
57. Bentley B, De Jong MJ, Moser DK, Peden AR. Factors related to nonadherence to low sodium diet recommendations in heart failure patients. *Eur J Cardiovasc Nurs.* 2005;4(4):331-336.
58. Kumanyika SK, Van Horn L, Bowen D, et al. Maintenance of dietary behavior change. *Health Psychol.* 2000;19(1s):42-56.
59. Belza A, Toubro S, Stender S, Astrup A. Effect of diet-induced energy deficit and body fat reduction on high-sensitive CRP and other inflammatory markers in obese subjects. *Int J Obes (Lond).* 2009;33(4):456-464.
60. Salas-Salvado J, Bullo M, Garcia-Lorda P, et al. Subcutaneous adipose tissue cytokine production is not responsible for the restoration of systemic inflammation markers during weight loss. *Int J Obes (Lond).* 2006;30(12):1714-1720.

61. Magkos F, Fraterrigo G, Yoshino J, et al. Effects of Moderate and Subsequent Progressive Weight Loss on Metabolic Function and Adipose Tissue Biology in Humans with Obesity. *Cell Metab.* 2016;23(4):591-601.
62. Tam CS, Covington JD, Ravussin E, Redman LM. Little evidence of systemic and adipose tissue inflammation in overweight individuals(dagger). *Front Genet.* 2012;3:58.
63. Campbell KL, Foster-Schubert KE, Makar KW, et al. Gene expression changes in adipose tissue with diet- and/or exercise-induced weight loss. *Cancer Prev Res (Phila).* 2013;6(3):217-231.
64. Bastard JP, Jardel C, Bruckert E, et al. Elevated levels of interleukin 6 are reduced in serum and subcutaneous adipose tissue of obese women after weight loss. *J Clin Endocrinol Metab.* 2000;85(9):3338-3342.
65. Clement K, Viguerie N, Poitou C, et al. Weight loss regulates inflammation-related genes in white adipose tissue of obese subjects. *Faseb j.* 2004;18(14):1657-1669.
66. Cancello R, Henegar C, Viguerie N, et al. Reduction of macrophage infiltration and chemoattractant gene expression changes in white adipose tissue of morbidly obese subjects after surgery-induced weight loss. *Diabetes.* 2005;54(8):2277-2286.
67. Thomson CA, Ravia J. A systematic review of behavioral interventions to promote intake of fruit and vegetables. *J Am Diet Assoc.* 2011;111(10):1523-1535.

Figures and Tables

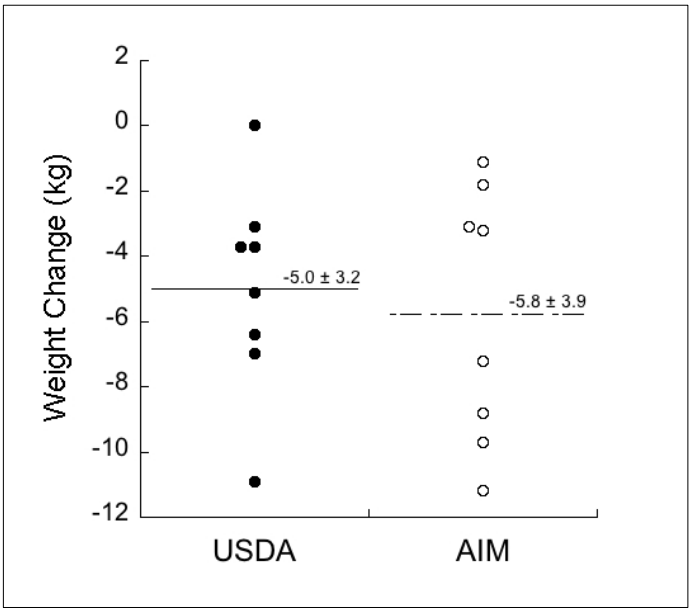

Figure 1 - Weight change during the 12-week intervention period measured in kg. Each point represents an individual subject, there are 8 subjects in each diet group. The mean weight loss of the subjects in each diet group is shown as a solid line for the USDA group and a dashed line for the AIM group.

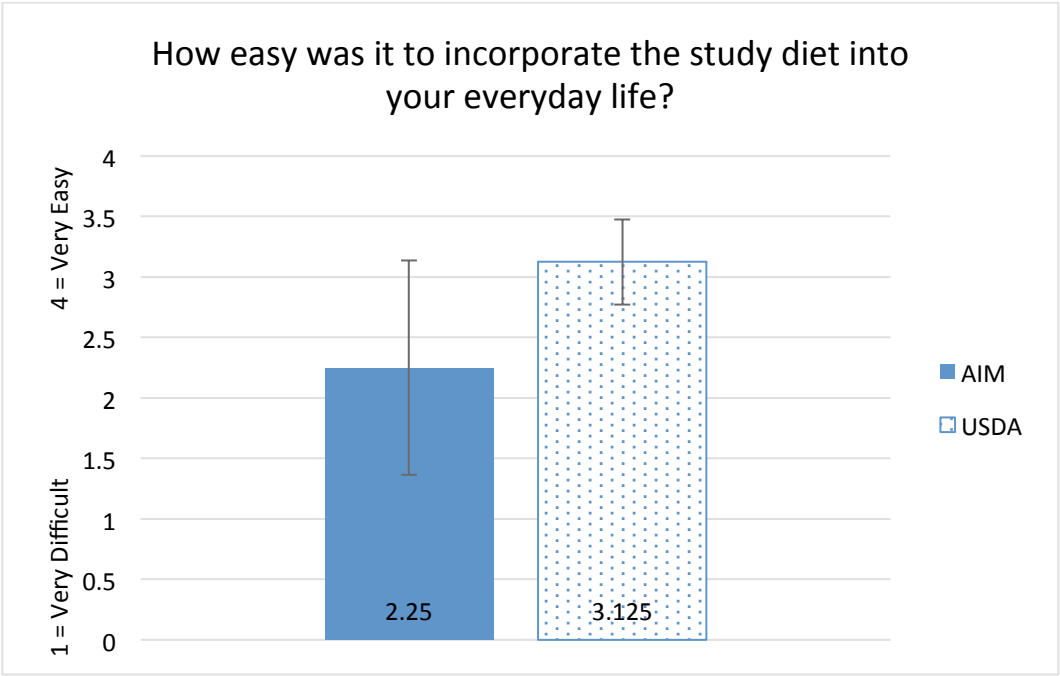

Figure 2 - Mean response of the 8 subjects in each group reporting the ease of incorporating the diet into everyday life. Progressive scale from 1 = very difficult to 4 = very easy. Error bars indicate standard deviations.

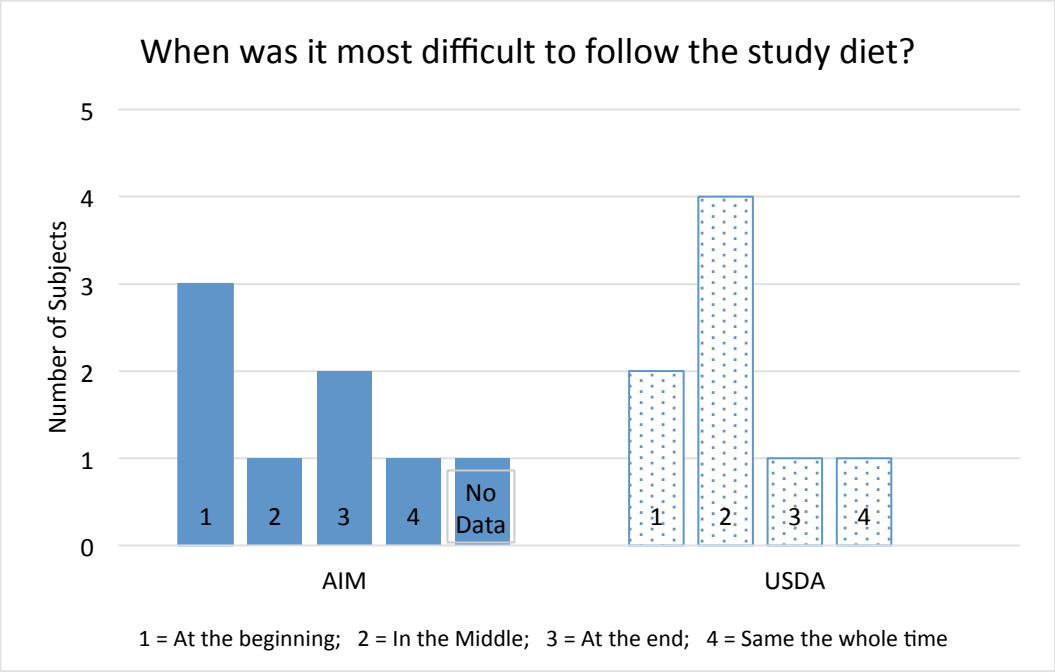

Figure 3 - Count of subject responses in each group reporting at what point during the intervention it was most difficult to follow the study diet. 1 = at the beginning; 2 = in the middle; 3 = at the end; 4 = same the whole time.

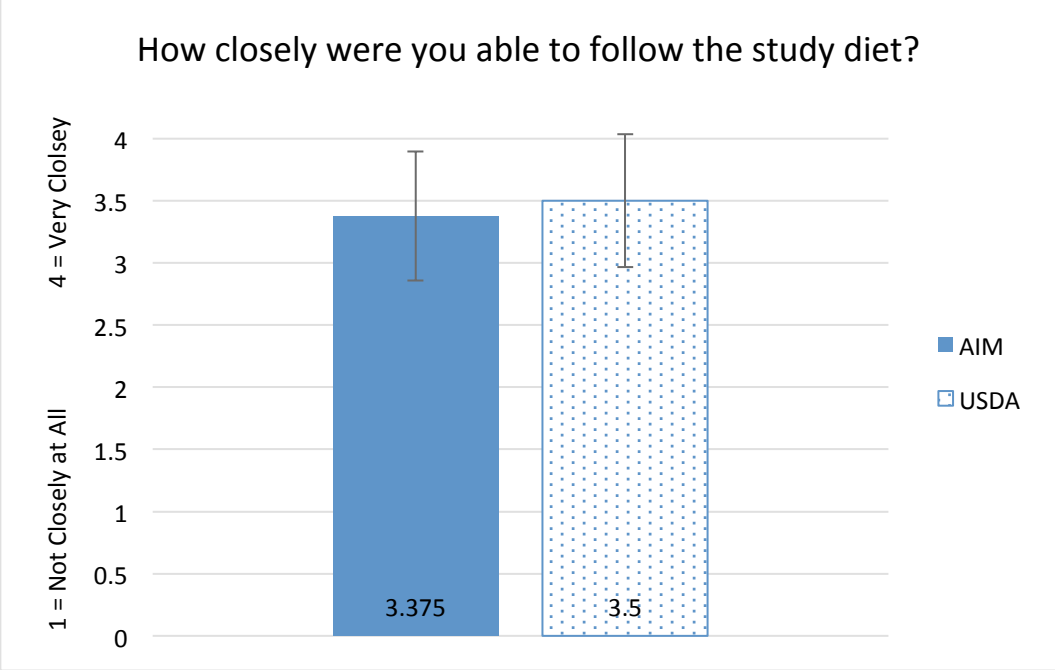

Figure 4 - Mean response of the 8 subjects in each group reporting the how closely they were able to follow the study diet. Progressive scale from 1 = not closely at all to 4 = very closely. Error bars indicate standard deviations.

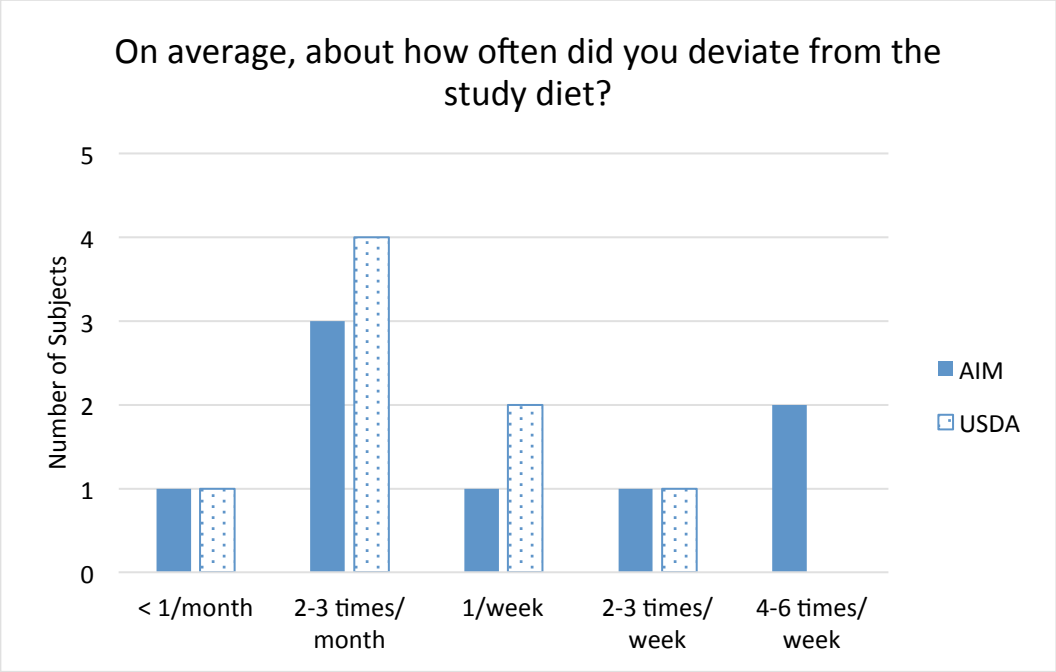

Figure 5 - Count of subject responses in each group reporting average deviation from the study diet.

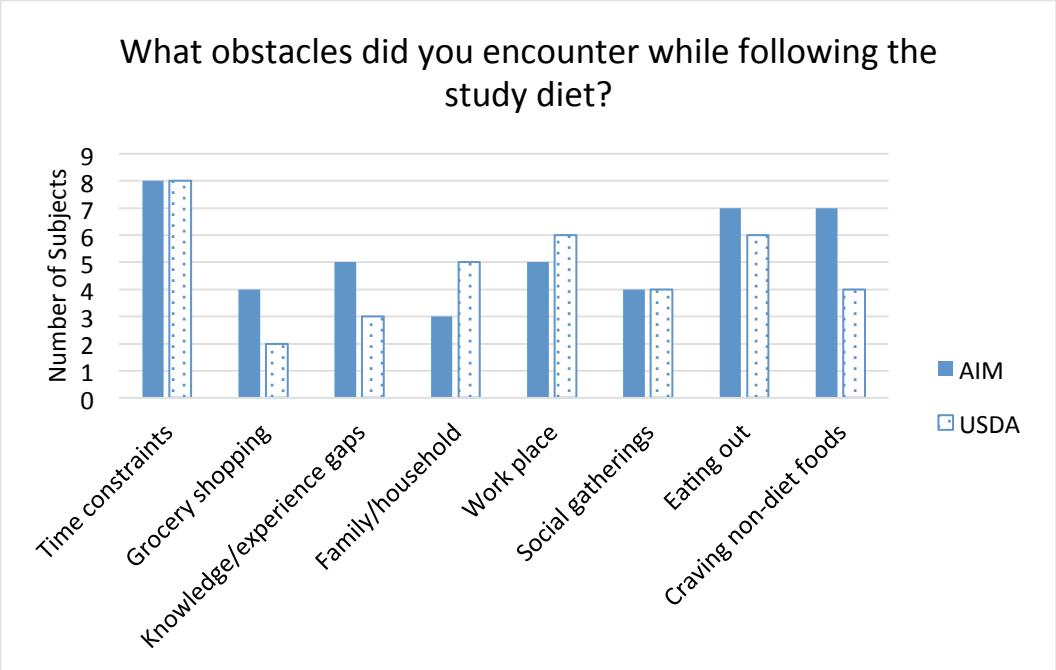

Figure 6 - Count of subject responses in each group reporting various obstacles to following the study diet.

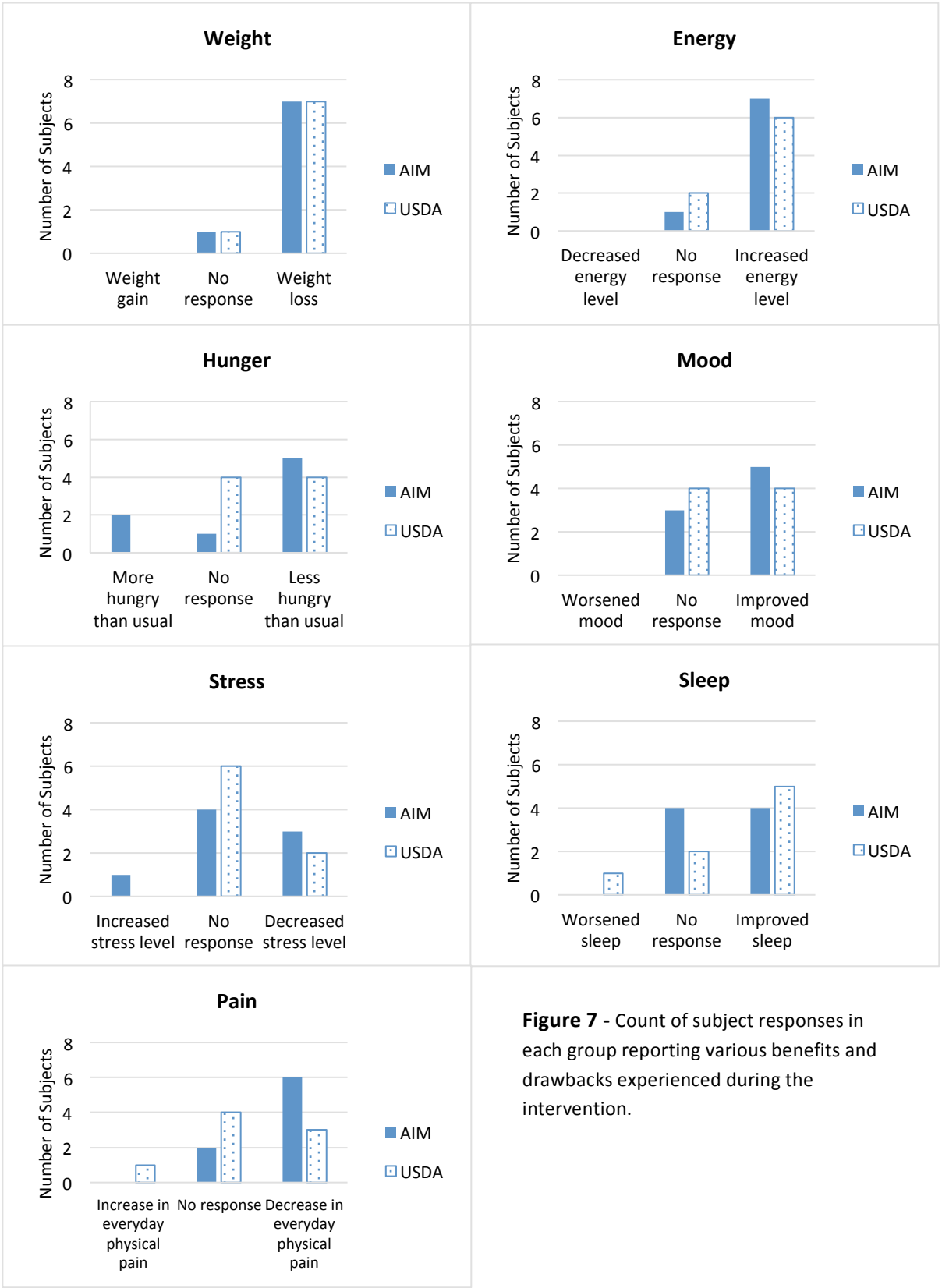

Figure 7 - Count of subject responses in each group reporting various benefits and drawbacks experienced during the intervention.

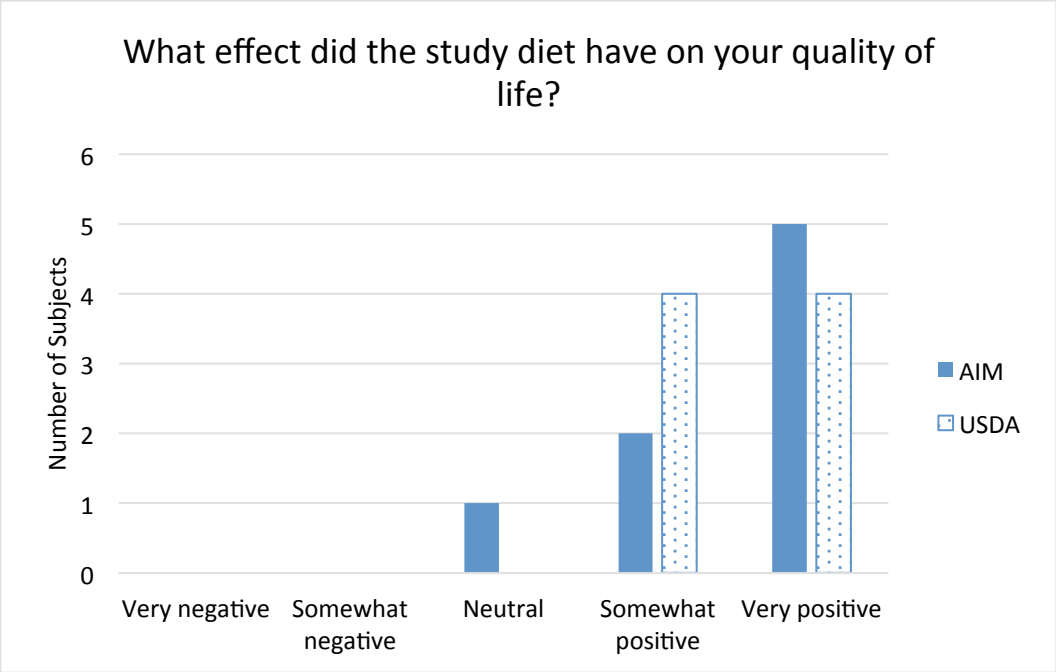

Figure 8 - Count of subject responses in each group reporting effect of the study diet on quality of life.

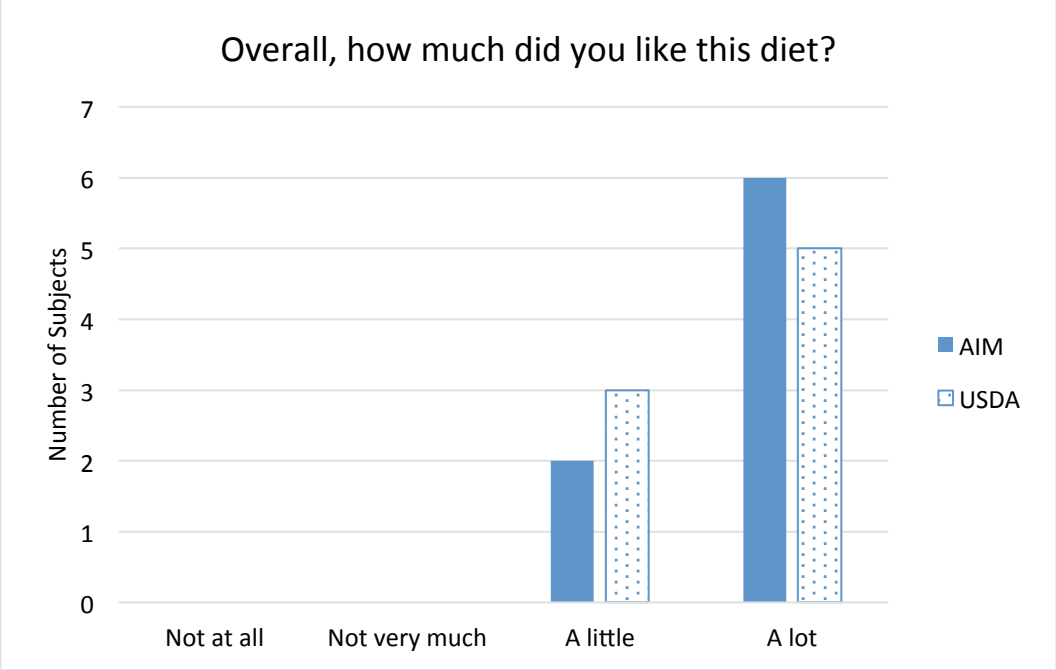

Figure 9 - Count of subject responses in each group reporting like or dislike of the study diet.

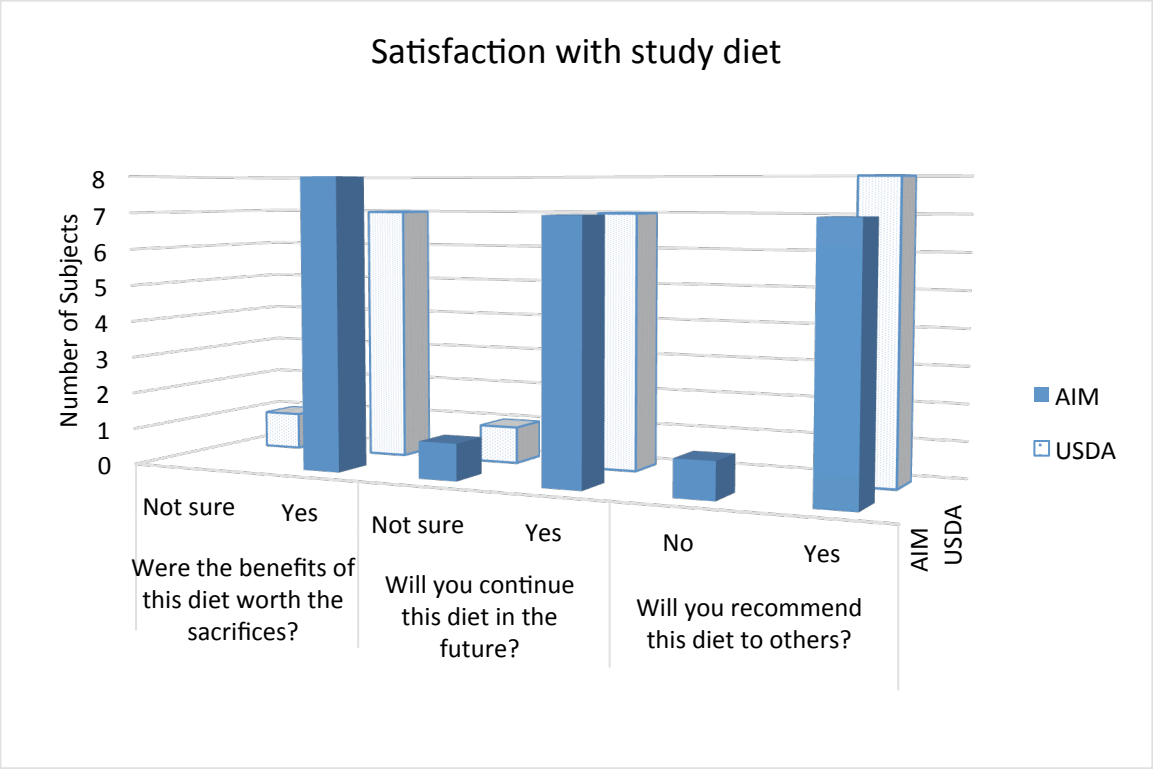

Figure 10 - Count of subject responses in each group reporting various measures of satisfaction with the study diet.

Table 1. Clinical characteristics of the study population at baseline in participants randomized to the Anti-Inflammatory Milieu (AIM) diet or the U.S. Department of Agriculture's "Dietary Guidelines for Americans" (USDA)[1].

	All (n=16)	**USDA (n=8)**	**AIM (n=8)**	**USDA vs. AIM P-value**
Gender (female / male)	14 / 2	7 / 1	7 / 1	1.0
Age (y)	47.6 ± 13.8	46.9 ± 13.2	48.4 ± 15.3	0.695
Body mass index (kg/m^2)	36.4 ± 6.0	35.9 ± 6.2	37.0 ± 6.2	0.923
Fasting glucose (mmol/L)	102.3 ± 9.7	102.9 ± 11.9	101.8 ± 7.7	0.511
Fasting insulin (mU/mL)	11.4 (10.1, 14.4)	11.8 (9.4, 12.9)	12.2 (10.7, 16.0)	0.841
HOMA-IR	3.0 (2.4, 3.6)	2.8 (2.2, 3.3)	3.2 (2.6, 3.9)	0.859
Hemoglobin A1c (%)	5.4 ± 0.4	5.4 ± 0.4	5.3 ± 0.4	0.799
Fasting high-sensitivity C-reactive protein (mg/L)	1.8 (0.9, 4.1)	1.3 (0.5, 2.1)	3.9 (1.1, 7.5)	0.681

[1]Values are means ± standard deviations or medians (25th, 75th percentiles). P-values are based on independent samples t-tests.

Table 2. Number of ATM and relative mean fluorescence intensity (rMFI) of pro-inflammatory markers CD36 and ABCA1 at baseline (pre-intervention) and at follow up (completed 12-week diet)[2].

	Baseline (pre-intervention)	Follow-up (completed 12-week diet)	RM-ANOVA	
			P (time)	P (time x intervention)
ATM number as cells per g of tissue				
USDA (n=7)	54,395 (25,092 – 70,659)	41,790 (24,120 – 46,628)	0.123	0.942
AIM (n=8)	42,877 (29,779 – 81,825)	36,115 (18,935 – 70,016)		
ABCA1 rMFI				
USDA (n=6)	1.8 ± 0.49	2.4 ± 0.67	0.004	0.979
AIM (n=8)	1.9 ± 0.34	2.4 ± 0.56		
CD36 rMFI				
USDA (n=8)	5.6 (4.6 – 6.5)	6.8 (5.1 – 8.4)	0.976	0.794
AIM (n=8)	6.8 (5.2 – 8.2)	5.2 (4.3 – 10.7)		

[2]Values are mean ± SD or medians (25^{th}, 75^{th} percentiles), in mRNA copy number of the respective target gene per ng of total RNA, normalized by a normalization factor based on the housekeeping gene, β-glucuronidase. RM-ANOVA: Repeated measures analysis of variance.

Table 3. Adipose tissue expression of genes encoding mediators of inflammation at baseline (pre-intervention) and at follow up (completed 12-week diet)[3].

	Baseline (pre-intervention)	Follow-up (completed 12-week diet)	RM-ANOVA	
			P (time)	P (time x intervention)
Tumor necrosis factor α (*TNFα*)				
USDA (n=8)	4.3 (3.9 – 5.1)	5.0 (3.6 – 6.9)	0.184	0.570
AIM (n=8)	3.8 (3.5 – 4.9)	5.5 (3.5 – 6.9)		
Interleukin-1β (*IL1β*)				
USDA (n=8)	1.2 (1.1 – 1.6)	1.8 (1.2 – 2.4)	0.138	0.941
AIM (n=8)	1.8 (1.5 – 2.6)	2.7 (1.1 – 4.3)		
Interleukin-6 (*IL6*)				
USDA (n=8)	14.1 (10.4 – 15.4)	12.7 (9.6 – 20.7)	0.751	0.689
AIM (n=8)	10.0 (7.4 – 11.8)	10.6 (6.3 – 19.9)		
Adiponectin (*ADIPOQ*)				
USDA (n=8)	12,676 ± 2,955	14,659 ± 2,353	0.085	0.462
AIM (n=8)	11,802 ± 4,707	12,620 ± 4,531		

[3]Values are mean ± SD or medians (25^{th}, 75^{th} percentiles), in mRNA copy number of the respective target gene per ng of total RNA, normalized by a normalization factor based on the housekeeping gene, β-glucuronidase. RM-ANOVA: Repeated measures analysis of variance.

Appendix 1

Date:_____ Subject #: _____

Diet and Metabolic Inflammation Study
Exit Survey

Your responses to the following questions about your experiences during this study will be used to improve the design of future dietary studies.

There is no penalty or loss of benefits for answering honestly. We greatly appreciate your feedback!

1. How easy was it to incorporate the study diet into your everyday life?
   - □ Very easy
   - □ Somewhat easy
   - □ Somewhat difficult
   - □ Very difficult

2. When was it most difficult to follow the study diet?
   - □ At the beginning
   - □ In the middle
   - □ At the end
   - □ The same the whole time
   - □ Not difficult at all

3. How closely were you able to follow the study diet?
   - □ Very closely
   - □ Somewhat closely
   - □ Not very closely
   - □ Not closely at all

4. On average, about how often did you deviate from the study diet? For example, how often did you eat a meal that contained some foods from the red column of foods to limit?
   - □ < 1/month
   - □ 2-3 times/month
   - □ 1/week
   - □ 2-3 times/week
   - □ 4-6 times/week
   - □ 1+ times/day

5. What obstacles did you encounter while following the study diet? Check all that apply:

- Time constraints:
  - □ Lack of time to plan ahead for meals
  - □ Lack of time to prepare/cook food
  - □ Lack of time to shop for food

- Grocery shopping:
  - □ Difficulty finding foods that were recommended on the study diet
  - □ Need to travel further to find stores that carry study diet foods
  - □ Expense of foods that were recommended on with the study diet

- Knowledge/experience gaps:
  - □ Not knowing what to prepare
  - □ Not sure which foods were allowed/not allowed

- Family/household:
  - □ Family or housemates not able or willing to eat the study diet as well
  - □ Having to prepare different meals for different people
  - □ Lacking support/understanding from family or housemates

- Work place:
  - □ Availability of other foods at work
  - □ Lack of support/understanding among co-workers
  - □ Work-related events with food

- Social gatherings:
  - □ Lack of support/understanding among friends
  - □ Pressure to eat what others are eating
  - □ Feelings of being isolated or left out
  - □ Feelings of embarrassment about your diet

- Eating out:
  - □ Difficulty finding establishments that serve study diet foods
  - □ Establishments unable or unwilling to modify foods to fit the diet
  - □ Temptation of foods that were not allowed on the study diet

- Craving non-diet foods:
  - □ Desire to eat foods that were not allowed on the study diet because normally you enjoy them
  - □ Desire to eat 'forbidden' foods simply because they were not allowed

Please list any other obstacles you encountered while following the study diet:

_____

_____

_____

_____

6. Did you experience any of the following benefits or drawbacks during the study? Check all that apply:

- □ Less hungry than usual
- □ More hungry than usual

- □ Increased energy level
- □ Decreased energy level

- □ Weight loss
- □ Weight gain

- □ Reduction in any everyday physical pain
- □ Increase in any everyday physical pain

- □ Improved mood: For example, feeling more content, happier, at peace
- □ Worsened mood: For example, feeling more irritable, grumpy, depressed

- □ Decreased stress levels
- □ Increased stress levels

- □ Improved sleep
- □ Worsened sleep

Please list any other benefits or drawbacks you encountered while following the study diet:

_____

_____

_____

_____

7. What effect did the study diet have on your quality of life?
    - □ Very positive
    - □ Somewhat positive
    - □ Neutral
    - □ Somewhat negative
    - □ Very negative

8. Overall, how much did you like this diet?
    - □ A lot
    - □ A little
    - □ Not very much
    - □ Not at all

9. Were the benefits of this diet worth the sacrifices?
    - □ Yes
    - □ No
    - □ Not sure

10. Will you continue this diet in the future?
    - □ Yes
    - □ No
    - □ Not sure

11. Will you recommend this diet to others?
    - □ Yes
    - □ No
    - □ Not sure

12. What other feedback would you like to share about your experience following the study diet?