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Uric Acid Level Is Associated With Postprandial Lipemic Response To A High Saturated Fat Meal

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Walden University
2015

Abstract

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MPH, Walden University, 2012

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Dissertation Submitted in Partial Fulfillment

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Abstract

Hyperlipidemia caused by a diet high in saturated fat can lead to visceral fat weight gain, obesity, and metabolic syndrome. Being over-weight from visceral fat has been linked to increased risk of developing most age-related diseases and disability, along with a lower income potential and quality of life. However, researchers are just beginning to understand the biological mechanisms that regulate the conversion of excess calories into visceral fat storage rather than glycogen or muscle. Epidemiological studies have repeatedly shown a comorbid association between age-related diseases involving hyperlipemia and circulating levels of uric acid, but not a direct association. This study utilized archival data from 31 healthy, middle-aged adults, who participated in a randomized, double-blind, crossover clinical trial on blood markers of lipidemia and inflammation following a high saturated fat (HSF) verses a "healthy" polyunsaturated fat (PUFA) meal. This primary study was conducted and funded by the National Institute on Aging. A secondary analysis of this data using Pearson's correlation with least squares (2-tailed) regression modeling found that when stratified by gender, baseline uric acid level was an independent and significant predictor of the lipemic response from the HSF, but not the PUFA meal. The linear regression plots indicated that males with uric acid levels above 4.5, and females above 3.0 mg/dL, had a progressively increased lipemic response to the HSF meal. The public health utility of this finding may include the clinical use of the gender-specific linear regression plots of uric acid values to identify and advise individuals at risk for hyperlipidemia from a diet high in saturated fats.

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Chapter 1: Introduction

Because of the recent epidemic in weight gain, today's youth are the first generation to have a shorter and less productive projected lifespan with more years of disability than their parents (Piernas & Popkin, 2011a). Epidemiological data show that the increase in weight gain started around 1985, due to the accumulation of drastic environmental changes in the availability of affordable high sugar and high fat foods that make up what has been termed, "the Western diet" (Schlosser, 2002). These changes in increased caloric density of the American diet, along with decreases in physical exercise, are thought to be the main contributors to the highest level of visceral fat weight gain ever recorded within a general population, with nearly 68% of the adults in the United States being overweight or obese in 2010 (Fortuna, 2012).

One animal model for the development of visceral fat weight gain towards obesity, insulin resistance, and metabolic syndrome using a single variable is the feeding of a 33% sucrose diet to a common lab rat (strains: Wistar, Lewis, or Sprague Dawley; Stranahan et al., 2008). The initial metabolic changes from the sucrose diet are elevations in insulin levels, followed by elevations in de novo lipid synthesis (Stranahan, Cutler, Button, Telljohann, & Mattson, 2011). It is the increase of endogenous lipogenesis that initiates the expansion of visceral fat with paralleled increases in stored and circulating levels of sphingolipids, triglycerides, and cholesterol. The most consistent diet used to cause weight gain in animal models utilizes a combination of high sugar (particularly fructose) and saturated fats (Dekker, Su, Baker, Rutledge, & Adeli, 2010).

Uric acid (UA) levels have been positively associated as a possible independent risk factor for developing lipidemia and associated diseases (e.g. insulin resistance, diabetes, cardiovascular disease; Nakagawa et al., 2006). However, epidemiological studies have only focused on the association of UA after weight gain, and not its role, if any, in the mechanism initiating weight gain. In this study, UA was assessed for its potential effect in enhancing postprandial endogenous lipogenesis (lipemic response) after a single high in saturated fats (HSF) fast food modeled meal (a known inducer of *de novo* triglyceride and cholesterol synthesis; Lin et al., 2005). By utilizing archival data from normal weight (e.g. body mass index, BMI< 25 kg/m^2), insulin sensitive [2-hour oral glucose tolerance test (OGTT) blood glucose level below 140 mg/dL], and healthy renal functioning [blood creatinine < 1.4 mg/dL] middle-aged adults (Table 1), this study tested if baseline UA levels affect their postprandial lipemic response to a single HSF meal. Each participant in the study longitudinally served as his or her own control (Table 2), which included a crossover low in saturated fat meal, consisting of an equal nutritional profile (i.e., protein, carbohydrates, and fat) as the HSF meal, but substituting saturated fats with polyunsaturated fats (PUFA) as a negative control (Table 3). The rationale for this study was from previous reports that UA correlated with hyperlipidemia diseases, including cardiovascular disease, type 2 diabetes, and metabolic syndrome. The mechanism through which UA is thought to increase *de novo* lipid synthesis is through the AMP-activated protein kinase (AMPK) pathway, but no clinical studies have been published to support these liver cell culture findings (Lanaspa, Cicerchi, et al., 2012).

The hypothesis tested in this study was that baseline levels of UA predict the postprandial lipemic response (i.e., percent change of VLDL over time) after a single

high fat meal. The next morning, fasted serum was also analyzed for a possible link between postprandial lipemic response and an acute change in insulin sensitivity (HOMA2 test from fasted insulin/glucose levels; Levy, Matthews, & Hermans, 1998). Regression modeling was used to analyze the predictive power of baseline levels of UA verses other previously studied factors involved in the regulation of postprandial lipemic response (i.e., baseline: lipids, markers of inflammation, insulin hormones, and insulin sensitivity). Correlation of all independent variables was also tested for codependence with UA for the presence of potential serial correlation bias errors.

Background

Approximately 68% of adults in the United States were overweight or obese. Over the past 25 years, the prevalence of obese adults (i.e. BMI > 30) has grown from 14.5% to 35.7% (Fortuna, 2012). Many epidemiological studies have shown that excess body fat leads to decreases in work productivity and long-term quality of life, and increased risk for developing cancer, cardiovascular disease, diabetes, and most of the other age-related degenerative diseases (Liu et al., 2013). Epidemiological survey data show that an over-eating epidemic started in the United States around 1985 (Blasbalg, Hibbeln, Ramsden, Majchrzak, & Rawlings, 2011).

History of the Western Diet

Factors that led to the development of the Western diet include the industrial revolution, mechanized farming, fertilizers, pesticides, and agricultural genetic engineering (Blasbalg et al., 2011). In reaction to the Great Depression and World War II, the U.S. government established laws for agriculture and consumer food shopping subsidies (e.g. corn, meat, and dairy) to establish food security for public health. These

initiatives resulted in a glut of available and inexpensive agricultural products that have been pushed onto consumers by an ever-growing food industry (Eichhorn & Nagel, 2010; Fields, 2004). This era marks the beginning of the recent and drastic environmental changes in the availability of affordable, high sugar and fat foods that make up the Western diet. In addition to the changes in dietary choices towards higher processed and calorically dense foods, caloric consumption also increased (Blasbalg, et al., 2011). One contributing factor for increased caloric consumption has been the environmental changes in mass communication (i.e. television and radio commercials) from the food industry. These advertisements most often encourage purchasing unhealthy foods, which over time has led many consumers to believe that a cheeseburger, French fries, and a milk shake are a good meal choice (Grotz, 2006). Since the 1970s, the U.S. population has increased their average consumption of sugar by 36% (i.e. 80 to 109 pounds per year) and meat by 80% (i.e. 112.5 to 203.7 pounds per year), where women had an average increase of 335, and men 168 calories per day (Daniel, Cross, Koebnick, & Sinha, 2011). Most of these extra calories have come from increased consumption of high fructose corn syrup found in sweetened beverages, which currently accounts for about 25% of daily caloric intake in the United States. Since 1977, the daily consumption of fructose has gone up from a mean of 37 to 54.7 grams/day, an increase of 49% (Vos, Kimmons, Gillespie, Welsh, & Blanck, 2008). High fructose consumption is associated with increased *de novo* hyper-lipogenesis, insulin resistance, and weight gain. Dietary fructose has been found to increase blood triglycerides by 186%, whereas intake of the same amount of glucose did not have this effect (Janevski et al., 2012). High dietary consumption of fructose has also been shown to increase UA levels through increased purine (e.g. ATP) catabolism, and as

a competitive inhibitor of kidney excretion through the Glut-9 receptor. Fructose consumption has been found to be a risk factor linked to the recent increased incidence rate of gout (i.e. high uric acid [> 7.0 mg/dL] levels form crystals and inflammatory response; Nakagawa, et al., 2006).

Regulation of Lipogenesis

Excessive caloric intake activates transcription factor SREBP-1c in liver and adipose cells to initiate protein synthesis of all fatty acid and cholesterol synthesis enzymes, and constitutes the macro regulatory mechanism of endogenous lipogenesis (Shao & Espenshade, 2012). Postprandial induced endogenous lipogenesis is reflected by the acute percent changes in circulating VLDL (i.e. lipemic response). Activators of SREBP-1c transcription and maturation into mature protein include insulin hormones, pro-inflammation cytokines, and saturated fatty acids (e.g. C16:0; Lin, et al., 2005). The initial and rate-limiting step enzyme for fatty acid synthesis is acetyl-CoA carboxylase (ACC), and for cholesterol is 3-hydroxy-3-methyl-glutaryl-CoA reductase (HMG-CoA). Micro regulation of the activity of SREBP-1c, ACC, and HMG-CoA is achieved by phosphorylation of active sites by AMPK, which turns "off" transcription of the enzymes, and enzymatic activity of lipid synthesis (Hardie, 2004; Winder & Hardie, 1999). Insulin, TNFα (a pro-inflammation cytokine), and ceramides activate protein phosphatase-2A (PP2A) and glutamate-activated protein phosphatase (GAPP), which both dephosphorylate and activate transcription and enzyme activity (de Mello et al., 2009; Poppitt et al., 2008).

At the juncture of regulation for anabolic fatty acid lipid synthesis and catabolic beta-oxidation of fatty acids is acetyl-CoA carboxylase (ACC), which is the rate-limiting

step of saturated fatty acid synthesis. Activated ACC catalyzes the carboxylation of acetyl-CoA to form malonyl-CoA, which is the natural metabolic inhibitor of carnitine acyltransferase-mediated shuttling of fatty acids into the mitochondria for catabolic beta-oxidation of free fatty acids. Therefore, ACC is a key metabolic switch between anabolic fatty acid synthesis and catabolic fatty acid beta-oxidation (McCarty, 2001). Several animal models have shown that inhibition of ACC acts to protect mice from weight gain, insulin resistance, and lipotoxicity (e.g. fatty liver disease) from a high saturated fat diet. These ACC knockdown mice also had the advantage of increased spontaneous exercise and better performance in learning and memory tasks (Dzamko et al., 2008a; McCarty, 2001; Turdi et al., 2010)

Effect of Food Choices on Visceral Fat Weight Gain

A confounding issue is the discovery that elevated lipids (particularly saturated free fatty acid C16:0) actually act to self-perpetuate higher levels of *de novo* fatty acid synthesis due to the feed-forward genetic and biochemical mechanism of the SREBP-1c pathway (Lin et al., 2005). Excess caloric intake relative to energy expenditure is the primary modifiable risk factor for the latest observed increases in visceral fat weight gain. This is made worse by food choices of high caloric density saturated fats and fructose that acts independently to hyper-activate lipid synthesis via the SREBP-1c pathway (Magne et al., 2010). However, food choices high in polyunsaturated fatty acids (PUFA; C18:2 high in nuts), as used in this study's control diet, have been shown not to hyper-activate lipogenesis (Jimenez-Gomez et al., 2009).

The Pros and Cons of Uric Acid on Health and Visceral Fat Weight Gain

Urate oxidase is an enzyme involved in purine metabolism, which is found in all living organisms. Although humans have the urate oxidase gene, it has been rendered nonfunctional due to a series of independent mutational events during early primate evolution, making UA the end product of purine catabolism. It has been proposed that the biochemical pathway regressive loss of urate oxidase has been advantageous to hominids by increasing levels of UA, which has specific antioxidant and nitric oxide-buffering properties, along with a positive association in protection of brain damage in stroke and muscle damage from exercise (Cutler, 1984; Haberman et al., 2007; Pan et al., 2013; Wu et al., 2013). Epidemiological data suggests that UA may reduce the risk of multiple sclerosis, Parkinson's, amyotrophic lateral sclerosis, and cancers, and may also preserve muscle strength, bone density and cognition during aging (Kataoka, Kiriyama, Kobayashi, Horikawa, & Ueno, 2013; Macchi et al., 2008; Molino-Lova et al., 2013; Wu et al., 2013). However, the beneficial aspects of UA most often follow a bell-shaped curve, where long-term hyperuricemia has been linked to hypertension, cardiovascular disease, type 2 diabetes Mellitus, metabolic syndrome, and gout (Soltani, Rasheed, Kapusta, & Reisin, 2013).

UA is mainly derived from *de novo* metabolic catabolism of ATP into adenosine, RNA turnover, and DNA catabolism from cell death and mitochondria turnover, with approximately 30% coming from the diet (Nieto, Iribarren, Gross, Comstock, & Cutler, 2000; Rock, Kataoka, & Lai, 2013). Local and systemic levels of UA acutely spike as a result of released purines caused by tissue damage (e.g. physical injury, exercise), inflammation, chemotherapy and starvation catabolysis (Rock et al., 2013). That UA is

produced quickly and at the site of metabolic stress and injury indicates a potential for its use as a first response factor to buffer damage (i.e. antioxidant and nitric oxide), as an immune homing and repair signal, and at higher levels as an inducer of apoptosis. Previous mouse studies have shown roles for purine-mediated signaling (e.g. adenosine, guanosine) in the repair and apoptosis responses from ischemia or trauma, but little work has been done in models with inactive urate oxidase (Parkinson, Sinclair, Othman, Haughey, & Geiger, 2002).

Humans have also evolved a relatively high renal reabsorption (63%) of UA compared to other animals, due to differences in levels of URAT1, GLUT9, and OAT1, 3, & 4, suggesting that it may be biologically viewed as beneficial factor, rather than as a waste product (Alvarez-Lario & Macarron-Vicente, 2010). To protect against over-production and gout, humans have repressed both xanthine oxidase transcription and core promoter activity, resulting in lower production of UA, in addition to having higher albumin levels that increase UA solubility (Xu, LaVallee, & Hoidal, 2000). Circulating and storage levels of triglyceride and cholesterol are also strongly correlated with species and within species UA levels (Zhao, Huang, Song, & Song, 2013). The hypothesis for this proposed correlation, and the basis for this study, was that UA modulated metabolic efficiency by acting as sensitizer of postprandial lipemic response. The proposed mechanism of how UA acts acting to increase postprandial lipemic response is as an inhibitor of AMPK, the major negative regulator of SREBP-1c, resulting in an increased sensitivity of lipogenesis activation (Lin, et al., 2005; Nakagawa et al., 2006). However, direct measures of AMPK and SREBP-1c in response to levels of UA were outside of the scope of this research study.

The Inflammation and Visceral Fat Weight Gain Cycle

Chronic inflammation is commonly observed with obesity and fatty liver disease, where the major source of circulating C-reactive protein and pro-inflammatory cytokines TNF-α, and interleukin 1 and 6 are released by inflamed adipose tissue (Harvie et al., 2011; Vgontzas, Bixler, Papanicolaou, & Chrousos, 2000). Chronic elevation of pro-inflammatory cytokines target the liver, pancreas, heart, or blood vessels, where it is thought to significantly contribute to the chronic inflammation observed in weight gain leading to metabolic syndrome and type 2 diabetes (Vgontzas et al., 2000). Inflammatory cytokines (i.e. TNF-α) have been shown to increase ceramide levels and cause insulin resistance (Boon et al., 2013; Dekker et al., 2013). High insulin levels and glucose from insulin resistance activate SREBP-1c and increase sensitivity towards visceral fat weight gain from excess caloric intake as reflected in postprandial changes in VLDL (C. Liu et al., 2013). The cyclic nature between the release of visceral fat pro-inflammatory cytokines and hyper-lipogenesis is perpetually fueled by the continuation of excess caloric intake. Weight loss studies have shown rapid drops in these cytokines with subsequent improvements in all markers of associated diseases during fasting or a low calorie diet (Gerner, Wieser, Moschen, & Tilg, 2013). A better understanding of the mechanism and risk factors for visceral fat weight gain is needed by all stakeholders in the push to lower the public health burden of the current epidemic in visceral fat weight gain, obesity, and associated diseases.

Problem Statement

Prevention of diseases affecting public health has long focused on the reduction of modifiable risk factors, which depends on being able to accurately identify and rank the most influential and modifiable risk factors. The incidence and prevalence of weight gain leading to obesity has risen almost exponentially over the last 20 years (Toth, Potter, & Ming, 2012). The prevalence of this epidemic points to drastic increases in caloric intake and lower usage of calories from physical exercise. The current increases in incidence and prevalence of weight gain have led to parallel increases in obesity, as well as early onset and progression of most age-related diseases, which has impacted United States society by decreasing productivity and increasing the cost of healthcare (Akbaraly et al., 2013; Kahn, Robertson, Smith, & Eddy, 2008). Most experts in the field have considered this to be the most detrimental, but modifiable, public health issue currently affecting the United States (Carlezon & Chartoff, 2007; Fisher & Kral, 2008). A review of the literature has revealed that a major gap in knowledge was the underlying mechanism and risk factors initiating lipemic response and weight gain in healthy people (Akbaraly, et al., 2013). This study was designed to utilize a healthy middle-aged adult population (i.e. normal BMI, insulin sensitivity, blood lipid levels, inflammation) with no comorbid health issues. The participants were subjected to a known risk factor for initiating lipidemia and weight gain (i.e. a high in saturated fat fast food modeled meal), where changes in serum triglycerides and cholesterol were measured at intervals for 8-hours post meal. The primary end-point (i.e. postprandial lipemic response: the percent of change from baseline with time of very low density lipoproteins [VLDL]) was used as the dependent variable to develop a scatter plot distribution of the data and one-way analysis

of variance (repeated measures; ANOVA). The postprandial lipemic response data were also used to group participants into an ordered distribution tertile consisting of low, median, and high risk for developing lipidemia.

The next stage of the study tested the hypothesis that baseline (pre-HSF meal) levels of uric acid (UA) can predict the outcome of the postprandial lipemic response. Independent/predictor variables (i.e. baseline: UA, lipids, markers of inflammation, insulin hormones and insulin sensitivity) utilized the Pearson's correlation with least squares (two-tailed) regression modeling for the predictive power of the postprandial lipemic response (the dependent variable). In addition, UA was analyzed for a Pearson's correlation with all of the other independent variables for the presence of potentially cooperative or confounding interrelationships.

Purpose of the Study

Prevention is the primary goal of public health initiatives. This study examined data on UA, lipids, insulin hormones, and markers of inflammation from healthy middle aged human blood serum samples measured before and at time points after ingestion of a HSF meal, and a crossover negative control, high in PUFA meal. The HSF meal consisted of 2 times the amount of lipogenic activating palmitic acid (C16:0) over the PUFA diet, which substituted palmitic acid with non-lipogenic activating linoleic acid (C18:2). The purpose of this study was to quantitatively measure (via regression modeling) the effects that baseline serum UA levels (the independent variable) have on the peak and sustained changes in circulating blood lipids (i.e. lipemic response; the dependent variable) and insulin sensitivity (the covariant variable) after a single HSF meal. Comparisons of the correlative predictive power of UA levels with the

postprandial lipemic response were compared to other known risk factors involved in the development of visceral fat weight gain, obesity, and metabolic syndrome (i.e. baseline levels of: lipids, markers of inflammation, insulin hormones, and insulin sensitivity).

Uric Acid as a Sensitizer of Postprandial Lipid Synthesis Response

A vast number of clinical studies have reported a positive association of UA with circulating levels of triglyceride, cholesterol, and glucose (Soltani et al., 2013). One hypothesis for the metabolic advantage to animals that lack functional urate oxidase is to increase UA during stress (e.g. exercise), where it acts as an antioxidant and enhances anabolic repair and recovery processes by suppressing AMPK-mediated catabolic pathways (i.e. mTOR autophagy, and suppression of SREBP-1c). However, the cost for blocking autophagy could potentially explain why humans and closely related primates are more susceptible to certain age-related neurological diseases such as stroke (via atherosclerosis), Alzheimer disease, and Parkinson disease, which are not naturally found in animals with active urate oxidase (LaFerla & Green, 2012). Furthermore, other human neurological diseases (i.e. Amyotrophic lateral sclerosis) has been shown to occur in lab animals when infected with viruses that harbor a reservoir in neurons (e.g. herpes simplex virus 1 and 2). These viruses act to preserve their presence by shutting down autophagy, the main mechanism for flushing out viral infections in neurons (Santana, Bullido, Recuero, Valdivieso, & Aldudo, 2012; Santana, Recuero, Bullido, Valdivieso, & Aldudo, 2012). Further support for the role of autophagy in neurological diseases is the finding that the incidence of a dementia symptomatically similar to Alzheimer disease has been found to be significantly higher in humans infected with neuron reservoir viruses (e.g.

HSV-1 and HIV), thereby giving evidence that autophagy is a common factor in the development of these diseases (Cutler et al., 2004; Salminen et al., 2013).

Theoretical Framework for the Study

The theoretical basis for this archival study was to test the hypothesis that the driving evolutionary advantage for the mutational of functional urate oxidase in humans was to increase UA, which, in turn, acts to increase metabolic postprandial visceral fat synthesis and storage efficiency (i.e. VLDL lipemic response). The proposed biological mechanism of how UA increases sensitivity of postprandial lipemic response was by suppression of AMPK-mediated catabolism and autophagy (Lanaspa, Sanchez-Lozada, et al., 2012). Direct measures of the effect of UA on AMPK and SREBP-1c were outside the scope of this study, but are key for future study. The modeled framework used to test this hypothesis utilized blood serum UA data from a healthy middle-aged population to see if the differences in levels could statistically predict lipemic response after eating a single HSF meal.

The theoretical framework of this study proposal was for the analysis of archival serum sample data from a completed double blind longitudinal crossover clinical trial, consisting of 31 participants who completed all six visits. The study design was broken up into three periods, where the repeated measures of the independent, dependent and covariates were collected at baseline, 4 hours post intervention meal, and the next day after intervention meal. The key independent and dependent variables were baseline blood levels of UA and acute changes in *de novo* lipemic response, respectively, after a HSF and PUFA test meal. Baseline blood levels of lipids, insulin hormones, 2-hour OGTT glucose insulin sensitivity, and markers of inflammation were analyzed as

secondary independent variables or possible covariates with UA. The rationale for the design was to increase the statistical accuracy of the repeated measures by using the participant as his/her own longitudinal control, thereby limiting confounding covariates. Changes due to the intervention meals were analyzed as the percent change from their baseline levels. The rationale for randomizing the participants and the 10-day washout period interval between the cross-over intervention meals was designed to limit possible carry-over effects of first meal on the outcome of the second cross-over meal. The analysis methodology of the data looked at the relationships between the independent and dependent variables using one-way repeated measures ANOVA, and Pearson's correlation with least squares (two-tail) regression modeling to test the research questions of this dissertation.

Nature of the Study

The archival serum sample data from a completed primary double blind randomized longitudinal crossover clinical trial conducted and funded by the National Institute on Aging (NIA, a branch of the National Institutes of Health), who privately holds the data, were used for this study. As a full-time employee (research scientist) of the NIA, I obtained anonymized (participant code identification number, with no personally identifiable information) data from the completed study: Assessment of the Effects of Fast-Food on Inflammatory Markers (ClinicalTrials.gov ID: NCT00233311). The rationale for the longitudinal crossover design was to have each participant serve as his or her own control, so that differences could be presented as percent change from their baseline values. The benefit of this design was a lower number of participants required to reach statistical significance. Another key aspect of the original study design

was to use a healthy middle-aged adult population (i.e. normal BMI, insulin sensitivity, blood lipid levels, inflammation) with no comorbid health issues. The participants were subjected to a known method for initiating lipidemia and weight gain (i.e. a high in saturated fat fast food modeled meal), where changes in serum glucose, lipids, inflammation, and insulin hormones were measured at intervals for 8-hours post intervention meal (HSF and PUFA-control) and the next morning (fasted).

The primary independent variable for my secondary analysis of the data was the baseline levels of UA, which were used to calculate the power to predict the postprandial lipemic response (the primary dependent variable) from a single HSF meal. Lipemic response for each participant was the calculated area under the curve of the percent change in VLDL, which yielded a single value. VLDL is assembled in the liver from endogenously synthesized triglycerides, cholesterol, and phospholipids before being released into circulating blood. Acute changes in VLDL from the test meals were used in this study as a measure of endogenous lipogenesis in response to a meal and were not a direct measurement of the exogenous lipids absorbed from the diet (e.g. LDL; Faeh et al., 2005). Single time point blood serum VLDL levels were collected at timed intervals over an 8.5-hour time period postprandial of PUFA and HSF meals. Baseline levels of other previously published independent markers of lipemic response were analyzed for their predictive power verses UA, as well as a covariant.

These secondary independent markers included cholesterol, triglycerides, high density lipoprotein (HDL), low density lipoprotein (LDL), very low density lipoprotein (VLDL), HDL/LDL ratio, quantitative insulin sensitivity calculation (HOMA2), 2-hour OGTT glucose, inflammation (i.e. C-reactive protein (CRP), white blood cell count, and

albumin/globulin ratio), inflammation cytokines (i.e. IL-6 and TNF-α), and insulin hormones (insulin, C-peptide, and insulin growth factor-1 (IGF-1). The lipemic response from the PUFA meal was used as a negative control for each of the aforementioned research questions.

Definitions

Insulin hormones: Consist of insulin, C-peptide (pro-insulin protein), and IGF-1. Insulin and C-peptide were used as independent variables and to test for changes in next-day insulin responses from the diets. C-peptide was substituted for insulin in HOMA2 formula to validate the insulin data. IGF-1 is a hormone similar in structure and activity to insulin, but does not fluctuate with diet like insulin. It was used in this study as an independent and possible confounding variable.

Lipemic response: Defined for this study as the percent change from baseline with time of serum VLDL levels. The area under the curve gave a single value that was used to indicate *de novo* lipogenesis as the dependent variable in the analysis of the data. VLDL is a lipoprotein made in the liver that transports only endogenously produced triglycerides, cholesterol, and phospholipids to cells for energy use or storage. Therefore, VLDL was a good indicator to measure *de novo* lipid synthesis.

Lipogenesis: The *de novo* lipid synthesis of free fatty acids, phospholipids, triglycerides, and cholesterol that is mediated by activation of the SREBP-1c pathway. SREBP-1c is activated by insulin through mTOR, and by saturated fatty acids (e.g. C16:0) through the PGC-1β and LXRα pathway. Down regulation of mTOR, ACC, and HMG-CoA activity is through AMPK phosphorylation. Postprandial-induced lipogenesis was directly reflected by acute changes in circulating VLDL.

Markers of inflammation: C-reactive protein, cytokines (i.e. tumor necrosis factor-α, interleukin-6), white blood cell count, and the albumin/globulin ratio, were analyzed as secondary independent variables and as possible confounding variables in this study.

Homeostatic model assessment (HOMA) 2: A new and improved computer model of the original HOMA calculation used to quantify insulin resistance and beta cell function. HOMA-IR and HOMA-β are calculated from fasted glucose and insulin serum values. It was measured from fasted morning draw data, pre and post (next morning) meal interventions as an outcome dependent variable, possibly linking lipemic response with insulin resistance. The nomenclature for output from the calculator, which was also used in this study's tables and figures, is as follows: %B = steady state beta cell function; %S = insulin sensitivity; and IR = insulin resistance.

Uric acid (UA): The end product of purine metabolism in humans due to mutational inactivation of urate oxidase. Baseline values of UA were used as the primary independent variable for its potential as a predictor of lipemic response.

Weight gain: Defined as the increase in visceral fat body weight from adipocyte expansion and hypertrophy caused by excess caloric intake activation of lipogenesis.

Research Questions and Hypothesis

The following research questions were derived from observed gaps in knowledge after an extensive review of the current literature in the field. Currently there is not agreement among scientists for the environmental advantages driving the mutational knockout of urate oxidase. Two of the leading hypotheses behind the driving evolutionary pressures for increasing UA have been as an antioxidant response to protect against metabolic stress (e.g. exercise), and as a 24-hour circadian rhythm regulator of

nitric oxide. However, more recent data indicated that UA may also act to enhance stress-induced anabolic recovery by suppression of AMPK-mediated catabolism and autophagy. The hypothetical model tested in this study was that UA increases the activation sensitivity of anabolic pathways (i.e. lipemic response). If true, then UA levels would be a prominent risk factor of lipidemia and visceral fat weight gain from eating excess calories. Diets high in purines and/or fructose also increase levels of UA, independent of exercise. However, with no exercise to burn off triglycerides, high UA and caloric intake leads to a net increase of circulating and stored lipids. The HSF fast food modeled meal used in this study has the same amounts of saturated fat as a typical (and the most popular) McDonald's corporation Big Mac sandwich. As a negative control, participants were also fed a crossover healthy meal, consisting of equal calories, carbohydrates, proteins, and fat as the HSF meal, but replaced saturated fats with PUFA.

Research Question 1: Can baseline levels of UA be used in a regression model to predict postprandial lipemic response to a HSF meal? It was expected that UA is acting as a natural enhancer of VLDL synthesis (i.e. lipemic response) when activated by a HSF meal.

H_o1: There is no positive correlation between baseline UA levels and postprandial lipemic response to a HSF meal.

H_a1: There is a positive correlation between baseline UA levels and postprandial lipemic response to a HSF meal.

The following sets of questions were designed to confirm and compare previously reported independent and covariant variables of lipemic response and insulin sensitivity

relative to UA. The covariant variables were analyzed just like UA for their power to predict lipemic response and compared to UA.

Research Question 2: How do baseline levels of lipids (i.e. triglycerides, cholesterol, HDL, LDL, VLDL, and HDL/LDL ratio) compare with UA in a regression model to predict postprandial lipemic response to a HSF meal? Because saturated fatty acids are a known activator of VLDL synthesis through SREBP-1c in a feed forward mechanism, it was possible that, within normal range, variations of baseline circulating lipids may lead to a higher postprandial lipemic response to a HSF meal, and constitute a confounding variable.

H_o1: There is a positive correlation between baseline lipid levels and postprandial lipemic response to a HSF meal.

H_a1: There is no positive correlation between baseline lipid levels and postprandial lipemic response to a HSF meal.

Research Question 3: How do baseline levels of markers of inflammation (i.e. C-reactive protein and pro-inflammatory cytokines TNF-α, and interleukin-6, white blood cell count, and albumin/globulin ratio) compare with UA in a regression model to predict postprandial lipemic response to a HSF meal? Because increased inflammation-mediated oxidative stress and ceramides (primary causes of insulin resistance) have been shown to modulate VLDL lipogenesis, it was possible that upper normal range variations of baseline circulating inflammation may lead to differences in postprandial lipemic response to a HSF meal, and constitute a confounding variable.

H_o1: There is a positive correlation between baseline markers of inflammation and postprandial lipemic response to a HSF meal.

H_a1: There is no positive correlation between baseline markers of inflammation and postprandial lipemic response to a HSF meal.

Research Question 4: How do baseline 2-hour OGTT glucose levels compare with UA in a regression model to predict postprandial lipemic response to a HSF meal? Because insulin resistance (i.e. high circulating insulin and glucose; HOMA2) has been shown to modulate lipogenesis, it was possible that upper normal range variations in baseline 2-hours OGTT may lead to a higher postprandial lipemic response to a HSF meal, and constitute a confounding variable.

H_o1: There is a positive correlation between 2-hour OGTT glucose levels and postprandial lipemic response to a HSF meal.

H_a1: There is no positive correlation between 2-hour OGTT glucose levels and postprandial lipemic response to a HSF meal.

Research Question 5: Does postprandial lipemic response (now the independent variable) after a HSF meal correlate with next morning fasting insulin sensitivity (HOMA2 insulin/glucose test; the dependent variable)? Participants with a high postprandial lipemic response to the HSF meal were expected to have a negative correlation with next morning fasting insulin sensitivity. Although this research did not directly involve UA, the outcome of this question was key for linking lipemic response (which may be correlated with UA in this study) with risk for diabetes type 2, a major concern in public health and implications for positive social change from this study.

H_o1: There is no negative correlation between postprandial lipemic response to a HSF meal and next morning fasting insulin sensitivity.

H_a1: There is a negative correlation between postprandial lipemic response to a HSF meal and next morning fasting insulin sensitivity.

Research Question 6: Are there any co-dependence correlations between the baseline levels of UA and the other independent variables that could indicate the presence of a serial correlation bias error in the results from Research Questions 1 - 5? Each independent variable was tested against UA for a Pearson's correlation, which could have revealed a co-dependence relationship. There was the potential for this analysis to also show that a multivariate combination of the independent variables might provide a higher power of prediction with postprandial lipemic response than UA alone.

H_o1: There are correlations between baseline UA and the other independent variables.

H_a1: There are no correlations between baseline UA and the other independent variables.

Assumptions

Caloric and fat loads from the intervention meals were the same for each participant and assumed to be the same dose. However, because of the differences in body weights (e.g. male verses female), the dose responses from the meals were likely to be different. Therefore, in the analysis of the data, I split male and female, as well as normalized the meal dose per body weight by multiplying lipemic response with BMI. Clinical chemistry values found to be below the detection limit of the assay were assumed to be at the set detection limit (e.g. < 4 would be changed to = 4) for the data analysis. It was assumed that changes in inflammation and/or UA levels were not due to acute infection or sudden changes in exercise routine between the baseline date and meal

dates. However, if time-0 right before meal was 20% higher or lower than the first visit baseline value for any of the inflammation markers or UA, the participant's data were considered as an outlier and dropped from the analysis.

Scope and Delimitations

This study's aim was to add to the knowledge of the mechanism and regulating agonists of lipemic response. The focus was to test if UA is a sensitizer of feeding-induced lipemic response. To date, there is a gap in knowledge as to why UA is correlated with being overweight, and gaps as to why some people have a propensity to gain visceral body fat, while others do not. The boundaries of the proposed study were to use a healthy middle-aged population to test if baseline blood serum UA levels can statistically predict lipemic response and, therefore, propensity to gain weight from over-eating during a single meal. The participant inclusion, eligibility, exclusion, and withdraw criteria followed by the primary study is briefly described below.

Limitations

For reasons still being investigated, ingesting high amounts of saturated fatty acids does not always result in the acute induction of inflammation pathways. For example, when given as a dietary supplement along with moderate to heavy exercise, saturated fats did not result in increased inflammation (Roberts et al., 2007). However, critics of this study pointed out that the heavy exercise (as done in the cited study) could have plateaued the release of stored fats, such that additions of circulating fats from the diet were negligible. However, in the majority of the studies in the field, high amounts of saturated fats has been shown to fuel inflammatory pathways in sedentary old-aged people, and people with type 2 diabetes, obesity, cardiovascular disease, arthritis, auto-

immune disorders, and other conditions where preexisting inflammation was present (Calder, 2008).

Significance

Cost and Benefit Analysis of Preventing Visceral Fat Weight Gain

Disability-adjusted life year (DALY) is a tool yielding a single number that was developed in 1990 at Harvard University for the World Bank to measure overall disease burden (Arnesen & Nord, 1999). A DALY score is calculated by the sum of years lost from illness, disability, or early death relative to current Japanese life expectancy statistics (Saika & Matsuda, 2013). DALYs can reflect the economic productivity of an individual or group. A similarly used tool is Quality-adjusted life year (QALY), developed in 1956 by Cundell and McCartney, which is also a measure of disease burden, but includes both quality and the quantity of life for the individual (Reidpath, Allotey, Kouame, & Cummins, 2003). Both of these weighted matrix tools can be used to produce a single value reflecting the public health impact (morbidity and mortality) of a current health issue, and the value for money in performing informational, legal, or medical interventions. Many countries with socialized medicine programs use DALY and QALY in the cost-utility analysis to allocate limited healthcare resources (Kahn, et al., 2008). In one study, the burden of disease caused by obesity in the United States from 1993 to 2008 showed that QALYs lost due to obesity has more than doubled. During the same time period, the prevalence of obesity increased by 89.9% (Jia & Lubetkin, 2010a). The latest data showed that overweight and obesity, particularly when associated with type 2 diabetes, cardiovascular disease, disability, and premature death, have surpassed smoking as the leading modifiable health risk in the United States (Jia &

Lubetkin, 2010b). The cost of Medicare and Medicaid spending on obesity-related conditions, which is largely preventable, is currently over $61 billion per year (Finkelstein, DiBonaventura, Burgess, & Hale, 2010). QALYs have been used by policy makers to calculate the economic impact and gains (particularly when applied to children) from developing an effective intervention to prevent visceral fat weight gain. Trasande (2010) showed that even the most conservative interventions in childhood obesity of a 1% decrease is cost effective, saving over $1 billion annually in attributable medical expenses for every QALY gained. However, effective interventions in prevention have not been readily identified. This is because the problem is likely multifaceted, with genetics, affordability (i.e. time and money) of healthy food choices, media marketing pressures, and nutritional education, all playing a role (Trasande, 2010).

The implications for my study to cause social change lie in providing the research community with a better understanding of the ranking of risk prediction factors in regulating lipogenesis and visceral fat weight gain. This added knowledge could lead to the development of a public health awareness campaign to disseminate information about the relationship between blood serum UA levels and postprandial lipemic response to dietary saturated fats.

Summary

The aim of this study was to test the utility of using baseline UA levels as a predictor of lipemic response to a single dose of a dietary model of a typical fast food high in saturated fats meal. One-way repeated measures ANOVA and Pearson's correlation with regression modeling were used to test for the most significant relationships between lipemic response with pre-intervention baseline blood levels of

UA, lipids, markers of inflammation, insulin hormones, and insulin sensitivity (i.e. HOMA2). At the end of this study, the analysis was able to identify and rank the power of each independent variable to predict postprandial lipemic response, and, therefore, risk factors for visceral fat weight gain from excess caloric intake. The public health implications from this study: (a) provide evidence to the public health and medical community about specific blood components (i.e. UA) that could be used to assess risk for weight gain; and (b) provide evidence to scientific researchers regarding potential mechanisms that initiate weight gain to identify dietary risk factors, and screen natural food factors for the purpose of prevention. In the next chapter, a comprehensive literature review highlights the current state of knowledge in the field and the gaps of knowledge needed to be answered in order to help the public prevent visceral fat weight gain and risk of associated diseases.

Chapter 2: Literature Review

Introduction

The aim of this study was to test the hypothesis that the driving evolutionary advantage for the mutational inactivation of urate oxidase in humans was to increase UA, which in turn acts to increase the metabolic efficiency and enhance anabolic recovery by suppression of AMPK-mediated catabolism and autophagy (Lanaspa, Sanchez-Lozada, et al., 2012). Over the past 40 years, most modern societies have been experiencing dramatic increases in visceral fat weight gain of their population, causing overweight and obesity levels to trigger an epidemic status in weight gain (e.g. 64% of US population). The result is that today's youth are the first generation to have a shorter and less productive projected lifespan than their parents (Piernas & Popkin, 2011a). Many factors

are thought to be involved in this common public health issue which include environmental changes in the availability of affordable high-sugar and high-fat foods and increasingly sedentary work and home environments (Fortuna, 2012; Schlosser, 2002). Previously published epidemiology studies have discovered other contributing factors, including many polymorphic changes in genes that contribute to a higher propensity of weight gain and development of associated diseases.

The next chapter discusses my literature search strategy for choosing, reading, and citing the most accepted as high quality research studies, key search terms, theoretical foundations, background and scope of the problem. This chapter details the current state of knowledge regarding the biological risk factors for visceral fat weight gain. This study's aim was to try to replicate a cell culture finding that UA is an inhibitor of AMPK and thereby increases metabolic efficiency as measured by lipemic response to a high in saturated fat meal in a human clinical trial.

Literature Search Strategy

Methods for Reading and Citing the Most Accepted as High Quality Research Studies

Over the past few years, the number of open access on-line scientific journals has exploded and, with it, fraud and the publication of low-quality papers that should not have been accepted for publication (Butler, 2013). There are over 4,000 journals that are now on many search engines' (e.g. PubMed) block list due to little to no peer review process, and an almost 100% acceptance rate as long as the fees are paid (Beall, 2013; Kolata, 2013). In doing literature searches using engines like Google Scholar, it can become problematic to know if the study one is reading and citing is accepted as high

quality by one's peers in the field (e.g. did one miss an obvious flaw that affected the results and conclusions). Recently, I came up with an approach to filter out poor quality studies. By utilizing the Web of Knowledge search engine, one can sort the results by the number of citations; the higher number of citations indicates that most experts in the field agree with the methods and conclusions of the study. However, this method only really works well for publications that are over 2 years old. For newly published studies one has to know the reputation (e.g. impact factor) of the peer-reviewed journal and fully read and evaluate the study results for one's self before citing. Unfortunately, Walden University's licensed version of Web of Knowledge only goes back to 2005, so my method was handicapped and not very useful with this database. Google Scholar does show the number of times cited, and cite number is heavily weighted in the Sort By Relevance algorithm, but one cannot sort the results by number of times cited. In my literature review and citations, I used the Web of Knowledge search engine through my NIH licensed account. The databases included: Web of Science™ Core Collection, MEDLINE®, and SciELO Citation Index, with the Timespan setting of all years (i.e. 1900 – current e-pub before publication).

The key search terms used included *visceral fat weight gain; lipemia; lipemic response; saturated and polyunsaturated fat diets; SREBP; triglycerides; cholesterol; HDL; LDL; VLDL; obesity; diabetes; inflammation; metabolic syndrome; aging; atherosclerosis; uric acid; insulin resistance; HOMA; OGGT; creatinine; omega-3 and 6; linoleic acid (C18:2n6); polyunsaturated; motivation to exercise; PGC-1beta; gout; evolution; adenosine; urate oxidase; lipid metabolism; hypertension; antioxidant; oxidative stress; nitric oxide; peroxynitrite; circadian rhythm; U.S. public health issues;*

Alzheimer's; ALS; Parkinson's; disability; economics and disability; cancer; palmitic acid; arachidonic acid; diet nuts and meat; exercise; endurance fuel; fructose; AMPK; portion control; over eating; CRP; cytokines; inflammation; autophagy; phosphofructokinase; BMI; Western diet; fast food industry; endocannabinoids. I used the method described above to choose which primary data and meta-analysis studies to cite, and only cited reviews for well-accepted concepts and background in the field. I also self-cited many of my own publications to show that this study is a continuation of my ongoing research interests.

Theoretical Foundation

UA has been found to be independently correlated with visceral fat weight gain and the diagnosis of cardiovascular disease. One hypothesis for the driving evolutionary pressure for genetic mutations aimed at increasing UA in humans (i.e. knockout of UOX and increase of URAT1) is to increase metabolic efficiency and enhance anabolic recovery by suppression of AMPK-mediated catabolism and autophagy (Lanaspa, Sanchez-Lozada, et al., 2012). The hypothetical model tested in this study was that UA decreases the activation energy of anabolic pathways (i.e. lipemic response). The proposed increase in anabolic response would act to increase the risk of lipidemia and visceral fat weight gain from excess caloric intake, thereby making UA a potential indicator and modifiable risk factor for visceral fat weight gain.

The environmental selection for mutations leading to knocking out functional urate oxidase in humans is also thought to be for its benefits as an antioxidant and cognitive response to stress (Cutler, 1984; Sutin et al., 2013). While higher UA levels were clearly beneficial in low to moderate caloric density environments, with today's

Western diet, it may be acting to predispose the population to weight gain, obesity, and metabolic syndrome (Wang et al., 2012; Zhao et al., 2013).

One practical outcome and utility of this study is preliminary evidence supporting the clinical practice of checking patient UA levels as an indicator of likely response to a diet and exercise program. If UA does decrease the energy of activation for fatty acid synthesis and storage, then it would provide more insight as to why some people have an increased propensity towards visceral weight gain (Gallant, Lundgren, & Drapeau, 2012; Spaeth, Dinges, & Goel, 2013). Increased understanding of the factors involved in UA, AMPK, and SREBP-1c feed forward mechanism of lipid synthesis and storage will identify points of interventions that could be used to break the cycle of weight gain (e.g. lowering UA, AMPK agonists, SREBP-1c antagonists).

Literature Review Related to Key Variables

Antagonistic Pleiotropy of the Western Diet

During the evolutionary emergence of Homo sapiens sapiens about 200,000 years ago, fat, sugar, and salt were scarce in available food sources. This drove the selection of genes to seek and biologically store these nutrients for survival. With the advent of modern farming and global trade, access to these sought-after items has become pervasive throughout modern societies. This is particularly the case in Western cultures, where daily diets have increased percentages and portions of saturated fats, simple sugars, and salt, as they have become more accessible (Fields, 2004). The birth of the fast food industry in the 1950s has made access to these items unprecedentedly and unnaturally available to the U.S. population (Grotz, 2006). Although humans have been very successful in changing the environment towards increased food availability, the

genes that drove us to seek high fat, sugar, and salty foods have now become maladaptive (i.e. antagonistic pleiotropy) to our health (Meisel & Wardle, 2013). Excess consumption of saturated fats, simple sugars, and salt, as found in the most sought-after fast food menu items, has been blamed as a primary cause for the current epidemic of heart disease, obesity, diabetes, and many cancers (Cotti & Tefft, 2013; Daniel, Cross, Koebnick, & Sinha, 2011; Mattson, Duan, & Guo, 2003).

Genetic Factors Affecting Risk of Visceral Fat Weight Gain

Recent changes in our environment (e.g. dietary intake of fructose, meat, milk, and decreased physical exercise), are thought to be the leading cause behind the current epidemic of visceral fat weight gain over the last 27 years (Schlosser, 2002). Epidemiological studies have shown that there are several genes with polymorphisms that make people more susceptible to weight gain and developing type 2 diabetes (e.g. leptin, Mrap2, Transcription factor 7-like 2 (T-cell specific, HMG-box), also known as TCF7L2)(Halaas et al., 1995; Liu, Elmquist, & Williams, 2013; Meisel & Wardle, 2013). The unanswered questions of what genetic factors produce increased visceral fat weight gain in the majority of the population and how these factors are interacting with our new environmental changes signify a large gap in knowledge of the field (Meisel & Wardle, 2013).

Age-Related Increases in Inter- and Extra-Cellular Lipidemia

Diets high in saturated fatty acids are associated with visceral fat weight gain, obesity, insulin resistance, metabolic syndrome, and a higher risk, onset and progression of most age-related degenerative diseases (e.g. cardiovascular, type 2 diabetes, cancer, macular degeneration, Alzheimer disease)(Liu, et al., 2013). Circulating blood and

cellular levels of triglycerides and cholesterol have been shown to increase linearly with normal aging (Jousilahti, Vartiainen, Tuomilehto, & Puska, 1996). Central to the mechanism for excess caloric intake and the age-related increase of these lipids is the transcription factor SREBP-1c, and subsequent activation of acetyl-CoA carboxylase (ACC) and 3-hydroxy-3-methyl-glutaryl-CoA reductase (HMB-CoA)(Shao & Espenshade, 2012). The two major activating factors for SREBP-1c are glucose via the insulin, AKT pathway, and free fatty acid oleate (C18:1) from dietary saturated fats and *de novo* synthesis. The observed increased activation of SREBP-1c that leads to cellular triglyceride and cholesterol loading with aging has been shown to be caused by the presence of a micro RNA (i.e. miR-33b) intron within the SREBP-1c gene that is spliced out during RNA maturation. miR-33b has been shown to directly inhibit transcription levels of 5' adenosine monophosphate-activated protein kinase (AMPK; which deactivates SREBP-1c) and 5' adenosine triphosphate-binding cassette transporter (ABCA1: cholesterol and phospholipid efflux) causing a feed forward mechanism of increasing SREBP-1c protein, activation, and cellular lipid loading (Najafi-Shoushtari, 2011). Stearoyl-CoA desaturase-1 (SCD1) desaturates C18:0 into C18:1, which is necessary for triglyceride, cholesterol ester, and ceramide synthesis. Down regulation of SCD1, through genetic or drug inhibitors has been shown to reduce the weight gain, fatty liver, and insulin resistance caused by a high calorie saturated fat diet. Contrary to *in vivo* studies, dietary intake high in saturated fatty acids (i.e. C10:0 to C16:0) are the most potent activators of SREBP-1c, through intercellular elongation and desaturation pathways (Miyazaki et al., 2004).

The Role of Saturated Fats in the Development of Insulin Resistance and Metabolic Syndrome

Epidemiology studies have identified metabolic syndrome as a combination of medical disorders that, when combined together, multiply rather than add towards the risk of developing cardiovascular and type 2 diabetes (Grundy et al., 2005). Metabolic syndrome, as defined by the International Diabetes Federation, is clinically diagnosed when a patient presents with obesity and any two of the following four conditions: raised triglycerides [>150 mg/dL]; reduced HDL cholesterol [<40 mg/dL] male, [<50 mg/dL] female; raised blood pressure (systolic>130 or diastolic>85 mm Hg); or raised fasting blood glucose [>100 mg/dL](Alberti, Zimmet, & Shaw, 2006). Recent studies indicate that the prevalence of metabolic syndrome in the United States is about 25% of the population. The risk for developing metabolic syndrome increases with age, where it affects 44% of people over 50 years old. The prevalence of patients diagnosed with cardiovascular disease who are also diagnosed with metabolic syndrome is approximately 50% (Ford, 2005).

The mechanisms that lead to metabolic syndrome have only been partially elucidated. The two major independent risk factors are age and obesity. Insulin resistance and type 2 diabetes have been shown to be independent, but common pathophysiological consequences of aging and obesity (Ford, Giles, & Dietz, 2002). Both of these health states corresponds to increased levels of ceramides, which have been shown to be a common mediator (Yang et al., 2009). Sphingolipids (e.g. ceramides, sphingomyelins, and gangliosides) play a central role in the mechanisms linking lipidemia with inflammation-induced insulin resistance (Holland et al., 2007). Known as

the antisocial lipids, sphingolipids are very hydrophobic, which causes them to bond together to make-up cellular membrane lipid rafts that bring together and house cell surface receptor components (e.g. insulin receptor) for activation. Under conditions when plasma membrane sphingolipid levels are high (i.e. lipidemia or inflammation), these cell surface rafts enlarge, thereby expanding the distance and energy needed for assembly and activation of receptor components (i.e. lower insulin receptor response resulting in insulin resistance)(Gill & Sattar, 2009). Ceramide has also been shown to be able to directly activate protein phosphatase-2A (PP2A), which inhibits AMPK, therefore turning on the SREBP-1c lipid synthesis pathway (Dobrowsky, Kamibayashi, Mumby, & Hannun, 1993). A recent report has indicated that the rate-limiting factor regulating *de novo* synthesis of sphingolipids is the level of available saturated free fatty acids (e.g. C16:0)(Watt et al., 2012). Therefore, one mechanism to decrease visceral body fat due to dietary and age-related increases of free fatty acids would be by inhibition of SREBP-1c, and/or activation of AMPK.

The Role of Inflammation in Perpetuating a Cycle of Insulin Resistance and Lipogenesis

Inflammation markers (C-reactive protein, NFκβ, and TNFα) have been used as markers to predict the development of cardiovascular disease, Type 2 diabetes, and metabolic syndrome (Gerner, et al., 2013). During acute infections, the rise of inflammatory cytokine TNFα has been shown to activate neutral sphingomyelinase causing increases in ceramides and insulin resistance (Brindley, Wang, Mei, Xu, & Hanna, 1999). This effect transiently increases circulating levels of glucose and free fatty acids that the immune system has been shown to effectively utilize for energy in

combating the infection. After clearance of the infection, cytokine levels drop and lipid raft size and receptor responses go back to basal homeostatic levels. Currently, there is little evidence that supports a direct relationship between increases in SREBP-1c pathway activity from over-eating or diets high in saturated fats initiating inflammation. However, there is evidence that once activated, inflammation perpetuates the SREBP-1c pathway along with the onset and progression of lipidemia and metabolic syndrome (Biddinger et al., 2005).

Excess Intake of Saturated Fats, Inflammation, and Risk for Age-Related Diseases

The simultaneous availability of high calorically dense food choices (e.g. pizza) with increases in portion size, particularly in the fast food industry (e.g. super sizing soft drinks and French fries), has been strongly associated with the recent and dramatic increases in obesity (Piernas & Popkin, 2011a, 2011b). Evidence has shown significant increases in blood proinflammatory cytokines (i.e. interleukins (IL)-1β and 6), tumor necrosis factor (TNFα), plasminogen activator inhibitor-1 (PAI-1), and C-reactive protein (CRP) shortly (i.e. 1 to 6 hours) after ingesting a single, large (i.e. ≥ 800 calorie), high saturated fat meal (Manning et al., 2004). High levels of these inflammatory markers have been directly related to a number of serious health problems, including heart disease, type 2 diabetes, insulin resistance, asthma, and obesity (Manning et al., 2008; Wood, Garg, & Gibson, 2011). It has been suggested that the types of food we eat on a regular basis can influence the median levels of inflammatory markers (Wood, et al., 2011). In the United States, almost one third of meals are now from a fast food restaurant, and most often consist of foods high in simple carbohydrates and saturated fats; where consumption of pizza has increased over 3-fold and typically consists of 46%

of the recommended daily value of saturated fat intake (Margioris, 2009). However, the identity of the specific lipid species causing the observed postprandial acute inflammation response has not been identified. Agreement of the known harmful components of fast food (e.g. cholesterol, trans-fats) by experts in the field has helped mold public health policies and public awareness campaigns (e.g. food label listings and recommended daily allowances). However, more studies are needed to identify other harmful components and doses contained within our food supply and in particular the fast food menu.

One of the most studied lipids associated with an increase of dietary meat and inflammation is the polyunsaturated omega-6 fat lipid arachidonic acid, as well as its saturated counterpart, arachidic acid. We get high amounts of arachidonic acid from dietary meats and some vegetable oils (e.g. peanut oil). One clinical intervention study reported a significant increase of arachidonic acid in participants two hours after ingestion of a single fast food meal (i.e. McDonald's Big Mac meal McDonald's Corp., London, England)(Gopaul, Zacharowski, Halliwell, & Anggard, 2000). Arachidonic acid is a precursor of endocannabinoid synthesis via the acyltransferase pathway, which has been shown to be a primary activator of the limbic forebrain's hunger response and associated with a propensity to consume excess calories (Matias, Bisogno, & Di Marzo, 2006). Arachidonic acid is also a precursor that can go down the cyclooxygenase pathway to produce eicosanoids (e.g. prostaglandins, prostacyclin, and thromboxanes), which activate inflammation, vasodilatation and thrombosis (Calder, 2002). Both of the biological effects of arachidonic acid, i.e., activating the hunger response and acute

inflammation, place it as a pivotal player for risk in over-eating weight gain and metabolic syndrome (Simopoulos, 2002).

The Role of Uric Acid in Exercise Performance

Most animals excrete nitrogenous waste through a pathway ending in allantoin and urea. However, humans are part of a small group of species, which includes birds and bats, that has evolved biochemical regression by mutational deactivation of urate oxidase, making uric acid (UA), rather than allantoin and urea, the endpoint of the purine degradation pathway (Johnson, Titte, Cade, Rideout, & Oliver, 2005). One hypothesis for the driving force behind the regression of an established biochemical pathway is a proposed gain in exercise endurance and training response (Sutin, et al., 2013). Aerobic exercise increases nitric oxide and the respiratory production of free radicals, particularly superoxide, which leads to the formation of peroxynitrite, hydroxyl-, carbon dioxide-, nitric oxide-, and nitrogen dioxide radicals (Davies, Quintanilha, Brooks, & Packer, 1982). UA has been shown to have a high specific antioxidant activity towards quenching these radicals thereby buffering their negative effects of inhibiting eNOS activity, myoglobin and erythrocyte oxygen exchange, hemolysis, and in decreasing muscle contraction efficiency (Gersch et al., 2008; Kondo, Takahashi, & Niki, 1997; Waring et al., 2003). UA reacts with superoxide, nitric oxide, and peroxynitrite to produce allantoin, 6-aminouracil, and triuret respectively (Kim et al., 2009; Robinson, Morre, & Beckman, 2004). Knocking out urate oxidase also has the benefit of sparing the utilization of 1-oxygen and 1-water that could then be used for muscle respiration, and in sparing the production of 1-hydrogen peroxide and 1-carbon dioxide that would have otherwise acted to decrease muscle contraction efficiency. Human studies have

shown that during rigorous exercise, skeletal muscle UA levels decrease (i.e. consumed) as non-enzymatic metabolic products of UA reacting with superoxide (i.e. allantoin) increase. During post exercise recovery, human muscle and circulating levels of UA increased significantly higher than pre-exercise levels (Hellsten, Tullson, Richter, & Bangsbo, 1997). Similar data on increases of UA with exercise and VO2 max has been shown in birds, which, like humans, also lack functional urate oxidase (Tsahar, Arad, Izhaki, & Guglielmo, 2006).

Uric Acid Protects Against Traumatic Stress Induced Methemoglobin and Hemolysis Anemia

Exercise causes physical injury and metabolic and oxidative stress, with increases in catabolic by-products of purines, carbon dioxide, nitric oxide, superoxide, hydrogen peroxide, and peroxynitrite (Davies, et al., 1982). Post-exercised mice have a high incidence of hemolysis, which has been reported to be negatively correlated with UA levels (Suzuki et al., 2006; Theodorou et al., 2010). Peroxynitrite and other oxidizing radicals have been shown to directly reduce the oxygen carrying and exchange efficiency of erythrocytes and myoglobin (i.e. methemoglobinemia and hemolysis (Kondo, et al., 1997; Meadows & Smith, 1987). UA has also been reported to protect against peroxynitrite and ROS mediated increases in methemoglobin and hemolysis (Ames, Cathcart, Schwiers, & Hochstein, 1981; Smith, Gore, & Roland, 1988). Further supporting these studies, clinical treatment of patients transfused with functional urate oxidase protein (i.e. rasburicase) has numerous reported cases of causing methemoglobin and hemolysis anemia emergencies. This effect has been attributed to increases in urate

oxidase-derived hydrogen peroxide with simultaneous decreases in antioxidant protection from UA (Ng, Edwards, & Egelund, 2012; Zaramella et al., 2013).

Neuroprotective Role of Uric Acid Against Stroke and Ischemia

Stroke is a major cause of age-related morbidity and mortality. Strenuous exercise and ischemia increases nitric oxide and free radical production (Gravier et al., 2013). Peaks of nitric oxide can cause degeneration of neurons via inactivation of protein disulfide isomerase and S-nitrosylation mediated neuropeptide misfolding (Obukuro et al., 2013). Increases in levels of carbon dioxide during exercise or ischemia enhance peroxynitrite-mediated protein tyrosine nitration (Gow, Duran, Thom, & Ischiropoulos, 1996). Buffering nitric oxide levels has been shown to reduce ischemic reperfusion damage in mouse models (D. H. Liu et al., 2013). Animal model studies have reported that treatment with exogenous UA or more soluble UA analogs can be beneficial in animal stroke models (Haberman, et al., 2007). However, in humans both positive and negative associations between levels of UA and the outcome of ischemic stroke have been reported (Chiquete et al., 2013).

Effects of Uric acid in Buffering AMPK Activation

Exercise of skeletal muscle activates AMP-activated protein Kinase (AMPK), which plays roles in cellular energy homeostasis (e.g. fatty acid beta-oxidation), activation of catabolysis, and autophagy (Ruderman et al., 2003). Activation of AMPK during stress has been identified as an inhibitory regulator of renal UA efflux excretion, resulting in increased reabsorption and circulating levels (Bataille, Maffeo, & Renfro, 2011). Sprint training in humans has been shown to stimulate URAT1 mediated kidney reuptake of UA (Stathis, Carey, Hayes, Garnham, & Snow, 2006). Unexpectedly, UA

has been reported to inhibit activation of AMPK in a human liver cell line (Lanaspa, Sanchez-Lozada, et al., 2012). However, during exercise of skeletal muscle, regulation of fatty acid oxidation utilizes AMPK-independent pathways (Dzamko et al., 2008b; Jeppesen et al., 2013). AMPK activation has protective functions in cell survival during transient energy depletion, but prolonged activation can lead to apoptosis via activation of Bcl-2 family member Bim, and by hyper-activation of autophagy via inhibition of mTOR (Weisova et al., 2011). Therefore, increases in UA may provide advantages during metabolic stress by buffering prolonged activation of AMPK (Venna, Li, Benashski, Tarabishy, & McCullough, 2012).

Increases in UA may also be advantageous for exercising by increasing circulating and stored triglycerides to be utilized for fuel. Higher numbers of mitochondria and levels of triglycerides in muscle have been shown to increase exercise performance and hyperactivity (Pandareesh & Anand, 2013; Turner et al., 2007). Repatterning of energy metabolism by increasing muscle triglycerides has been shown to increase running performance and maximum life span in a phosphoenolpyruvate carboxykinase over-expressing mouse model (Hakimi et al., 2007). Farber et al. (1991) has shown that circulating triglycerides are a major energy source in endurance athletes, and others have shown it is the preferred fuel used by long-distance migrating birds (Farber, Schaefer, Franey, Grimaldi, & Hill, 1991; Jenni-Eiermann et al., 2002). Increased lipids increase transcriptional coactivator peroxisome proliferator-activated receptor-gamma coactivator 1-beta (PGC.1beta), with concomitant increases in muscle mitochondria numbers and oxidative energy metabolism capacity (Lin, et al., 2005; Song et al., 2012).

Uric Acid as a Stress Signal in Response to Starvation

Epidemiology and mouse studies have shown that UA is strongly correlated with increased disinhibition, risk taking, impulsivity, positive outlook, and a high energy drive for voluntary exercise (Lorenzi, Borba, Dutra, & Lara, 2010; Sutin, et al., 2013). This evidence indicates that it may act on neurons to change cognitive behavior that would be beneficial in a drive for migration towards new food sources during starvation conditions. In times of famine, it is conceivable that increased UA levels would be advantageous in increasing motivation, risk taking behaviors, exercise endurance, and anabolic recovery in the search for food. UA's ability to buffer nitric oxide and raise blood pressure may be life saving during famine conditions when blood pressure and cognitive performance drop (Alvarez-Lario & Macarron-Vicente, 2010). Even after food is found, UA may be further beneficial in its ability to increase insulin secretion and triglyceride synthesis (i.e. metabolic efficiency) in the utilization and fat storage of excess food.

Acute Increases in Uric Acid Associated with Lower Levels of Oxidative Stress

Many of the physiological responses during rigorous physical exercise (e.g. utilization of glucose, glycogen, and fat, and muscle catabolism) are acutely mimicked by caloric fasting. In previous work, I have reported on the progressive increase in plasma UA with a concurrent decrease in oxidative damage levels during an eight-week intermittent fasting clinical study. This study also showed acute increases of UA and decreases of oxidative damage markers on fasting days verses the previous feeding day (Johnson et al., 2007). Uric acid makes up 53 to 60% of the fast-acting oxidative radical absorption capacity in human serum (Benzie & Strain, 1996; Cao, Alessio, & Cutler,

1993), and has been shown to play a key role in protecting nitric oxide synthase and antioxidant enzyme activities (Hink et al., 2002; Lee et al., 2013).

Uric Acid as a Regulator of an Antioxidant and Nitric Oxide 24-hour Circadian Rhythm

Blood levels of UA follow a 24-hour circadian rhythm that parallels melatonin and antioxidant levels, and is inverse of nitric oxide and markers of oxidative stress (Andreoli et al., 2010; Arguelles, Gomez, Machado, & Ayala, 2007; Kanabrocki et al., 2000; Sennels, Rgensen, Goetze, & Fahrenkrug, 2012). The rise in UA, which peaks at 4:00 AM, is likely due to the "dumping" of purines (e.g. adenosine, cAMP) in resetting the circadian clock, but may also be part of an unrecognized oxidative stress and nitric oxide circadian system (Edgar et al., 2012; Kalinchuk, McCarley, Porkka-Heiskanen, & Basheer, 2011; O'Neill, Maywood, Chesham, Takahashi, & Hastings, 2008; PorkkaHeiskanen et al., 1997). Knock out of urate oxidase, rather than changes in regulation, acts to tightly couple the circadian rhythm of purine signaling metabolism with the peroxiredoxin antioxidant/redox cycle (Stangherlin & Reddy, 2013). The finding that the peak of UA does not correlate with peaks of blood pressure gives some evidence that UA levels may not be directly related to blood pressure, as suggested by other researchers (Hermida, Ayala, & Portaluppi, 2007). However, the nightly peak of UA levels do parallel with the peaks of *de novo* triglyceride and cholesterol synthesis (Jones & Schoeller, 1990; Parker et al., 1982). This observation may be due to UAs suppression of AMPK, which is a major negative regulator of *de novo* lipid synthesis. More work is needed to uncover this proposed mechanism.

The Negative Health Effects of Diet-Induced Increases in Uric Acid

Due to increases in meat and fructose consumption, the average U.S. adult blood UA levels have increased approximately 30% over the past three decades, and the epidemiological prevalence of gout has gone up over 2-fold (Rho, Zhu, & Choi, 2011). Increasing levels of UA by dietary fructose has been shown to increase blood insulin and triglyceride levels (Lanaspa, Sanchez-Lozada, et al., 2012). Uric acid levels naturally increase in response to starvation or physical exercise, where it is proposed to enhance motivation to migrate, forage, and increase insulin response towards visceral fat storage (Green & Fraser, 1988; Lanaspa, Cicerchi, et al., 2012). However, diet-induced increases of UA can act as a misdirected stress response, where increased sensitivity in insulin response and lipogenesis raise the risk of visceral fat weight gain from excess calories (W. T. Lin et al., 2013; Rock, et al., 2013).

Potentially Confounding Factors from Natural Dietary VLDL Synthesis Inhibitors

Participant levels of natural VLDL synthesis (e.g. SREBP-1c) pathway inhibitors from their previous diet (e.g. lycopene) are a variable that was not measured in this study, and could be a confounding factor in the results and interpretation of the data. Utilization of human liver cell culture (e.g. HepaRG™, Life Technologies Corporation; Grand Island, NY) and clonal rodent models would give a cleaner background and, therefore, more confidence in the relationship between cause and effect for this study.

Levels of Inflammation and Basal Metabolic Rate Mediating Arachidonic Acid Metabolites

The effect of other lipids in a typical fast food meal was not evaluated in this study and could constitute a confounding factor in the results and interpretation of the

data. A meal high in saturated fat meats contains large amounts of arachidonic acid (Li, Ng, Mann, & Sinclair, 1998). Arachidonic acid is not only a lipid precursor for many inflammatory signaling molecules, but also a precursor for endogenous cannabinoid synthesis. Food-induced activation of endocannabinoids (i.e. anandamide, oleoylethanolamide, and noladin) has recently been implicated as a possible causal factor for over-eating and food addiction (E. K. Jones & Kirkham, 2012; Petersen et al., 2006). The endocannabinoid system is normally silent and only becomes transiently activated during stressful conditions (e.g. exercise, fasting, and physical injury)(Raichlen, Foster, Gerdeman, Seillier, & Giuffrida, 2012). Chronic activation of the endocannabinoid system by nonsynonymous genetic polymorphisms in the cannabinoid receptor CB2 or over-production of endocannabinoids through eating large doses of lipid precursors has been shown to increase over-eating and sedentary behaviors (Ishiguro et al., 2010; Kirkham, Williams, Fezza, & Di Marzo, 2002). As expected from a stress response of starvation, the activation of the endocannabinoid system during fasting conditions acts to conserve energy homeostasis by decreasing metabolic rate (Banni & Di Marzo, 2010). Endocannabinoids are also activated during and after physical trauma as a natural painkiller and anti-inflammation agent (Esposito & Cuzzocrea, 2013; Rettori, De Laurentiis, Zorrilla Zubilete, Rettori, & Elverdin, 2012). The anti-inflammatory activity of endocannabinoids may help counteract the inflammation-inducing effects of a meal high in saturated fats (Batetta et al., 2009; Gopaul, Zacharowski, Halliwell, & Anggard, 2000). However, because endocannabinoids are mainly produced locally (e.g. limbic forebrain that controls hunger, and the neurons of injured tissue), the beneficial anti-inflammatory effects have not been reported to be systemic enough to counteract the

inflammatory effects of a large high saturated fat meal. Endocannabinoid regulatory pathways are regulated by the hormones and neuropeptides involved in energy homeostasis, which can be directly affected by diet (You, Disanzo, Wang, Yang, & Gong, 2011). Endocannabinoids mediate the actions of nutrient intake and processing through modifications in behavior and caloric metabolism, where perturbation by dietary fat precursors could lead to over-eating and propensity to store visceral fat (Heyman, Gamelin, Aucouturier, & Di Marzo, 2012).

Gaps and Barriers

Epidemiology studies on weight gain, obesity, and metabolic syndrome have reported many relationships regarding the possible causes and resulting effects on health. However, most clinical trials on this topic use obese subjects who already have many confounding variables (e.g. gout, cardiovascular disease, and type 2 diabetes)(Manning, et al., 2008; Peairs, Rankin, & Lee, 2011). The resulting gap in knowledge from these studies could be filled with the use of healthy populations to discover the factors involved in regulating the sensitivity of initiating lipogenesis. The discovery of predicting markers of an individual's sensitivity of initiating lipogenesis from excess caloric intake would be a valuable clinical tool in evaluating and managing his/her risk of visceral fat weight gain, obesity, metabolic syndrome, and associated age-related diseases.

Implications for Social Change

The aim of this study was to increase the knowledge of UA's role in health and disease, in order to identify targets of intervention that enhance the positive aspects of UA and limiting the negative. UA has a bell shaped curve associated with many beneficial health outcomes (Fini, Elias, Johnson, & Wright, 2012). However, with excess

caloric intake, particularly with foods high in saturated fats, UA is positively associated with the onset and progression of many age-related diseases (Zhao, et al., 2013). This study tested if UA acts to increase the sensitivity of inducing lipogenesis after a high in saturated fat meal. The potential relevance for social change from this study is to increase the knowledge of risk factors for visceral fat weight gain.

Summary and Conclusions

This literature review depicts my strategy and utilized search engines for choosing the most accepted as high quality research study findings by my peers. Utilizing this strategy, I was able to layout the history of previous work on the factors that lead to the development of the "Western diet" and sedentary work environment that make up the theoretical basis behind the leading causes of the current weight gain and obesity epidemic facing most modern societies today. A detailed understanding of the behavior and biochemical pathways regulating hunger response triggers, satiation, and regulators of visceral fat synthesis has been reviewed. However, there remains a large gap of knowledge as to the factors of why some people quickly gain visceral fat, while others with similar caloric diets and physical exercise are resistant.

This study provides evidence as to whether UA acts to increase lipemic response and, therefore, visceral fat synthesis from excess caloric consumption in humans. Because of the novelty of the hypothesis tested in this study, the research model does not reach the standard of "theory." The aim of this study was to test the hypothesis that pre-meal blood UA levels can predict lipemic response to a single high in saturated fats meal. If the null hypothesis can be rejected, blood UA levels may be a novel and useful factor

to predict risk of visceral fat weight gain from excess caloric consumption - particularly from meals high in saturated fats.

Chapter 3: Research Design And Methods

Introduction

This chapter describes the study design, rationale, setting, sampling method, sample size and power analysis, data collection, instrumentation, and statistical methods. This study examined levels of UA, lipids, insulin pathway hormones, markers of inflammation, and insulin sensitivity data as a secondary study from archival data. The primary study selected a general representation of healthy middle-aged adults and collected blood serum samples measured at baseline and time points after ingestion of a HSF meal, and a PUFA meal. The purpose of my secondary study using this data was to quantitatively measure the effects that baseline serum UA levels have on the acute peak and sustained changes in endogenously-synthesized circulating blood lipids and insulin sensitivity after a single HSF meal.

Comparisons of the predictive value of UA levels on postprandial lipemic response (i.e. percent change in VLDL), were compared to other known blood risk factors involved in the development of visceral fat weight gain, obesity, type 2 diabetes, and metabolic syndrome. The predictive power of the independent variables on lipemic response post ingestion of a HSF meal was determined using one-way repeated measures ANOVA, and Pearson's correlation with least squares (two-tail) regression modeling to test the research questions of this dissertation.

A brief description of the instruments and criteria for inclusion and exclusion of participants used in the primary study is briefly described in this chapter to disclose that the participant protocol methods followed ethical and legal guidelines.

Research Design and Rationale

This study analyzed archival serum sample data from a completed double blind randomized longitudinal crossover clinical trial. The rationale for the design was to have each participant serve as his or her own control, so that differences could be presented as percent change from their baseline values. The benefit of this design was a lower number of participants required to reach statistical significance. However, one drawback may have been a bias in the population, where the findings and conclusions may not be generalizable to all people.

In the study, 68 participants were recruited, with 31 completing all six visits. The schedule and procedures performed at each visit are briefly depicted below. This study was funded by and conducted at the National Institute on Aging (NIA; 251 Bayview Blvd., Baltimore, MD 21224). The Protocol had obtained Institutional Review Board (IRB) approval by the NIA IRB and MedStar Research Institute, NIA/Astra Unit. The principal investigator was Luigi Ferrucci, MD PhD (Clinical and Scientific Director of NIA). The NIA IRB approval and outline of this study is provided at http://clinicaltrials.gov/; identifier: NCT00233311.

Methodology

Participant Population and Recruitment Methods Used in the Primary Study

The participants used in the primary study were from a convenience sample of recruited male and female volunteers. Recruited subjects were from the following NIA

screening protocol that operates within local clinics and health care facilities in the Baltimore-Washington area. Brochures and flyers were used to advertise recruitment for the study. Recruitment letters were mailed to healthcare providers and to those participants who requested information. This included ads and/or public service announcements on websites, in radio broadcasts, television, as well as newspapers, newsletters, and magazines. Direct mailing through established marketing and advertising organizations were also used.

Inclusion Criteria

A narrow age criterion of healthy male and female participants between the ages of 50 and 65 was used to remove the known effects of age from the experiment. Previous reports have shown that aging causes plasma levels of pro-inflammatory cytokines to increase (Hager et al., 1994), where the production of IL-6, TNF-alpha and IL-1 from stimulated polymorphonucleated leukocytes increases with the age of the donor (Roubenoff et al., 1998). Most important to this study are the findings of correlations between age and the postprandial lipemic response to a fatty meal (Cohn, McNamara, Cohn, Ordovas, & Schaefer, 1988).

Participant Eligibility

Participants needed to meet all of the following criteria. Failure to meet any of the eligibility criteria would have rendered the participant ineligible for participation in the study.

- Body mass index (BMI) must be $\geq$ 19-kg/m^2 and $\leq$ 30 kg/m^2.

- Waist circumference less than or equal to 40 inches for men and 35 inches for women (measured at natural waist just above the navel). These criteria are based on guidelines of the American Heart Association.
- CRP level less than 3mg/L.
- Report no difficulties or need for help in performing self-care or instrumental activities of daily living
- Able to walk for at least 10 minutes without needing to stop or without symptom onset
- No substantial cognitive impairment based on mental status screening tests (score ≤24 on Mini-Mental Status Exam)
- No history of a cardiovascular event over the last 3 months (including angina, myocardial infarction, CABG, congestive heart failure, cerebral-vascular diseases), cancer, diabetes mellitus, neurological disease, thyroid disease, birth defect, kidney or liver disease, gastrointestinal (G.I.) diseases (including gallbladder disease), musculoskeletal disorder (if they cause pathological weakness and/or chronic pain), important sensory deficits.

Exclusion Criteria

- Blood pressure > 160 mmHg systolic or >95 mmHg diastolic with or without treatment
- WBC > 12,000/: l
- Platelets < 100,000 or >600,000 /: l
- Hemoglobin < 11 gm./dl
- Creatinine >1.4 mg/dl or calculated creatinine clearance < 50 cc/min

- Bilirubin > 1.5 mg/dl unless higher levels can be ascribed to Gilbert's disease
- Abnormal liver function (alanine transaminase > 56 IU/L; aspartate transaminase > 47 IU/L, and alkaline phosphatase > 120 IU/L)
- Corrected calcium < 8.5 or > 10.7 mg/dl
- Albumin < 3.4 g/dl
- Shortness of breath while performing normal activities of daily living, such as walking or climbing stairs
- Use of any hormones (e.g. estrogen, testosterone)
- Absolute need for long-term treatment with anti-inflammatories, aspirin (>100 mg per day per physician orders), statins, antibiotics, corticosteroids, immunosuppressors, H2 blockers and pain medications. Non-steroid anti-inflammatory use is allowed, but should be stopped three days before the study
- Use of herbal supplements are not permitted during the study (multivitamin is permitted, but other vitamin supplementation is not permitted during the study)
- Any medication/drug that acts on lipid metabolism (e.g. Xenical)
- Any severe psychiatric condition
- Any infections requiring use of antibiotics within the past 3 months
- Alcoholic intake > 30 grams while on this study is not permitted.
- Any marked increase in exercise regimen in the last 2 weeks
- Allergies to nuts
- Any condition that may preclude informed consent

- Recent blood donation (past 3 months)
- Pregnant

Criteria for Withdrawal of Participants from the Study

Participants were considered for removal from study for any of the following reasons:

- Participant decided to withdraw from the study
- General or specific changes in the participant's condition which rendered the participant unsuitable for continuation in the study per the judgment of the investigator
- Participant was non-compliant. If the subject was determined to be non-compliant with the protocol, counseling would be indicated. Evidence of repeated noncompliance may have resulted in further counseling or possible removal from the study with no further monetary compensation.

Table 1

Primary Study Participant Demographics and Physical Screen Data

Participant ID #	Age (yrs.)	Race	Sex	BMI	Visit 3 Meal	Visit 5 Meal	Serum Creatinine (mg/dL)	2-hour OGTT (mg/dL)
FF-003	50.2	Ca	M	21.7	HSF	PUFA	0.8	68
FF-007	55.7	Ca	M	25.6	PUFA	HSF	1.0	74
FF-010	58.5	AA	M	29.5	PUFA	HSF	1.3	84
FF-015	54.1	Ca	M	23.6	PUFA	HSF	1.0	109
FF-018	55.4	AA	M	21.8	HSF	PUFA	0.9	134
FF-022	65.5	Ca	M	24.2	HSF	PUFA	1.0	121
FF-024	54.8	AA	M	25.3	PUFA	HSF	0.9	111
FF-025	51.7	Ca	F	23.0	HSF	PUFA	0.7	94
FF-026	65.2	Ca	M	24.1	PUFA	HSF	1.0	122
FF-028	60.9	Ca	M	25.3	PUFA	HSF	1.0	127
FF-030	56.6	Ca	M	22.7	HSF	PUFA	1.2	48
FF-034	56.5	Ca	M	27.8	HSF	PUFA	0.8	62
FF-035	56.1	Ca	F	23.2	HSF	PUFA	0.9	86
FF-039	61.8	Ca	M	19.8	PUFA	HSF	0.9	100
FF-040	55.6	Ca	F	27.5	HSF	PUFA	0.8	76
FF-044	56.0	AA	F	24.2	HSF	PUFA	1.0	76
FF-046	56.7	Ca	F	21.2	HSF	PUFA	0.9	73
FF-047	64.2	Ca	F	30.0	HSF	PUFA	1.0	164
FF-049	60.9	Ca	M	28.4	HSF	PUFA	1.3	108
FF-050	59.6	Ca	M	22.9	PUFA	HSF	1.3	92
FF-051	60.9	Ca	F	26.1	HSF	PUFA	0.8	111
FF-052	52.8	Ca	F	21.4	PUFA	HSF	0.7	118
FF-053	50.9	AA	F	27.9	PUFA	HSF	1.1	92
FF-055	64.2	Ca	F	27.0	HSF	PUFA	1.0	104
FF-056	60.5	Ca	F	27.6	HSF	PUFA	0.8	136
FF-057	57.9	AA	F	23.7	HSF	PUFA	0.9	176
FF-059	56.7	AA	M	28.1	PUFA	HSF	0.9	132
FF-062	53.1	Ca	F	29.8	HSF	PUFA	0.9	108
FF-063	56.8	Ca	F	28.0	PUFA	HSF	0.9	166
FF-066	54.3	AA	F	25.9	PUFA	HSF	1.0	68
FF-068	52.4	AA	M	29.2	HSF	PUFA	1.2	74

Table 2

Primary Study Participant Visit Schedule

Procedure	Screening Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Total Procedures
Blood draw	X	X	X	X	X	X	6
History/physical exam	X						1
Informed consent	X						1
Study meal			X		X		2
Mini-mental exam	X						1
Food Record Diary (blue booklet)	X	X	X		X		4
Oral glucose tolerance test		X					1
Creation of participant file	X						1
Reimbursement check distribution	X	X		X		X	4
Urine pregnancy test	X					X	2
Vital signs	X	X	X	X	X	X	6
Body measurements	X						1
ECG	X						1

Table 3

Nutritional Makeup of Intervention Meals

	HSF meal	PUFA meal	HSF/ PUFA		
Water (g)	138.3	324.3	0.4		
Energy (kcal)	887.0	845.0	1.0		
Protein (g)	46.3	46.8	1.0		
Fat, total (g)	50.4	41.7	1.2		
Carbohydrate (g)	59.7	75.7	0.8		
Sugars, total (g)	7.2	27.5	0.3		
Fiber, total dietary (g)	3.9	8.4	0.5		
Cholesterol (mg)	149.0	73.0	2.0	HSF contribution to total fat	PUFA contribution to total fat
Saturated fatty acids, total (g)	18.6	7.2	2.6	36.8%	17.2%
4:0 (g)	0.3	0.0	28.8	0.6%	0.0%
6:0 (g)	0.2	0.0	118.5	0.5%	0.0%
8:0 (g)	0.2	0.0	14.1	0.3%	0.0%
10:0 (g)	0.4	0.0	18.4	0.8%	0.1%
12:0 (g)	0.4	0.1	6.7	0.9%	0.2%
14:0 (g)	2.0	0.1	21.6	3.9%	0.2%
16:0 (g)	9.2	4.4	2.1	18.2%	10.5%
18:0 (g)	5.1	1.9	2.7	10.1%	4.5%
Monounsaturated fatty acids, total (g)	20.1	19.5	1.0	40.0%	46.7%
16:1 (g)	1.1	0.2	4.9	2.2%	0.6%
18:1 (g)	18.2	19.0	1.0	36.1%	45.5%
20:1 (g)	0.3	0.2	1.4	0.6%	0.5%
22:1 (g)	0.0	0.0	0.1	0.0%	0.0%
Polyunsaturated fatty acids, total (g)	6.1	12.3	0.5	12.2%	29.4%
18:2 (g)	5.4	11.5	0.5	10.8%	27.6%
18:3 (g)	0.6	0.6	0.9	1.1%	1.5%
18:4 (g)	0.0	0.0	2.0	0.0%	0.0%
20:4 (g)	0.1	0.1	1.1	0.1%	0.1%
20:5 n-3 (g)	0.0	0.0	1.8	0.0%	0.0%
22:5 n-3 (g)	0.0	0.0	5.0	0.0%	0.0%
22:6 n-3 (g)	0.0	0.0	1.0	0.0%	0.0%
Trans Fatty Acids (g)	0.0	0.0	15.4	0.0%	0.0%
omega 3	0.6	0.7	0.9	1.2%	1.6%
omega 6	5.5	11.6	0.5	10.9%	27.7%
ratio 6/3	9.1	17.7	0.5		

Sampling Method and Randomization

All participant samples were taken during separate baseline exam, oral glucose tolerance test (OGTT), at timed intervals over 8.5 hours post feeding of intervention meal (HSF or PUFA), and the next morning (fasted). Who received the first of the two intervention meals was randomly selected, and the crossover meal given 10 days later. The order of assignment to each of the meal groups was randomly selected by the NIA pharmacy. This sample collection method was used to establish before intervention baseline levels for each participant. Changes due to the study intervention meal are relative to within-subject comparison to their baseline; i.e. each participant served as his/her own control.

Sample Size and Power

A power analysis using Raosoft (2012; http://www.raosoft.com/samplesize.html) sample size calculator revealed that for a 95% confidence level, response distribution of 50%, and a 5% margin of error, the study will need 29 participants, assuming a normal distribution curve. This study had 31 participants who finished all 6 visits, and met the recommended sample size for achieving a high statistical power of analysis. A secondary analysis for sample size was based on a minimum clinically important difference in CRP, one of the outcome variables, following consumption of a test meal, where a change of 1.0 mg/L in serum levels of CRP constituted a clinically meaningful change. This value was derived from previously published data from the InChianti study, which revealed that the mean serum level of CRP from an unusually healthy population is 1.4 mg/L [standard deviation = 0.75 mg/L](Cesari et al., 2004). According to recent American Heart Association guidelines, persons with a CRP level greater than 3.0 mg/L are considered to

be at high risk for cardiovascular disease (Sabatine et al., 2007). Therefore, for the purposes of this study, I was only interested in those people with CRP levels below high cardiovascular risk. Based on this information, the sample size calculator determined that a sample size of 12 participants, who act as their own controls, would give a Power of 83% to yield a statistically significant result. An accrual ceiling of 100 subjects was established in order to assure that 15 men and 15 women were eligible and able to complete the study.

Data Collection and Access

The data used in this secondary analysis study is from a double-blinded longitudinal crossover clinical trial that was conducted by and at the National Institute on Aging (NIA; a branch of the National Institutes of Health, under the Department of Health and Human Services of the U.S. federal government), who privately holds the data. The methods described below pertain to my usage of the primary study's data in a secondary analysis as my dissertation study.

As a full time employee (research scientist) of the NIA, I obtained anonymized (participant code ID#, with no personally identifiable information) data from the completed study: Assessment of the Effects of Fast-Food on Inflammatory Markers (ClinicalTrials.gov ID: NCT00233311). The specimens were not collected specifically for this current research study and the data given to me was anonymized; therefore, my study does not involve human subjects. I was given this data as a known colleague and employee of the National Institute on Aging, who funded and conducted the primary study. Since the primary study has already been conducted, my secondary research study analysis of the existing data does not pose any additional safety risks to the participants.

My research study is in the general scope and pretense of the original study that the participants originally signed up for under the primary studies protocol and, therefore, does not breach their participation agreement. My secondary analysis of the data represents minimal risk to the participants and involves no procedures or access linking personal identifiers or information for which written consent is normally required outside of the research context. The data has been used to investigate the relationship between baseline blood levels of UA, lipids, insulin hormones, 2-hour OGTT glucose, insulin sensitivity, and markers of inflammation, with acute changes in *de novo* lipemic response, after a HSF and PUFA test meal.

Labeling of Data Samples

The data was sent to me electronically via email and stored on an encrypted and secure computer hard drive. The participants' stored data are labeled with no identifying information (such as name); only the participants' code numbers were given to link the data for this study. Any identifying information about the subject has been kept confidential and only accessible by the IRB to the extent required by HIPAA laws.

Specimen Data Banking and Tracking

Specimen data have been stored and tracked utilizing the NIA Biological Sample Inventory system following NIH guidelines. The data was stored on my computer hard drive at the National Institute on Aging building; 251 Bayview Blvd., Baltimore, MD 21224. My work computer and backup server are only accessible by my Department of Health and Human Services (HHS) ID Badge Smart Card and pin number. The data on my computer is protected by a Federal Information Processing Standard (FIPS) 140-2 compliant encryption software package. All efforts have been made to protect the

subjects' personal information to the extent reqired by law. Medical records of research study subjects are to be stored and kept according to legal requirements. Subjects will not be identified in any reports or publications resulting from this study. Organizations that may request, inspect and/or copy research and medical records for quality assurance and data analysis include groups such as: National Institute on Aging, the Department of Health and Human Services (DHHS) agencies, The Office of Human Research Protection, Food and Drug Administration, and the MedStar Research Institute Institutional Review Board (IRB).

Operationalization of the Instruments and End-Point Variables of the Study

The following information is provided to convey that the generation of data from the primary study followed ethical and procedural standards. All participant blood draws, clinical blood chemistry (Table 4), oral glucose test, and physical (i.e. sex, race, body mass index) data were generated on site at the NIA Clinical Research Unit at Harbor Hospital Baltimore, MD 21225. They operate under the MedStar Health Research Institute's (MHRI's) biomarker, biochemical, and biorepository core, and are a College of American Pathologists (CAP)- and Clinical Laboratory Improvement Amendments (CLIA)-certified central laboratory. The instrumentation used to collect the data for the original primary study is regulated and monitored for accuracy by U.S. Federal Clinical Laboratory Improvement Amendments (CLIA) under the Center for Clinical standards and Quality program (Clinical Laboratory Improvement Amendments, 2014). The utility of using the hour 2 glucose reading as a measure of insulin sensitivity during the oral glucose tolerance test is in the public domain and the standard established by the American Diabetes Association (Tuomilehto, J. 2002). The quantitative insulin

sensitivity calculator (HOMA2) instrument is in the public domain (Hines, et al., 2013; Levy, et al., 1998).

Table 4

Blood Serum Clinical Data Used for the Study

Lipids	Analytes
cholesterol	uric acid
triglycerides	glucose (fasting and OGTT)
high density lipoproteins	creatinine
low density lipoproteins	
very low density lipoproteins	Markers of inflammation
	C-reactive protein
insulin hormones	albumin
insulin	total proteins
C-peptide (insulin precursor)	globulins
insulin like growth factor 1	white blood cell count
	tumor necrosis factor - α
	interleukin 6

Development of Instrument for Measuring Lipemic Response

Very low-density lipoproteins are assembled in the liver from endogenously synthesized triglycerides, cholesterol, and phospholipids before being released into circulating blood. Acute changes in VLDL were used in this study as a measure of endogenous lipogenesis in response to a meal (Faeh et al., 2005). Single time-point

blood serum VLDL levels were collected at timed intervals over an 8.5-hour time period postprandial consumption of PUFA and HSF meals. I designed the instrument of postprandial lipemic response to the intervention meal as the percent change of blood serum VLDL from the baseline at timed intervals for 480 minutes after ingestion of the test meal. The area under the curve of the percent change from time-0 was used to calculate the net lipemic load response from the meal (the dependent variable) for each participant. This single value was used to analyze each individual's response and for grouping participants into an ordered distribution tertile consisting of low, median, and high lipogenesis responders. Individual and group medians of lipemic response were analyzed using regression modeling against the independent variables.

Pre-meal intervention visit collection of morning fasting serum levels of UA, cholesterol, triglycerides, HDL, LDL, VLDL, HDL/LDL ratio, quantitative insulin sensitivity calculation (HOMA2), 2-hour OGTT glucose, inflammation (CRP, white blood cell count, and albumin/globulin ratio), inflammation cytokines (IL-6 and TNF-α), and insulin hormones (insulin, C-peptide, and IGF-1) were used as independent variables to test correlative prediction power with lipemic response from the HSF meal. The lipemic response from the PUFA meal was used as a negative control for each research question below. Analysis of the data included separating male and female, as well as test normalization of the meal dose per body weight by multiplying lipemic response with BMI. Clinical chemistry values found to be below the detection limit of the assay were assumed to be at the set detection limit for the purposes of inclusion in the data analysis.

Research Questions of the Study

Research Question 1: Can baseline levels of UA be used in a regression model to predict postprandial lipemic response to a HSF meal? It is hypothesized that UA is acting as a natural sensitizer of postprandial lipogenesis when activated by a HSF meal.

H_o1: There is no positive correlation between baseline UA levels and postprandial lipemic response to a HSF meal.

H_a1: There is a positive correlation between baseline UA levels and postprandial lipemic response to a HSF meal.

Research Question 2: How do baseline levels of lipids (i.e. triglycerides, cholesterol, HDL, LDL, VLDL, and HDL/LDL ratio) compare with UA in a regression model to predict postprandial lipemic response to a HSF meal? Because saturated fatty acids are known modulators of lipemic response (percent change VLDL), it was hypothesized that within normal range variations of baseline circulating lipids may correlate with a higher postprandial lipemic response to a HSF meal, and constitute a confounding variable.

H_o1: There is a positive correlation between baseline lipid levels and postprandial lipemic response to a HSF meal.

H_a1: There is no positive correlation between baseline lipid levels and postprandial lipemic response to a HSF meal.

Research Question 3: How do baseline levels of markers of inflammation (i.e. C-reactive protein and pro-inflammatory cytokines TNF-α, and interleukin-6, white blood cell count, and albumin/globulin ratio) compare with UA in a regression model to predict postprandial lipemic response to a HSF meal? Because increased inflammation-mediated

oxidative stress and ceramides (primary causes of insulin resistance) have been shown to modulate postprandial lipogenic response, it was hypothesized that upper normal range variations of baseline circulating inflammation may correlate with a higher postprandial lipemic response to a HSF meal, and constitute a confounding variable.

H_o1: There is a positive correlation between baseline markers of inflammation and postprandial lipemic response to a HSF meal.

H_a1: There is no positive correlation between baseline markers of inflammation and postprandial lipemic response to a HSF meal.

Research Question 4: How do baseline 2-hour OGTT glucose levels compare with UA in a regression model to predict postprandial lipemic response to a HSF meal? Because insulin resistance (i.e. high circulating insulin and glucose) has been shown to modulate postprandial lipemic response, it was hypothesized that upper normal range variations in baseline 2-hour OGTT may be correlated with a higher postprandial lipemic response to a HSF meal, and constitute a confounding variable.

H_o1: There is a positive correlation between 2-hour OGTT glucose levels and postprandial lipemic response to a HSF meal.

H_a1: There is no positive correlation between 2-hour OGTT glucose levels and postprandial lipemic response to a HSF meal.

Research Question 5: Does postprandial lipemic response (now the independent variable) after a HSF meal correlate with next morning fasting insulin sensitivity (HOMA2 insulin/glucose test; the dependent variable)? Participants with a high postprandial lipemic response to the HSF meal are expected to have a negative correlation with next morning fasting insulin sensitivity. IGF-1 and c-peptide values

were used to substitute insulin in the HOMA2 algorithm equation to validate the accuracy of the primary insulin data; as the HOMA2 results using c-peptide, insulin, or IGF-1 have been previously shown to be nearly identical. The outcome of changes in insulin sensitivity the day after a single high in saturated fat meal is the key question for linking lipemic response with risk for diabetes type 2, a major concern in public health.

H_o1: There is no negative correlation between postprandial lipemic response to a HSF meal and next morning fasting insulin sensitivity.

H_a1: There is a negative correlation between postprandial lipemic response to a HSF meal and next morning fasting insulin sensitivity.

Research Question 6: Are there any co-dependence correlations between the baseline levels of UA and the other independent variables that could indicate the presence of a serial correlation bias error in the results from Research Questions 1 - 5? Each independent variable was tested against UA for a Pearson's correlation, to reveal if a co-dependence relationship exists. After rank ordering the highest predictive power of all independent variables, analysis of combining 2 and 3 variables was tried to increase predictive power. This analysis was performed to show if a multivariate combination of these variables might provide a higher power of prediction with postprandial lipemic response than UA alone.

H_o1: There are correlations between baseline UA and the other independent variables.

H_a1: There are no correlations between baseline UA and the other independent variables.

Statistical Analysis of the Data

The primary end-point postprandial lipemic response, (i.e. the percent change from baseline with time of very low density lipoproteins (VLDL)) was used as the dependent variable to develop a scatter plot distribution of the data and 1-way analysis of variance (repeated measures) analysis (ANOVA) Pearson correlation with regression. The postprandial lipemic response data was also analyzed by grouping participants into an ordered distribution tertile consisting of low, median, and high risk for developing lipidemia (Andersen, Holst, Michaelsen, Baker, & Sorensen, 2012).

Because of normal variations in general population weight and body composition between participants, the fixed caloric and lipid dose from the intervention meal may have resulted in having different effects (e.g. hypo-caloric for a large, muscular male, and hyper-caloric for a petite female). In the data analysis, splitting male and female, and normalization by body size (i.e. multiplying the lipemic response by creatinine (reflects muscle mass) and BMI) were tested to determine if it would decrease the standard deviation (Lagerpusch et al., 2013).

The next stage of the study analysis tested the research questions regarding the hypothesis that baseline (pre-HSF meal) levels of UA can predict the outcome of the postprandial lipemic response from a single HSF meal. Pearson's correlation with least squares (two tailed) regression modeling was utilized to assess the predictive power of UA, in addition to other independent/predictor variables (i.e. baseline: lipids, insulin sensitivity, insulin hormones, and markers of inflammation), on the postprandial lipemic response (the dependent variable). In addition, UA was analyzed for a Pearson's correlation with all of the other independent variables for the presence of potentially

cooperative or confounding inter-relationships. Results have been expressed as mean ± standard error of the mean. Values of $P<0.05$ were considered statistically significant, where differences of $P< 0.1$ were denoted as "*" near significant, $P< 0.05$ as "**" significant, and $P< 0.01$ as "***" very significant. Analyses were performed using Prism software package 5.0 (GraphPad Software, San Diego, CA, USA).

Threats to Validity

The archival data that obtained for this study was anonymized (participant code ID#, with no personally identifiable information) from the completed clinical trial entitled Fast Food Study: Assessment of the Effects of Fast-Food on Inflammatory Markers (ClinicalTrials.gov ID: NCT00233311). The specimens were not collected specifically for my current research study; therefore, this current study does not involve human subjects. I was given this data as a known colleague and employee of the National Institute on Aging, who funded and conducted the primary study. Since the primary study has already been conducted, my secondary research study analysis of the existing data did not pose any procedural safety risks to the participants. My research study is within the general scope and pretense that the participants originally signed up for under the primary study's protocol and, therefore, does not breach their participant agreement. My secondary analysis of the data represents minimal risk to the participants and involves no procedures or access linking personal identifiers or information for which written consent is normally required outside of the research context. I will maintain the data and analysis for 5 years following the publication of my dissertation. Although the data was anonymized, it will be stored on an encrypted and secure computer hard drive.

I was given the data by an IRB-approved researcher involved in the primary study, who is a Staff Scientist at the National Institute on Aging, who funded and conducted the primary study. The NIA privately holds the data from the primary study. Authorized approval that I may use the data for this proposed dissertation study has been agreed upon in a signed Data Use Agreement (Appendix A). The data was sent to me electronically (email) and stored on an encrypted and secure computer hard drive (i.e. my computer located at the National Institute on Aging building; 251 Bayview Blvd., Baltimore, MD 21224). This computer and backup server is only accessible by my United States Department of Health and Human Services (HHS) ID Badge Smart Card and pin number. The data on this computer is protected by a Federal Information Processing Standard (FIPS) 140-2 compliant encryption software package. Safety and security checks in place to facilitate accuracy of the data include mirrored hard drive copies of the original data set, and dated copies of each major step in the analysis. All data and analysis from my study will be securely maintained for 5 years after publication of my dissertation and any resulting peer reviewed journal publication. Disposal of the data will occur from wiping and destroying of the hard drive by and under U.S. Federal government guidelines. No adverse events are perceived to affect the participants under the design of this research study because I have only received anonymized data for a secondary analysis from a study that has already been completed.

An overview of the previsions taken to insure participant safety, ethical treatment, and accuracy of the results from the instruments used to generate the data from the primary study will be briefly discussed. Participants' blood draws, clinical blood chemistry, oral glucose test, and physical (i.e. sex, race, body mass index) data were

generated at the NIA Clinical Research Unit at Harbor Hospital Baltimore, MD 21225. They operate under the MedStar Health Research Institute's (MHRI's) biomarker, biochemical, and biorepository core, and are a College of American Pathologists (CAP)- and Clinical Laboratory Improvement Amendments (CLIA)-certified central laboratory. The instrumentation used to collect the data from the original study is regulated and monitored for accuracy by U.S. Federal Clinical Laboratory Improvement Amendments (CLIA) under the Center for Clinical standards and Quality program (Clinical Laboratory Improvement Amendments, 2014). The utility of the hour 2 glucose reading during the oral glucose tolerance test is in the public domain and the standard established by the American Diabetes Association (Tuomilehto, 2002). The quantitative insulin sensitivity calculator (HOMA2) instrument is in the public domain (Hines, et al., 2013; Levy, et al., 1998). I designed the instrument of postprandial lipemic response to the intervention meal as the percent change of blood plasma VLDL from the baseline at timed intervals for 480 minutes after ingestion of the test meal, (i.e. area under the percent change curve).

The rationale for the sample size utilized Raosoft (Roasoft Inc., Seattle, WA) power analysis for 95% confidence level, 50% response distribution, and a 5% margin of error, which determined that I needed 29 participants. The data set that I received has 31 participants who have fully completed the study. Each participant has served as his/her own control, where the baseline data was used to compare their postprandial lipemic responses to a single high in saturated fat (experimental), followed by a washout period and crossover high in polyunsaturated fat (control) test meal. To insure I have a foundational understanding of the laws regarding human research protections, I have

completed the National Institutes of Health (NIH) Human Research Protections training and received a certificate (Appendix B).

Discontinuation of Participation

If the subject decided to discontinue participation in the primary study, he/she was given the option to request that any samples with his/her name on them be destroyed. If this occurred, the subject was not to be asked for further information or samples. However, it was agreed upon by the participants that data already collected from the study would not be destroyed.

Ethical Considerations

Careful consideration was given to the health and well being of the participants during the primary study. Each participant was required to pass the Mini-Mental Status Exam to insure competency of understanding the potential risks of participation in the study before being given the Informed Consent form. Participants were also given a flier describing the risks and benefits of participating as well as the schedule and activities of each of the six visits (Table 1).

Risks and Comfort Considerations

Blood and urine tests. Participants were informed of the known risks and discomforts associated with each procedure scheduled during the study. There was a slight risk of pain, bruising around the site where blood was drawn, and rarely bleeding or infection. To minimize this risk, the routine blood draw protocol was followed and pressure was applied to the site. Some people can experience feelings of lightheadedness or dizziness after having blood drawn. To reduce the risk of falling, the subject was closely monitored and asked about these symptoms before allowing them to stand. A

total of 430.5 mL of blood was collected over the 6-week course of the study. This amount of blood does not exceed the amount of blood taken by the American Red Cross during a single donation of 473 mL. A healthy person can safely donate this amount of blood every 8 weeks. The samples collected were used to monitor the subject's current medical condition and for the research purposes described for this study. There were no perceived risks to giving a urine sample.

Oral glucose tolerance test. Drinking the sugar solution may have lead to a temporary sense of abdominal fullness and discomfort, nausea, vomiting, or diarrhea, but it is a rare occurrence and was not reported by any participants during the primary study.

Body composition. Height, weight and waist circumference were obtained by non-invasive techniques.

Summary

This chapter reviewed the study design, rationale, setting, sampling method, sample size and power analysis, data collection, instrumentation, and statistical methods. The aim of this study was to quantitatively measure the effects that baseline serum uric acid (UA) levels have on the acute peak and sustained changes in endogenously synthesized circulating blood lipids and insulin sensitivity after a single HSF meal. Levels of UA, lipids, insulin pathway hormones, markers of inflammation, and insulin sensitivity data from healthy middle aged human blood serum samples were measured at baseline and time points after ingestion of a high in saturated fat (HSF), and a high in polyunsaturated fat (PUFA) meal. Statistical analysis determined the predictive power of UA levels on postprandial lipemic response (i.e. percent change in VLDL), which in the same manner was compared to other known risk factors involved in the development of

visceral fat weight gain, obesity, type 2 diabetes, and metabolic syndrome. The predictive power of the independent variable/s on lipemic response post ingestion of a high saturated fat meal was determined with the use of one-way repeated measures ANOVA and Pearson's correlation with least squares (two tail) regression modeling. This chapter also briefly reviewed the participant protocol, including: selection criteria, participation protocol, and ethical considerations, in order to disclose the criteria used to conduct the primary study. In the next chapter, the results and findings from this proposed secondary study of archival data are given in context of the research questions and statistical data showing whether or not the null hypothesis can be rejected.

Chapter 4: Data Analysis

Introduction

The aim of this study was to test if baseline blood levels of UA can be used as a predictor of the rise in endogenously synthesized blood lipids from a single HSF meal. Because the increase of blood lipids from a meal are mostly from endogenous liver synthesis and not from the meal itself, the propensity towards lipogenesis rather than protein or glycogen synthesis is important in assessing an individual's risk of hyperlipemic diseases (e.g. atherosclerosis, metabolic syndrome; Lin et al., 2005). This chapter describes the steps taken to collect, organize, and analyze that data in testing the research questions for this dissertation study.

Data Collection

This study used archival serum sample data from a completed, double blind, randomized, longitudinal, crossover, and clinical trial. In the primary study titled: Assessment of the Effects of Fast-Food on Inflammatory Markers (ClinicalTrials.gov ID:

NCT00233311), 68 participants were recruited, with 31 completing all six visits. The schedule and procedures performed at each visit are briefly depicted in Appendix B. Each participant has served as his or her own control, so that differences could be presented as percent change from baseline values. The benefit of this design was a lower number of participants required to reach statistical significance. After receiving the data for this study, is was discovered that Participants 10 and 30 could not be used because of missing data. Participant 51 had to be taken out because her C-reactive protein level and liver enzymes went over the exclusion trigger limit that was established in the primary study, briefly described in Chapter 3, page 49 of this study. Therefore, the data set used for this secondary analysis utilized the data from 28 participants (14 male, 14 female).

The dependent variable used to measure endogenous lipid synthesis directly after eating a meal was the relative percent changes in blood VLDL over time. Single time point blood serum VLDL levels were collected at timed intervals over an 8.5-hour time period postprandial consumption of the HSF and "healthy" control PUFA meals. Using the area under the curve (AUC) of the percent change post meal (where time 0 = 0%) as an instrument to derive a single value as the postprandial lipemic response to the intervention meal. The relative percent changes from baseline in VLDL from a single HSF or "healthy" control (PUFA) meal were used as a measure of endogenous lipogenesis in response to the meal (i.e. lipemic response; Faeh et al., 2005).

The major independent variable of the study was fasted baseline blood levels of UA, taken just before administration of the intervention meals. Additional independent variables of blood lipids, inflammation, insulin sensitivity, and liver function were used to test for their independent and/or co-association between lipemic response and UA.

The demographic of the study participants has been previously described in Table1. The means of all baseline clinical values from the primary study participant pool, which have been stratified by gender in this dissertation study, are depicted in Table 5. The tables have been organized by the following categories: lipids, markers of inflammation, insulin sensitivity, and liver function.

Table 5

Baseline Clinical Values Used in Study

	Baseline clinical values			
	All	Male	Female	M/F
Age (yrs.)	56.82 ± 1.19	57.64 ± 1.21	56.00 ± 1.18	3%
BMI (kg/m2)	25.45 ± 0.78	25.15 ± 0.77	25.74 ± 0.79	-2%
Uric acid (mg/dL)	4.71 ± 0.22	5.49 ± 0.22	3.94 ± 0.21	39%
Creatinine (mg/dL)	0.96 ± 0.04	1.02 ± 0.04	0.90 ± 0.03	13%
VLDL (mg/dL)	17.54 ± 2.48	23.93 ± 4.16	11.14 ± 1.38	115%
Triglycerides (mg/dL)	95.65 ± 10.78	105.80 ± 10.32	85.50 ± 11.24	24%
Cholesterol (mg/dL)	201.51 ± 8.83	191.73 ± 6.93	211.29 ± 10.73	-9%
HDL (mg/dL)	60.04 ± 4.57	53.80 ± 4.21	66.29 ± 4.92	-19%
LDL (mg/dL)	122.37 ± 7.13	116.73 ± 5.39	128.00 ± 8.86	-9%
HDL/LDL ratio	0.50 ± 0.04	0.47 ± 0.04	0.54 ± 0.04	-13%
C-reactive protein (ug/mL)	1.17 ± 0.23	1.15 ± 0.21	1.19 ± 0.25	-3%
TNFα (pg/mL)	6.19 ± 0.46	6.14 ± 0.32	6.24 ± 0.59	-2%
TNF RI (pg/mL)	1,706.31 ± 75.47	1,750.31 ± 62.48	1,662.32 ± 88.46	5%
TNF RII (pg/mL)	2,909.53 ± 131.89	2,949.93 ± 136.97	2,869.14 ± 126.81	3%
IL-6 (pg/mL)	2.44 ± 0.40	2.41 ± 0.34	2.48 ± 0.45	-3%
IL6sR (pg/mL)	48,188.57 ± 3,714.97	44,869.13 ± 2,789.97	51,508.00 ± 4,639.97	-13%
sGP130 (pg/mL)	277.97 ± 13.80	291.37 ± 12.37	264.56 ± 15.23	10%
White blood cells (billion/L)	5.78 ± 0.43	6.02 ± 0.43	5.54 ± 0.44	9%
Albumin to globulin ratio	1.48 ± 0.05	1.45 ± 0.04	1.52 ± 0.06	-5%
Glucose (mg/dL)	89.54 ± 2.63	88.87 ± 1.90	90.21 ± 3.35	-1%
C-peptide (ng/dL)	399.91 ± 31.29	387.26 ± 26.75	412.56 ± 35.82	-6%
Insulin (uLU/mL)	5.93 ± 0.83	5.76 ± 0.75	6.11 ± 0.90	-6%
IGF-1 (ng/mL)	155.80 ± 13.68	139.55 ± 20.35	172.04 ± 17.96	-19%
HOMA2 %B	78.37 ± 6.49	75.17 ± 6.94	81.57 ± 6.03	-8%
HOMA2 %S	166.95 ± 23.36	176.63 ± 27.51	157.27 ± 19.22	12%
HOMA2 IR	0.77 ± 0.11	0.75 ± 0.10	0.79 ± 0.12	-5%
2-hour OGTT (mg/dL)	105.49 ± 8.07	101.20 ± 6.26	109.79 ± 9.89	-8%
ALP (IU/L)	79.27 ± 5.30	84.47 ± 6.71	74.07 ± 3.90	14%
ALT (IU/L)	31.62 ± 2.17	36.60 ± 2.38	26.64 ± 1.97	37%
AST (IU/L)	27.35 ± 1.82	30.20 ± 1.75	24.50 ± 1.89	23%
GGT (IU/L)	30.69 ± 7.43	40.80 ± 13.13	20.57 ± 1.72	98%

Note. The mean baseline clinical values are followed by the standard error, stratified by gender, and include on the far right, the relative percent differences between the gender pools (i.e. percent difference of male over female).

Results

The primary research question of this study was to test if baseline levels of UA could be used in a regression model to predict postprandial lipemic response to a HSF meal. The first step in the analysis was to calculate the lipemic response value for each participant post HSF meal. The relative percent change for each participant was used to adjust for the person-to-person differences in the absolute VLDL mg/dL values at time 0. This was done by organizing the data in a Microsoft Excel spreadsheet and using the formula ((=time point cell value/time 0 cell value)-1) to derive the relative change in VLDL post meal, where time 0 = 0% (%ΔAUC). Because of the potential issue of a limited number of participants in the population pool to reach significance in this study, near-significant Pearson correlations P-alpha values ≤ 0.1 are depicted (*) to indicate trends that could be followed up in future studies. Figure 1 shows that the females in this study had a near-significant increased lipemic response after the HSF meal over the males. The lipemic response from the "healthy" control PUFA meal does not show this trend. However, as can been seen in Table 5, the males' mean baseline (i.e. time-0) VLDL levels starts out over 2-fold higher than the females'.

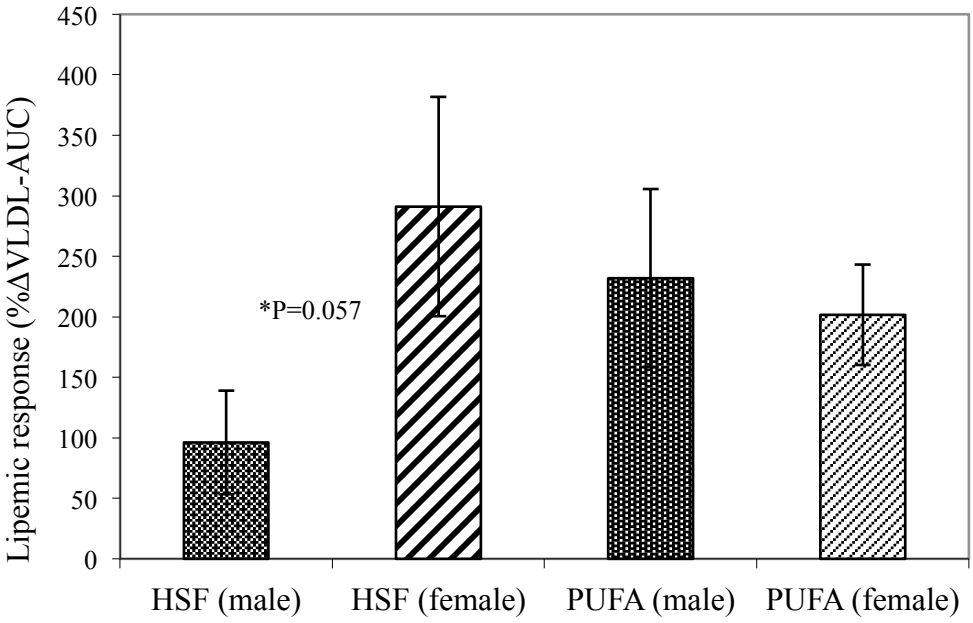


Figure 1. Post meal lipemic response. Mean percent change in the lipemic response is stratified by gender with standard error bars. There was a near-significant trend (P=0.057) of the lipemic response between genders for the HSF meal. "*" $P \leq 0.1$ near significant, "**" $P \leq 0.05$ significant, "***" $P \leq 0.01$ very significant.

These data were then transferred into the statistical analysis software GraphPad Prism 5.0, where the time points (minutes) were in the X-axis and the participants' corresponding percent change (Δ) in VLDL values was in the Y-axis. The Prism 5.0 software was then able to calculate a single value for the AUC, which is the relative lipemic response to the intervention meal. All of the participants' values for lipemic response were placed in the X axis and statistically analyzed using Pearson (two tailed) correlation using one-way analysis of variance (repeated measures) analysis ANOVA and linear regression modeling for associations with all baseline clinical chemistry and BMI values, including UA. The results showed that UA was statistically significant in correlating with lipemic response from the HSF meal for both genders independently (male P=0.045; female P=0.041), but not when they are merged together (P=0.438).

The HSF lipemic response also showed a near-significant correlation by gender for the inflammation marker TNFα (male P=0.097; female P=0.051), and a significant correlation with glucose response in females only (P=0.042). UA did not show any correlation with the lipemic response from the PUFA meal. However, there were several statistically significant correlations of the PUFA lipemic response with the HDL/LDL ratio, TNF receptor II, and liver function enzyme GGT activity for both genders. This data analysis showed the importance of separating genders as well as doing the analysis as a whole group. Interestingly, BMI showed significant correlations with the lipemic response for both HSF and PUFA meals for all participants and males after the HSF meal, but not for females. This association will be followed up on later in the analysis. Using the GraphPad Prism 5.0 software, a distribution plot with linear regression analysis of the data from Table 6, stratified by gender is shown in Figure 2. This plot shows that when stratified by gender, UA levels positively correlated with lipemic response from a HSF meal, and may have clinical utility in predicting risk of lipemic diseases in individual patients. These results answer the first research question of this study, which is that there is a positive correlation between baseline UA levels and postprandial lipemic response to a HSF meal.

Table 6

Post Meal Lipemic Response verses Clinical Baseline Values

A) HSF lipemic response vs. baseline clinical values

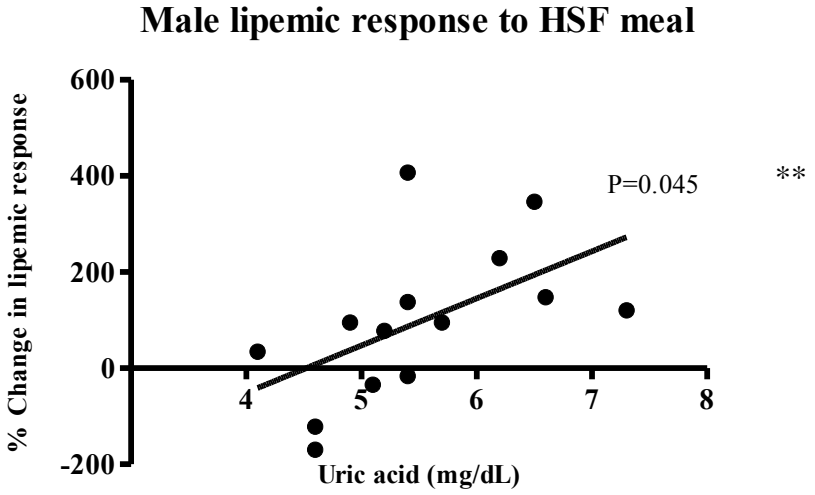
	All	Male	Female
Uric acid	0.438	****0.045**	****0.041**
BMI	***0.017	**0.045	0.102
Creatinine	0.591	0.688	0.890
Triglycerides	0.435	0.115	0.733
Cholesterol	0.330	0.692	0.457
HDL	0.909	0.217	0.206
LDL	0.348	0.366	0.757
HDL/LDL ratio	0.566	0.061	0.297
C-reactive protein	0.142	0.110	0.396
TNFα	0.573	*0.097	*0.051
TNF RII	0.420	0.609	0.159
TNF RIII	0.686	0.920	0.471
IL-6	0.273	0.977	0.100
IL6sR	0.613	0.542	1.000
sGP130	0.748	0.317	0.725
White blood cells	0.223	0.444	0.465
Albumin/globulin ratio	0.456	*0.079	0.743
Glucose	0.178	0.977	**0.042
Insulin	0.899	0.672	0.487
HOMA2 %B	0.580	0.566	0.957
HOMA2 %S	0.833	0.455	0.256
HOMA2 IR	0.865	0.677	0.462
2-hour OGTT glucose	0.378	0.985	0.173
ALP	0.949	0.636	0.632
ALT	0.740	0.635	0.442
AST	0.371	0.605	0.178
GGT	0.594	0.535	0.746

B) PUFA lipemic response vs. baseline clinical values

	All	Male	Female
Uric acid	0.943	0.880	0.202
BMI	**0.0141	*0.0747	0.164
Creatinine	0.371	0.634	*0.0939
Triglycerides	0.132	0.186	0.657
Cholesterol	0.386	0.171	0.836
HDL	**0.0334	*0.0825	0.393
LDL	0.857	0.359	0.542
HDL/LDL ratio	**0.0245	0.184	0.151
C-reactive protein	0.192	0.686	0.328
TNFα	0.524	0.280	0.883
TNF RII	*0.095	***0.0071	0.852
TNF RIII	**0.0382	**0.0273	0.568
IL-6	0.391	0.165	0.959
IL6sR	0.412	0.114	0.740
sGP130	0.004	0.138	0.033
White blood cells	0.133	0.318	0.440
Albumin/globulin ratio	0.629	0.160	0.613
Glucose	0.647	0.733	0.687
Insulin	0.909	0.844	0.749
HOMA2 %B	0.744	0.878	0.412
HOMA2 %S	0.423	0.422	0.712
HOMA2 IR	0.901	0.877	0.771
2-hour OGTT glucose	0.694	0.729	0.695
ALP	0.097	0.157	0.733
ALT	0.163	0.966	0.128
AST	**0.0155	0.150	0.146
GGT	0.358	0.519	0.962

Note. Pearson correlation *P alpha* value of post meal lipemic response (%ΔAUC) verses the mean baseline clinical values found in Table 5. "*" $P \leq 0.1$ near significant, "**" $P \leq 0.05$ significant, "***" $P \leq 0.01$ very significant.

A)



B)

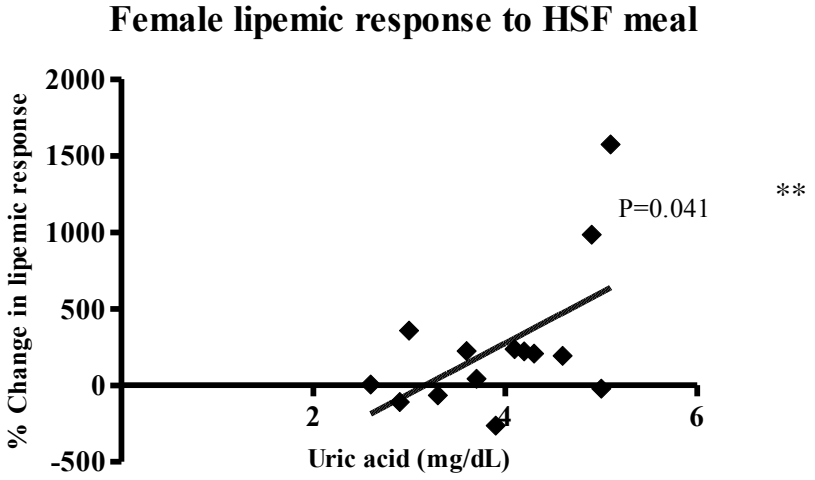


Figure 2. Post meal lipemic response verses baseline uric acid level. Distribution plot and linear regression analysis of: A) male lipemic response to HSF meal (%ΔAUC) verses baseline UA levels (***P*=0.045); B) female lipemic response to HSF meal (%ΔAUC) verses baseline UA levels (***P*=0.041). "*" $P \leq 0.1$ near significant, "**" $P \leq 0.05$ significant, "***" $P \leq 0.01$ very significant.

Research questions 2, 3, and 4 were designed to confirm and compare previously reported independent and covariant variables of lipemic response and insulin sensitivity relative to UA. Table 6A also answers how baseline lipids (question 2), markers of inflammation (question 3), and insulin response (question 4) compare to UA in correlating with the lipemic response to a HSF meal. The results show that there are no significant correlations between baseline lipids, markers of inflammation, or insulin sensitivity and postprandial lipemic response to a HSF meal. However, the results for TNFα (male, P=0.097; female, P=0.051) are trending toward significance, where a higher number of study participants may have yielded a significant difference.

In order to test for other post meal responses associated with UA, Pearson correlation analysis of baseline UA levels verses all measured post meal responses (i.e. lipids, markers of inflammation, and insulin response) were analyzed. Table 7 shows the *P alpha* value of the analysis. Because each gender was previously shown to have a strong independent correlation between baseline UA and %ΔAUC VLDL, but none when analyzed together, the lipemic response for each gender was adjusted to 100% maximum response and merged together. The results show that baseline UA levels are a strong predictor of lipemic response to a HSF meal (P=0.018), but still not for the PUFA meal. Interestingly, baseline UA levels strongly correlated with glucose response after the HSF meal in females (P=0.006), but not in males.

Table 7

Baseline Uric Acid Level verses All Post Meal Responses

A) UA vs. post HSF meal response

	All	Male	Female
VLDL	0.438	****0.045**	****0.041**
% of max VLDL	**0.018	**0.044	**0.042
Triglycerides	0.357	0.239	0.100
Cholesterol	0.677	0.932	0.355
HDL	0.312	0.073	0.458
LDL	0.536	0.845	0.603
ApoB	0.288	0.710	0.503
C-reactive protein	0.452	0.931	0.657
TNFα	0.702	0.188	0.271
TNF RIl	0.198	0.643	0.458
TNF RIIl	0.553	0.768	*0.054
IL-6	0.716	0.943	0.278
IL6sR	0.834	0.724	0.312
sGP130	0.989	0.477	0.353
Glucose	0.283	0.108	***0.006
C-peptide	0.912	0.285	0.311
Insulin	0.697	0.661	0.731
IGF-1	0.397	0.882	0.244

B) UA vs. post PUFA meal response

	All	Male	Female
VLDL	0.366	0.799	0.161
% of max VLDL	0.905	0.798	0.164
Triglycerides	0.371	0.346	0.870
Cholesterol	0.282	0.797	0.805
HDL	0.599	0.131	0.637
LDL	0.914	0.823	0.383
ApoB	0.890	0.550	0.921
C-reactive protein	0.261	0.869	0.843
TNFα	0.890	0.808	0.674
TNF RIl	0.640	0.450	0.581
TNF RIIl	0.685	0.111	0.922
IL-6	0.585	0.740	0.413
IL6sR	0.784	0.617	0.106
sGP130	0.163	0.756	0.812
Glucose	0.475	0.329	0.430
C-peptide	0.957	0.164	0.646
Insulin	0.409	0.539	0.821
IGF-1	0.779	0.413	0.119

Note. Pearson correlation *P alpha* value of UA verses post meal lipemic response (%ΔAUC) all and stratified by gender for: A) HSF meal; B) PUFA meal. "*" $P \leq 0.1$ near significant, "**" $P \leq 0.05$ significant, "***" $P \leq 0.01$ very significant.

Research question 6 asks if there are any co-dependence correlations between the baseline levels of UA and the other independent variables that could indicate the presence of a serial correlation bias error in the results from Research Questions 1 – 5. Each independent variable was tested against UA for a Pearson's correlation. The analysis shows that there are no correlations between baseline UA and any of the other independent variables. The finding that UA is not associated with BMI may be very relevant because it means that the correlations found between HSF meal lipemic response and UA and BMI are independent.

Table 8

Baseline Uric Acid Levels verses All Other Baseline Clinical Values

	UA vs. baseline clinical values		
	All	Male	Female
BMI	0.678	0.884	0.146
Creatinine	0.249	0.543	0.719
Triglycerides	0.230	0.418	0.135
Cholesterol	*0.087	0.943	0.228
HDL	0.226	0.159	0.209
LDL	*0.083	0.490	0.260
HDL/LDL ratio	0.968	*0.095	0.862
C-reactive protein	0.952	0.842	0.175
TNFα	0.991	0.196	0.344
TNF RIl	0.277	0.694	0.210
TNF RIIl	0.404	0.837	0.152
IL-6	0.974	0.495	0.430
IL6sR	0.425	0.710	0.851
sGP130	0.500	0.645	0.979
White blood cells	0.541	0.896	0.988
Albumin/globulin ratio	0.452	0.768	0.763
Glucose	0.855	0.784	0.383
Insulin	0.781	0.350	0.465
HOMA2 %B	0.409	0.329	0.622
HOMA2 %S	0.523	0.461	0.569
HOMA2 IR	0.808	0.357	0.460
2-hour OGTT glucose	0.618	*0.097	0.238
ALP	0.276	0.983	0.579
ALT	*0.081	0.738	0.994
AST	0.382	0.970	0.269
GGT	0.380	0.908	0.827

Note. Pearson correlation *P alpha* value of UA verses all other baseline clinical values, all and stratified by gender. HOMA2: %B = steady state beta cell function; %S = insulin sensitivity; and IR = insulin resistance. "*" $P \leq 0.1$ near significant, "**" $P \leq 0.05$ significant, "***" $P \leq 0.01$ very significant.

To address the observed correlation between HSF lipemic response and BMI, a Pearson correlation analysis was performed on BMI verses all post HSF meal responses

(%ΔAUC), Table 9A. The results show that, although BMI is significantly correlated with the all participants (P=0.017) as a whole and males (P=0.045), it is not a significant predictor of HSF lipemic response for females (P=0.102). Interestingly, like UA, BMI also significantly correlated with the glucose response from the HSF meal in females only (P=0.029). Pearson correlation analysis was also performed on BMI verses all baseline clinical values to test for independent and/or potential covariates. The results show that BMI has significant correlations with inflammation markers C-reactive protein (P=0.017) and sGP130 (P=0.045), as well as with insulin (P=0.026), and all HOMA2 insulin resistance markers when using all participants. These results corroborate previous study findings that BMI is a risk factor for inflammation, insulin resistance, and metabolic syndrome (Fortuna, 2012).

Table 9

Baseline BMI verses Post Meal Lipemic Response

	A) BMI vs. post HSF meal response		
	All	Male	Female
VLDL	**0.017	**0.045	0.102
% of max VLDL	***0.010	**0.045	0.102
Triglycerides	0.262	0.873	0.266
Cholesterol	0.994	0.618	0.596
HDL	0.544	0.523	0.883
LDL	0.156	0.150	0.804
ApoB	0.433	0.390	0.989
C-reactive protein	0.387	0.762	*0.057
TNFα	0.900	0.926	0.577
TNF RII	0.711	0.431	0.246
TNF RIII	0.597	*0.058	0.163
IL-6	0.326	0.930	0.378
IL6sR	0.653	0.621	0.660
sGP130	0.697	0.122	0.114
Glucose	**0.041	0.447	**0.029
C-peptide	0.200	0.480	0.291
Insulin	0.190	0.393	0.410
IGF-1	0.238	0.145	0.861

	B) BMI vs. baseline clinical values		
	All	Male	Female
Uric acid	0.678	0.884	0.146
Creatinine	0.162	0.282	0.133
Triglycerides	0.175	0.150	0.809
Cholesterol	0.737	0.318	0.168
HDL	0.796	0.700	0.264
LDL	0.884	0.121	0.335
HDL/LDL ratio	0.847	0.665	0.902
C-reactive protein	**0.017	*0.092	0.147
TNFα	0.167	0.424	**0.045
TNF RII	0.565	0.300	0.116
TNF RIII	0.833	0.202	0.231
IL-6	0.523	0.716	0.294
IL6sR	*0.063	***0.008	0.377
sGP130	**0.045	0.142	0.280
White blood cells	0.526	0.685	0.476
Albumin/globulin ratio	0.541	**0.018	0.403
Glucose	0.170	0.674	0.223
Insulin	**0.026	*0.060	0.216
HOMA2 %B	**0.050	*0.065	0.473
HOMA2 %S	**0.034	0.129	0.178
HOMA2 IR	**0.026	*0.064	0.207
2-hour OGTT glucose	0.443	0.640	0.280
ALP	0.508	0.599	0.970
ALT	0.464	0.919	0.658
AST	**0.014	0.132	0.105
GGT	0.243	0.346	0.791

Note. Pearson correlation *P alpha* value of BMI verses: A) post HSF meal lipemic response (%ΔAUC); B) all other baseline clinical values; all and stratified by gender. HOMA2: %B = steady state beta cell function; %S = insulin sensitivity; and IR = insulin resistance. "*" $P \leq 0.1$ near significant, "**" $P \leq 0.05$ significant, "***" $P \leq 0.01$ very significant.

Because baseline levels of blood UA and BMI have been shown to be independent predictors of the participants' lipemic response to a HSF meal, both were multiplied together to test if the resulting value was a stronger predictor than either used alone. Table 10A, shows the Pearson correlation *P alpha* value of UA x BMI verses all post HSF meal lipemic responses (%ΔAUC). Compared to UA alone (male, P=0.045; female P=0.041), UA x BMI (male, P=0.004; female P=0.02) increased the significance of the correlation over ten-fold for males and two-fold for females. Using percent of

maximum value for each gender to merge the data together, thereby increasing the n of the analysis, shows that factoring blood UA levels and BMI together is a strong predictor of lipemic response to a HSF meal (P=0.001). Females are still showing a significant correlation with glucose response from the HSF meal (P=0.001), and now males are nearing significance (P=0.081). The data distribution and linear regression modeling of UA x BMI verses post HSF meal lipemic response (%ΔAUC) for each gender is shown in Figures 3A & B. Pearson correlation analysis was also performed on UA x BMI verses all baseline clinical values to test for any unforeseen independent and/or potential covariates. As shown in Table 10B, the results are similar as seen for UA alone in Table 8, in that there are no significant correlations. However, compared to the results for BMI alone in Table 9B, factoring in UA has resulted in the loss all of associations between BMI with inflammation and insulin resistance. This analysis shows that a multivariate combination of the independent variables UA and BMI provides a higher power of prediction for the postprandial lipemic response from a HSF meal, than from using UA alone.

Table 10

Baseline Uric Acid x BMI verses Post Meal Lipemic Response

A) UA x BMI vs. post HSF meal response

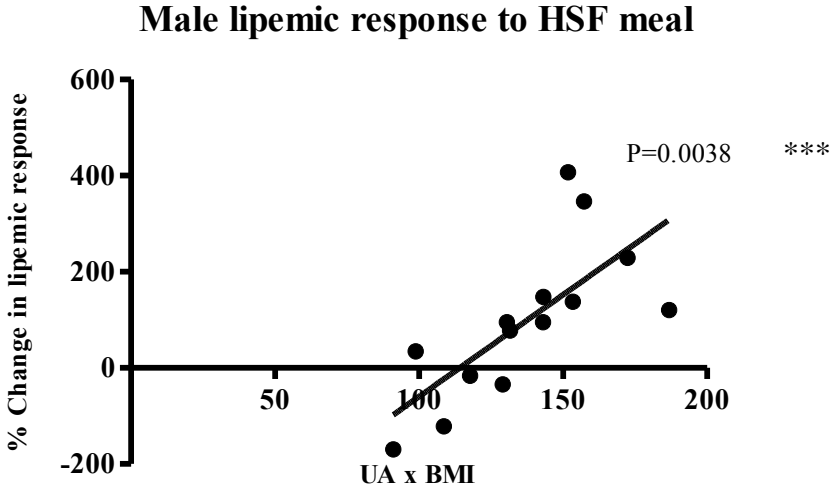
	All	Male	Female
VLDL	*0.091	***0.004**	****0.020**
% of max VLDL	***0.001	***0.004	**0.020
Triglycerides	0.201	0.319	*0.081
Cholesterol	0.661	0.913	0.337
HDL	0.148	**0.028	0.533
LDL	0.872	0.533	0.690
ApoB	0.505	0.952	0.595
C-reactive protein	0.369	0.795	0.294
TNFα	0.695	0.329	0.315
TNF RII	0.320	0.441	0.294
TNF RIII	0.900	0.399	**0.043
IL-6	0.898	0.983	0.202
IL6sR	0.938	0.537	0.293
sGP130	0.979	0.121	0.175
Glucose	*0.064	*0.081	**0.001
C-peptide	0.607	0.205	0.670
Insulin	0.920	0.436	0.630
IGF-1	0.193	0.512	0.481

B) UA x BMI vs. baseline clinical values

	All	Male	Female
Creatinine	0.151	0.919	0.416
Triglycerides	0.630	0.144	0.390
Cholesterol	0.159	0.487	0.177
HDL	0.282	0.165	0.193
LDL	0.225	0.715	0.264
HDL/LDL ratio	0.856	0.305	0.855
C-reactive protein	0.285	0.278	*0.097
TNFα	0.531	*0.095	0.114
TNF RII	0.186	0.312	*0.097
TNF RIII	0.495	0.382	0.103
IL-6	0.719	0.392	0.294
IL6sR	0.191	0.274	0.717
sGP130	0.897	0.242	0.723
White blood cells	0.359	0.863	0.738
Albumin/globulin ratio	0.899	0.148	0.616
Glucose	0.463	0.982	0.221
Insulin	0.483	0.909	0.311
HOMA2 %B	0.904	0.925	0.580
HOMA2 %S	0.689	0.907	0.343
HOMA2 IR	0.465	0.914	0.301
2-hour OGTT glucose	0.910	0.109	0.192
ALP	0.436	0.726	0.580
ALT	0.234	0.737	0.986
AST	0.785	0.402	0.155
GGT	0.829	0.529	0.775

Note. Pearson correlation *P alpha* value of UA x BMI verses: A) post HSF meal lipemic response (%ΔAUC); B) all other baseline clinical values; all and stratified by gender. HOMA2: %B = steady state beta cell function; %S = insulin sensitivity; and IR = insulin resistance. "*" $P \leq 0.1$ near significant, "**" $P \leq 0.05$ significant, "***" $P \leq 0.01$ very significant.

A)



B)

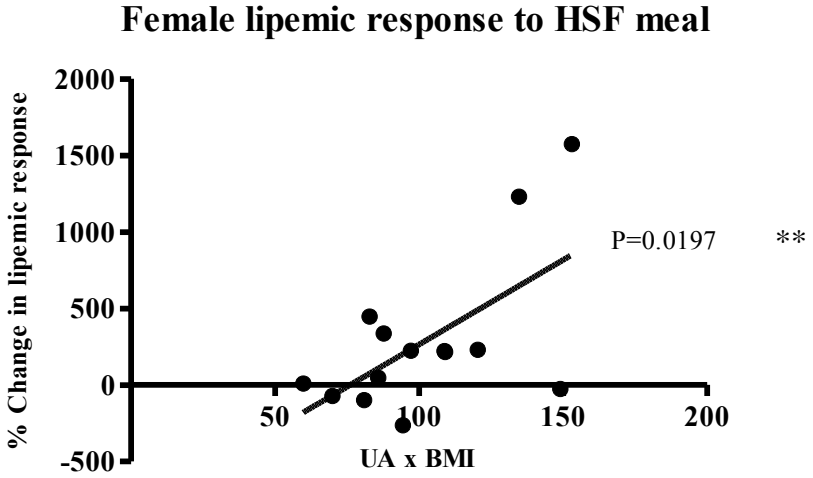


Figure 3. Post meal lipemic response verses baseline uric acid x BMI. Distribution plot and linear regression analysis of: A) male lipemic response to HSF meal (%ΔAUC) verses baseline UA x BMI (***P=0.004); B) female lipemic response to HSF meal (%ΔAUC) verses baseline UA x BMI (**P=0.02). "*" $P \leq 0.1$ near significant, "**" $P \leq 0.05$ significant, "***" $P \leq 0.01$ very significant.

The next statistical analysis was to test if the lipemic response (%ΔAUC) of VLDL has any covariant correlations with any of the other measured responses (i.e. %ΔAUC of: lipids, markers of inflammation, and insulin sensitivity) post HSF or PUFA meal. Table 11 shows the Pearson correlation *P alpha* values of %ΔAUC of VLDL verses all post meal lipemic responses (%ΔAUC) for: A) HSF meal, and B) PUFA meal. The results from all participants in the study after the HSF meal show significant associations between the changes in VLDL and triglycerides (P=0.003), and HDL (P=0.001), as expected because it replicates previous studies showing that HSF meals can activate the SREBP-1c pathway of *de novo* lipid synthesis (Lin, et al., 2005). Interestingly, as with UA and BMI, the changes in VLDL were very significantly correlated with glucose response from the HSF meal, but only in females (P=0.0001); the strongest correlation observed in this study. There was also the observation of a gender specific effect between changes in VLDL with insulin response factor IGF-1 that was seen only in males (P=0.044). The results between changes in VLDL with the other measured responses after the PUFA meal showed significant positive gender specific relationships with all markers of inflammation, except C-reactive protein, TNF RI, and IL-6. This result is not too surprising because PUFA fats (i.e. linoleic and arachidonic acid) are known precursors of inflammatory eicosanoids (Calder, 2002). Table 11B also showed a very significant correlation between changes in VLDL with changes in HDL (P=0.008) after the PUFA meal that was found when using all participants, but lost after gender stratification.

Table 11

Post Meal Lipemic Response verses All Post Meal Responses

A) Post HSF meal lipemic response (VLDL) vs. all other post HSF meal responses				**B) Post PUFA meal lipemic response (VLDL) vs. all other post PUFA meal responses**			
	All	Male	Female		All	Male	Female
Triglycerides	***0.001	*0.081	***0.008	Triglycerides	*0.064	*0.054	0.808
Cholesterol	0.655	0.899	0.608	Cholesterol	0.938	0.773	0.849
HDL	0.611	0.677	0.177	HDL	**0.044	*0.095	0.420
LDL	**0.012	***0.006	**0.047	LDL	**0.006	***0.001	0.627
ApoB	0.266	0.784	0.357	ApoB	0.283	0.607	0.196
C-reactive protein	0.965	0.780	0.683	C-reactive protein	0.843	0.511	0.720
TNFα	0.693	0.971	0.948	TNFα	**0.046	0.234	**0.048
TNF RIl	0.228	0.785	0.282	TNF RIl	0.865	0.749	0.440
TNF RIIl	0.989	0.737	0.972	TNF RIIl	0.114	0.450	*0.063
IL-6	0.907	0.931	0.847	IL-6	0.622	0.676	0.554
IL6sR	**0.019	0.716	*0.095	IL6sR	0.407	0.902	***0.003
sGP130	0.947	*0.075	0.776	sGP130	0.806	0.245	*0.064
Glucose	***0.003	0.252	***0.000	Glucose	0.373	0.211	0.667
C-peptide	0.828	0.187	0.438	C-peptide	0.797	0.155	0.151
Insulin	0.827	0.295	0.794	Insulin	0.820	0.308	0.574
IGF-1	0.701	**0.035	0.888	IGF-1	**0.033	**0.033	0.628

Note. Pearson correlation *P alpha* value of post meal lipemic response (%ΔAUC) verses all other post meal responses (%ΔAUC): A) HSF; B) PUFA; all and stratified by gender. "*" $P \leq 0.1$ near significant, "**" $P \leq 0.05$ significant, "***" $P \leq 0.01$ very significant.

Research question 5 asks if there are significant changes in next morning fasting insulin sensitivity (glucose, C-peptide, insulin, and calculated HOMA2 IR) following the HSF meal. Data for the absolute mean values of insulin sensitivity (i.e. glucose, C-peptide, insulin, and calculated HOMA2 insulin resistance values are presented in Table 12 for next morning post: A) HSF and B) PUFA meals. The percent change from the before meal baseline (Table 5) is given for all participants and stratified by gender. The percent difference between male verses female is presented on the right side of Table 12. Pearson's correlation analysis revealed that only males had a near-significant change in insulin and HOMA2 IR (insulin resistance) following the HSF meal (P=0.06). There were no such associations following the PUFA meal, or with the female participants.

This data therefore supports the null hypothesis that there are no changes in next morning fasting insulin sensitivity following the HSF meal.

Table 12

Changes in Next Morning Following Meal Insulin Sensitivity

A) Next morning after HSF meal fasted indicators of insulin sensitivity

	All	Male	Female	M/F
Glucose (mg/dL)	92.22 ± 2.28	92.73 ± 1.92	91.71 ± 2.64	1%
C-peptide (ng/dL)	438.83 ± 43.99	456.08 ± 45.08	421.59 ± 42.91	8%
Insulin (uLU/mL)	7.53 ± 1.15	8.20 ± 1.61	6.86 ± 0.70	20%
HOMA2 %B	87.82 ± 8.65	89.57 ± 10.05	86.07 ± 7.24	4%
HOMA2 %S	132.88 ± 17.24	137.31 ± 21.23	128.45 ± 13.24	7%
HOMA2 IR	0.98 ± 0.15	1.07 ± 0.21	0.89 ± 0.09	19%

Change from baseline	All	Male	Female
Glucose (mg/dL)	3% ± 3%	4% ± 2%	2% ± 3%
C-peptide (ng/dL)	10% ± 9%	18% ± 8%	2% ± 9%
Insulin (uLU/mL)	27% ± 15%	***42%** ± 16%	12% ± 13%
HOMA2 %B	12% ± 9%	19% ± 10%	6% ± 8%
HOMA2 %S	-20% ± 13%	-22% ± 16%	-18% ± 11%
HOMA2 IR	27% ± 15%	***42%** ± 16%	13% ± 13%

B) Next morning after PUFA meal fasted indicators of insulinsensitivity

	All	Male	Female	M/F
Glucose (mg/dL)	91.12 ± 2.15	93.47 ± 2.55	88.77 ± 1.74	5%
C-peptide (ng/dL)	468.27 ± 53.70	466.48 ± 49.20	470.05 ± 58.20	-1%
Insulin (uLU/mL)	6.47 ± 0.86	6.43 ± 0.87	6.50 ± 0.85	-1%
HOMA2 %B	81.31 ± 8.02	76.80 ± 8.29	85.83 ± 7.74	-11%
HOMA2 %S	168.78 ± 32.96	180.63 ± 41.49	156.92 ± 24.43	15%
HOMA2 IR	0.84 ± 0.12	0.85 ± 0.12	0.83 ± 0.12	2%

Change from baseline	All	Male	Female
Glucose (mg/dL)	2% ± 3%	5% ± 2%	-2% ± 3%
C-peptide (ng/dL)	17% ± 10%	20% ± 9%	14% ± 11%
Insulin (uLU/mL)	9% ± 14%	12% ± 13%	7% ± 14%
HOMA2 %B	4% ± 9%	2% ± 10%	5% ± 8%
HOMA2 %S	1% ± 17%	2% ± 19%	0% ± 14%
HOMA2 IR	8% ± 14%	12% ± 13%	4% ± 14%

Note. Mean values of insulin sensitivity markers from the next morning following the: A) HSF, and B) PUFA meals. The mean is from all participants, and stratified by gender, with corresponding standard errors. Directly below the means are the percent change from the before meal baseline (Table 5). To the right of the table are the relative percent

differences between the gender pools (i.e. male over female). HOMA2: %B = steady state beta cell function; %S = insulin sensitivity; and IR = insulin resistance. "*" $P \leq 0.1$ near significant, "**" $P \leq 0.05$ significant, "***" $P \leq 0.01$ very significant.

Summary

The purpose of this study was to test if blood levels of UA influenced the level of endogenous lipid synthesis after eating a HSF meal, a known activator of lipogenesis. The primary research question asked if baseline levels of UA could be used in a regression model to predict postprandial lipemic response to a HSF meal. The distribution plot with linear regression analysis in Figure 2 shows that, when stratified by gender, UA levels positively and significantly correlates with lipemic response from a HSF meal (male, P=0.045; female, P=0.041). These results answer the 1st research question of this study, which is that there is a positive correlation between baseline UA levels and postprandial lipemic response to a HSF meal. Figure 1 also shows that these correlations could have a clinical utility predicting risk of lipemic diseases in individual patients.

The answer as to how baseline lipids (question 2), markers of inflammation (question 3), and insulin response (question 4) compares to UA in correlating with the lipemic response to a HSF meal has all been answered in Table 6A. The results show that there are no significant correlations between baseline lipids, markers of inflammation, or insulin sensitivity and postprandial lipemic response to a HSF meal. However, the results for TNFα (male, P=0.097; female, P=0.051) are trending toward significance, where a higher number of study participants may show a statistically significant difference.

Research question 5 asks if there are significant changes in next morning fasting insulin sensitivity (i.e. glucose, C-peptide, insulin, and calculated HOMA2 IR) following the HSF meal. Because spikes in blood levels of saturated fatty acids have been shown to activate VLDL synthesis through a SREBP-1c feed forward mechanism, it is possible that within normal range variations of baseline circulating lipids may lead to a higher postprandial lipemic response to a HSF meal, and constitute a confounding variable. However, the Pearson's correlation analysis shown in Table 12 revealed that only males had a near-significant change in insulin and HOMA2 IR (insulin resistance) following the HSF meal (P=0.06). As expected, there were no such associations following the "healthy" control PUFA meal. This data therefore supports the null hypothesis that there are no changes in next morning fasting insulin sensitivity following the HSF meal.

Research question 6 asks if there are any co-dependent correlations between the baseline levels of UA and the other independent variables that could indicate the presents of a serial correlation bias error in the results from research questions 1 – 5. Baseline UA levels were tested against all of the other baseline independent variables using Pearson's correlation. The analysis shows that there are no correlations between baseline UA and any of the other independent variables. The finding that UA is not associated with BMI may be very relevant because it means that the correlations found between the HSF meal lipemic response and UA, and BMI are independent. Further interpretations of the results, implications for social change, and discussions on the limitations found with this study, plus recommendations for future research, will be discussed in Chapter 5.

Chapter 5: Conclusions And Recommendations

Introduction

Blood levels of UA have previously been reported to be associated with aging, high blood pressure, heart disease, type 2 diabetes, obesity, and metabolic syndrome (Soltani, et al., 2013). Using archival data from a completed double blind, randomized, longitudinal, crossover clinical trial, this study aimed to test if circulating UA levels could predict the lipemic response after eating a single, typical fast food modeled meal high in saturated fats. The study population consisted of healthy, middle-aged adults. The analysis showed that when stratified by gender, baseline UA levels could significantly predict the lipemic response (%ΔAUC) from a HSF, but not a "healthy" PUFA meal. The public health utility of this finding would be to use clinically obtained UA values to plug into the gender specific linear regression plot (Figure 2) to identify individuals at risk for hyperlipidemia from a HSF meal.

The results from this study also found that BMI was only a near-significant predictor of lipemic response from a HSF meal, but was independent of UA. However, when UA and BMI values were factored together, the power to predict the lipemic response from a HSF meal significantly increased (i.e. over 10-fold for males, and 2-fold for female). Therefore, the gender specific regression plot (Figure 3) may be a more accurate predictor of a patient's risk for hyperlipidemia from a HSF meal.

The health consequence of a strong lipemic response from a HSF meal is most evidently seen in the female population of this study. The female data showed that a high lipemic response from a HSF meal resulted in simultaneous insulin resistance (i.e. high and sustaining glucose levels immediately following the meal; P=0.0001). The observed

insulin resistance directly following the HSF meal was specific to the HSF meal, because the effect did not show up during the equal calorie and fat content PUFA meal. While UA and BMI predicted the lipemic response in both male and females, only the females showed a statistical significance of UA and BMI to predict insulin resistance following the HSF meal. However, when UA and BMI were factored together, the predictive power for insulin resistance following the HSF meal nears significance for the male population as well.

Interpretations of the Findings

Two of the major gaps in the current knowledge of hyperlipidemia are the risk factors and most sensitive targets for intervention in the prevention of lipidemia and weight gain in healthy people (Akbaraly et al., 2013). During feeding, the human body requires about 5 grams of glucose to bring blood glucose levels from a fasting state of ~60 mg/dL to a fed state of ~130 mg/dL. The calories from a typical meal (e.g. ~750 kcal.) are quickly stored into glycogen (e.g. liver, muscle, and brain), protein (e.g. muscle and liver), and fat (visceral and subcutaneous). During prenatal and early childhood, the fat storage is primarily subcutaneous fat; however, with aging, a progressively higher percent is stored as visceral fat (Schlosser, 2002). Visceral fat is also the type of fat that has been shown to be most associated with the development of most age-related and hyperlipemic diseases (Liu, et al., 2013). The factors that direct where excess calories are stored remains unknown, but are recognized as being critically important in public health prevention of age-related diseases.

The justification for factoring together UA with BMI in this study's data analysis (Table 10 and Figure 3) was to adjust for different body sizes given that the calories for

the meal were fixed. The number of calories ingested by each participant for the meal was fixed (i.e. 866 kcal.), but the dose/response of a low body weight/muscle mass participant is likely to be different than a much larger participant. BMI is calculated by age, height and weight measurements entered into a tabulated chart that is stratified by gender. The biggest limitation of the BMI calculation is that it does not reflect muscle mass, where many athletes (particularly weight lifters) can be diagnosed as being obese although they have a very low percent body fat. The response to caloric intake in stimulating lipogenesis and inflammation is very low in active individuals with high muscle mass, whereas, in most cases it is very high in individuals with a high percent body fat, even though they have the same BMI (Lagerpusch et al., 2013).

The HOMA2 calculation used in this study was to measure insulin sensitivity/resistance (Hines et al., 2013; Levy et al., 1998). Insulin resistance is defined as the physiological failure of cells to respond to insulin. Pancreatic beta-cells produce insulin in response to blood glucose levels to maintain homeostasis between 70 and 130 mg/dL. Chronically high blood glucose and insulin levels (e.g. type 2 diabetes) results in a response of the target cells to lower their production of glucose receptors (e.g. GLUT4), thereby setting up a self-perpetuating cycle. The results from Table 11A show that elevated levels of glucose (i.e. insulin resistance) occurred immediately following the HSF meal, but not the "healthy" control PUFA meal. The observed insulin resistance did not carry over to the next morning HOMA2 test, and also did not show up in the OGTT. This finding indicated that it may be clinically useful to conduct a HSF challenge test, using the same protocol as for the OGTT.

Fitting the Study Findings into the Theoretical Framework

The theoretical foundation for this archival study was based on the previous findings that UA has been found to be independently correlated with visceral fat weight gain and the diagnosis of cardiovascular disease (Soltani, et al., 2013). However, it there were no publications indicating whether UA is a cause of an effect of hyperlipidemia. One hypothesis for the driving evolutionary pressure for genetic mutations aimed at increasing UA in humans (i.e. knockout of UOX and increase of URAT1) was to increase metabolic efficiency and enhance anabolic recovery by suppression of AMPK mediated catabolism and autophagy (Lanaspa, Sanchez-Lozada, et al., 2012). The hypothetical model being tested in this study was that UA decreases the activation energy of anabolic pathways (i.e. lipemic response). The proposed increase in anabolic response would act to increase the risk of lipidemia and visceral fat weight gain from excess caloric intake, thereby making UA a potential indicator and modifiable risk factor for visceral fat weight gain. The modeled framework being used to test this hypothesis utilized blood serum UA data from a healthily middle aged population to see if the differences in baseline UA levels could statistically predict lipemic response after eating a single HSF meal. The findings from this study showed that baseline UA levels positively and significantly correlate with the lipemic response from a HSF, but not a PUFA meal (Figure 2 A and B). The data analysis also revealed that the observed phenomena are gender specific. This finding supported the theoretical foundation of previous studies, and adds to the base of knowledge by showing that the increase of risk for hyperlipidemia begins to increase in individuals with blood UA levels of 4.5 mg/dL for male and 3.0 mg/dL for female.

More work is needed to understand the mechanisms of why this effect is on different scales between the genders and why it is specific to saturated fats.

Limitations of the Study

The two major additions in the primary study design that would have greatly benefited this dissertation study would have been to include a full lipid profile and UA measurements for all of the time-point samples from the OGTT, and UA levels for the post intervention meal samples. This would have given valuable information towards understanding how different calorie sources (i.e. glucose from the OGTT, and saturated verses PUFA fats from the meals) were affecting the SREBP-1c lipogenesis pathway response. It would have also been interesting to see if UA changed during these dietary interventions. However, previously published studies have not shown UA to significantly change in HSF and PUFA meal intervention trials similar to this one (Jimenez-Gomez et al., 2009). However, UA has been shown to significantly raise post meal in studies using large doses of fructose or purines (e.g. RNA from yeast or sardines; Rock, et al., 2013).

This study may have benefited if the primary study recruited more participants in increasing the strength of the statistical correlations. The tables and figures from the study analysis included statistical designations of near-significance ($*P \leq 0.1$). This was to show trends in the data that may confirm previously reported findings, and also to show potential research questions for future studies with a more focused design or higher number of participants to reach significance. A recommendation for future studies on this data set will be to do a Pearson's correlation analysis of HOMA2 IR at baseline and post meals verses all baseline clinical values and verses all relative changes measured

(%ΔAUC) post HSF and PUFA meals. This analysis would give a better understanding of which factors are involved in causing insulin resistance.

Recommendations

Since 1985, there has been an alarming rise in visceral fat weight gain, with approximately 68% of adults in the U.S. currently diagnosed as being overweight or obese (Fortuna, 2012). Current gaps in the knowledge include the genetic and biological risk factors for visceral fat weight gain and, in particular, why some people are more prone to lipogenesis. These gaps have been a major stumbling block for public health organizations in being able to launch effective prevention and treatment campaigns.

According to the Adult Treatment Panel II and International Diabetes Federation criteria, a patient meets diagnostic criteria for metabolic syndrome when three or more of the following risk factors are present: abdominal obesity waist circumference for men greater than or equal to 102 cm, for women greater than or equal to 88 cm; fasting blood triglyceride levels greater than or equal to 150 mg/dL, HDL-cholesterol less than 40 mg/dL for men and 50 mg/dL for women; blood pressure greater than or equal to 130/85 mmHg; and/or a fasting blood glucose greater than or equal to 100 mg/dL (Alberti, Zimmet, & Shaw, 2006). Very high levels of UA can result in precipitation of soluble UA into needle-shaped crystals (i.e. gout) that can accumulate in joints and kidneys to cause physical damage, leading to further damage by chronic inflammation, and is a cause of arthritis and kidney disease (Rho, Zhu, & Choi, 2011). This dissertation study only focused on the phenomena associated with soluble UA [i.e. UA$\leq$ 6.0mg/dL]; however, the presence of gout symptoms interfering with the interpretation of the data cannot be rule out in participants with high levels. The observed increase in

inflammation markers after the "healthy" control meal triggered concerns that a diet high in PUFA, which are substrates of inflammation signals (i.e. arachidonic acid), could further antagonize inflammatory damage in gout patients. Further analysis of data from this study needs to test if baseline markers of inflammation are correlated with changes in inflammation markers immediately after eating the PUFA meal. A Pearson's correlation analysis of baseline C-reactive proteins verses all other baseline clinical values showed a significant association with BMI (P=0.017, Table 9A), but also with most of the other markers of inflammation (data not shown). This result indicates that preexisting levels of inflammation are correlated with an amplified increase in levels of inflammation post meal. This observation of a feed-forward self-cycling mechanism was also seen with BMI, in that higher BMI participants are more likely to have a higher lipemic response to the HSF meal with corresponding insulin resistance (P=0.017, Table 6A). In other words, the more visceral fat a participant had, the more geared their metabolic pathway was to synthesize more fat. The observation of the interactions between BMI and inflammation being self-perpetuating has been previously reported, and is an established component of the metabolic syndrome mechanistic hypothesis (Alberti, Zimmet, & Shaw, 2006). A future study design to better understand the factors involved in the self-perpetuating nature of the metabolic syndrome should include a more defined lipid challenge (e.g. just C16:0 or C18:2) that is able to dissect out which lipids and signaling pathways are most involved. The design of the follow-up study should also include increasing and decreasing UA levels before the dietary challenges to establish if UA is a direct effector of lipemic response sensitivity. Future work also needs to address the

factors underlying the gender differences observed in the outcome findings from this study.

Implications for Social Change

The implications for the results from this study to cause positive social change lies in providing the research community evidence that UA is an independent regulator of postprandial lipemic response that is specific to a diet high in saturated fats. The results from this study corroborate previously reported findings that BMI and inflammation are involved in increasing the sensitivity to postprandial lipemic response from either meal, but the association is independent and much weaker than that observed for UA in this study. The relatively weak correlations of BMI and inflammation with lipemic response from the HSF meal in this study are likely because the participant pool was selected to represent a healthy-weight population. The key finding that UA levels are an independent predictor of lipemic response from a HSF meal is summarized in Figure 2 and 3. After obtaining BMI and UA levels, these figures could be utilized by healthcare professionals to determine a patient's risk of visceral fat weight gain from excess caloric intake. These findings could be added to the public health awareness campaign of disseminated information so that it includes the relationship between blood serum UA levels and postprandial lipemic response to dietary saturated fats. Continuation of this line of research is also likely to uncover new targets for pharmaceutical drugs and natural dietary supplements directed toward prevention and treatment of hyperlipemia and age-related diseases.

This study found a significant and positive correlation between lipemic response and insulin resistance immediately following a HSF meal. Diets high in saturated fats are

known inducers of insulin resistance, where palmitic acid (C16:0), the major precursor of ceramides is the most potent inducer (Holland et al., 2007). Lowering ceramides has been shown to be a key in reversing the self-perpetuating cycle of insulin resistance and metabolic syndrome (Gill & Sattar, 2009). However, inflammation, mainly through the production of reactive oxygen species, is a key activator of ceramide production via activation of sphingomyelinases. Therefore, to break the cycle, the current clinical data indicates that a three-pronged approach would be most effective. One step is to lower ceramide synthesis precursor palmitic acid by maintaining a low saturated fat and simple sugar (e.g. fructose) diet, intermittent fasting and exercise. Our 24-hour hormone cycle favors peak endogenous fatty acid and steroid (i.e. cholesterol) synthesis in response to our nocturnal peak of growth hormone (Jones & Schoeller, 1990; Parker et al., 1982). This is why taking statins to lower cholesterol synthesis is most effective and therefore prescribed to be taken with the evening meal (Faeh et al., 2005). There is also building evidence that eating lighter meals at dinner may be an effective strategy in lowering endogenous lipogenesis (Faeh et al., 2005). The second step is to lower inflammation, which can be achieved by lowering infections activating inflammation, particularly chronic infections (e.g. hepatitis virus, oral plaque, etc.…) (Lin, et al., 2005). After clearing the primary activator of inflammation, anti-inflammatory agents (e.g. steroids, and non-steroid (ibuprofen, aspirin)) may be useful in breaking the inflammation cycle (Lin, et al., 2005). Lastly, a diet high in antioxidants has been shown to be useful in breaking a self-perpetuating inflammation cycle after the primary inflammatory trigger has been cleared. Dietary antioxidants have also been shown to be useful in increasing the threshold of the inflammation response, thereby lowering false antigen activated auto-

immune responses (Vgontzas, et al., 2000). Antioxidants like N-acetyl-cysteine have been shown to shorten the time in quenching the immune response back to baseline after clearance of antigen resulting in the prevention of initiating a self-perpetuating inflammation cycle and consequential collateral damage to surrounding healthy tissues (Boon et al., 2013; Dekker et al., 2013). Since UA has been shown to be a potent antioxidant that positively correlates with lipemic response to a HSF meal, it could be acting as a beneficial response to put on the "metabolic brakes" on the self-perpetuating cycle described above (Cutler, 1984; Sutin et al., 2013). However, more research is needed to support this hypothesis.

Conclusion

Utilizing data from a healthy middle-aged population, this study has found that when stratified by gender, baseline UA levels are an independent and significant predictor of the lipemic response (%ΔAUC) from a HSF, but not a "healthy" PUFA meal (Table 6). In most animals, the biochemical pathway of purine catabolism ends with allantoin as the end product, with many species having the biochemical catabolic pathway going further to ammonia. However, in apes, birds, bats, and some reptiles, the urate oxidase gene has accumulated multiple mutations resulting in knocking out enzymatic activity thereby making UA the final catabolic end product. The evolutionary advantage and driving force for the progressive knockdown and eventual knockout of urate oxidase in apes, birds, bats, and some reptiles remains unknown. Evidence that UA is not an unwanted waste product, but has some biologically desired function, is in the presence of enzymatic systems in the kidneys that expend energy to transport UA from the urine back into the blood stream. Blood serum UA levels increase with starvation and

physical injury (e.g. exercise), which is further amplified by an increase in kidney reabsorption via URAT1, indicating that UA is being utilized as a stress signal, or in a repair pathway. One of the proposed benefits of high UA is that it is acting to increase the metabolic efficiency of visceral fat synthesis and storage by inhibiting AMPK, thereby increasing the postprandial activation of the sterol regulatory element binding protein-1c (SREBP-1c) lipogenesis pathway (Lanaspa, Sanchez-Lozada, et al., 2012). However, UA levels can also be increased by a diet high in purines (e.g. meat) or fructose, which has been positively correlated with obesity, hypertension, cardiovascular disease, type 2 diabetes mellitus, metabolic syndrome, and gout (Soltani, Rasheed, Kapusta, & Reisin, 2013).

The concerning public health implication of high BMI found from this study is that having a high percent of visceral fat somehow makes one more prone to gain more fat from the same calorie meal. This result indicates that there is a sensitivity and/or feed-forward mechanism, where the existing visceral fat is influencing metabolism to synthesize more visceral fat. Fortunately, the effect of BMI in causing temporary insulin resistance directly after a HSF meal was specific to HSF and did not happen with the PUFA meal. These results confirm previous studies that found that Mediterranean (i.e. high PUFA) meals are beneficial in maintaining insulin sensitivity and weight loss as compared to HSF meal (Jimenez-Gomez et al., 2009). Other studies have shown that diets high in simple sugars (e.g. fructose), stimulate endogenous fatty acid synthesis that closely matches the blood lipid profiles (e.g. high palmitic acid) of people on a high in saturated fat diet (Lanaspa, Sanchez-Lozada, et al., 2012). However, this effect can be countered by insuring that 5 – 10% of fats in a meal are polyunsaturated omega-3 fatty

acids (e.g. DHA, EPA, ALA)(Jimenez-Gomez et al., 2009). Previous studies have shown that fasting and exercise can stop this cycle in muscle and brain, by increasing insulin sensitivity (Bataille, Maffeo, & Renfro, 2011).

Although the results from this study show that UA is significant and positively correlated with lipemic response after a HSF meal, it is not known if lowering UA (e.g., through use of allopurinol) would result in a lower lipemic response. The public health information and utility that can be taken away from this study are found in Figures 2 and 3, which can be used to advise patients with UA above 4.5 and 3.0 mg/dL for males and females, respectfully, about their increased sensitivity with HSF foods.

Building on the findings from this study, a future study should test if increasing and lowering UA levels affects the lipemic response to a HSF meal. Currently, there is no clinical diagnostic utility for measuring UA levels, besides assessing for gout (Nakagawa, et al., 2006). The continuation of this line of research should determine if UA has any diagnostic value and, if so, establish healthy ranges.

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Appendix A : Data Use Agreement

DATA USE AGREEMENT

This Data Use Agreement ("Agreement"), effective as of 12/12/2013 ("Effective Date"), is entered into by and between Roy G. Cutler ("Data Recipient") and National Institute on Aging ("Data Provider"). The purpose of this Agreement is to provide Data Recipient with access to a Limited Data Set ("LDS") for use in research in accord with the HIPAA and FERPA Regulations.

1. Definitions. Unless otherwise specified in this Agreement, all capitalized terms used in this Agreement not otherwise defined have the meaning established for purposes of the "HIPAA Regulations" codified at Title 45 parts 160 through 164 of the United States Code of Federal Regulations, as amended from time to time.

2. Preparation of the LDS. Data Provider shall prepare and furnish to Data Recipient a LDS in accord with any applicable HIPAA or FERPA Regulations

3. Data Fields in the LDS. No direct identifiers such as names may be included in the Limited Data Set (LDS). In preparing the LDS, Data Provider shall include the **data fields specified as follows**, which are the minimum necessary to accomplish the research (list all data to be provided): Participant number matched to: gender, age, race, body mass index; blood levels of: cholesterol, triglycerides, high density lipoproteins, low density lipoproteins, very low density lipoproteins, insulin, c-peptide, insulin like growth factor-1, uric acid glucose, c-reactive protein, albumin, total proteins, globulins, white blood cell count, tumor necrosis factor-alpha, and interleukin-6.

4. Responsibilities of Data Recipient. Data Recipient agrees to:

    a. Use or disclose the LDS only as permitted by this Agreement or as required by law;

    b. Use appropriate safeguards to prevent use or disclosure of the LDS other than as permitted by this Agreement or required by law;

    c. Report to Data Provider any use or disclosure of the LDS of which it becomes aware that is not permitted by this Agreement or required by law;

    d. Require any of its subcontractors or agents that receive or have access to the LDS to agree to the same restrictions and conditions on the use and/or disclosure of the LDS that apply to Data Recipient under this Agreement; and

    e. Not use the information in the LDS to identify or contact the individuals who are data subjects.

5. Permitted Uses and Disclosures of the LDS. Data Recipient may use and/or disclose the LDS for its Research activities only.

6. Term and Termination.

   a. Term. The term of this Agreement shall commence as of the Effective Date and shall continue for so long as Data Recipient retains the LDS, unless sooner terminated as set forth in this Agreement.

   b. Termination by Data Recipient. Data Recipient may terminate this agreement at any time by notifying the Data Provider and returning or destroying the LDS.

   c. Termination by Data Provider. Data Provider may terminate this agreement at any time by providing thirty (30) days prior written notice to Data Recipient.

   d. For Breach. Data Provider shall provide written notice to Data Recipient within ten (10) days of any determination that Data Recipient has breached a material term of this Agreement. Data Provider shall afford Data Recipient an opportunity to cure said alleged material breach upon mutually agreeable terms. Failure to agree on mutually agreeable terms for cure within thirty (30) days shall be grounds for the immediate termination of this Agreement by Data Provider.

   e. Effect of Termination. Sections 1, 4, 5, 6(e) and 7 of this Agreement shall survive any termination of this Agreement under subsections c or d.

7. Miscellaneous.

   a. Change in Law. The parties agree to negotiate in good faith to amend this Agreement to comport with changes in federal law that materially alter either or both parties' obligations under this Agreement. Provided however, that if the parties are unable to agree to mutually acceptable amendment(s) by the compliance date of the change in applicable law or regulations, either Party may terminate this Agreement as provided in section 6.

   b. Construction of Terms. The terms of this Agreement shall be construed to give effect to applicable federal interpretative guidance regarding the HIPAA Regulations.

   c. No Third Party Beneficiaries. Nothing in this Agreement shall confer upon any person other than the parties and their respective successors or assigns, any rights, remedies, obligations, or liabilities whatsoever.

d. Counterparts. This Agreement may be executed in one or more counterparts, each of which shall be deemed an original, but all of which together shall constitute one and the same instrument.

e. Headings. The headings and other captions in this Agreement are for convenience and reference only and shall not be used in interpreting, construing or enforcing any of the provisions of this Agreement.

IN WITNESS WHEREOF, each of the undersigned has caused this Agreement to be duly executed in its name and on its behalf.

DATA PROVIDER

Signed: [redacted]

Print Name: [redacted]

Print Title: Staff Scientist at the NIA

DATA RECIPIENT

Signed:

Print Name: Roy G. Cutler, MS, MPH

Print Title: Biologist at the NIA

Appendix B: Protecting Human Research Participants Certificate

Certificate of Completion

The National Institutes of Health (NIH) Office of Extramural Research certifies that **Roy Cutler** successfully completed the NIH Web-based training course "Protecting Human Research Participants".

Date of completion: 11/27/2013

Certification Number: 1335300

Curriculum Vitae

Roy G. Cutler, M.S., MPH
Business Address:
National Institute on Aging
251 Bayview Blvd.
Baltimore, MD 21224
Work: 410-558-8239
RCutler@nih.gov

Career Aim:
My goal is to be part of a research team whose goal is to understand the root cause of aging and to use this information to develop biomarkers and therapies to increase productive years of life by prevention of age-related degeneration and diseases.

Education:
Candidate for Doctor of Philosophy – Public Health, Epidemiology
Walden University, Minneapolis, Minnesota 55401

M.P.H. in Epidemiology; awarded January 2012
Walden University, Minneapolis, Minnesota 55401

M.S. in Applied Molecular Biology; awarded June 1992
University of Maryland Graduate School (UMAB), Baltimore, Maryland 21228

B.S. in Biochemistry; awarded June 1991
University of Maryland Baltimore County (UMBC), Baltimore, Maryland 21228

Work Experience:
Research Scientist August 2000 - Present
National Institute on Aging, Laboratory of Neurosciences, 251 Bayview Blvd., Baltimore, MD 21224

Director of Research
October 1992 – June 2000
Genox Corporation, 1414 Key Highway, Baltimore, MD 21230

Lab Manager October 1992 – June 2000
Genox Corporation, 1414 Key Highway, Baltimore, MD 21230

Visiting Research Scientist
June 1992 – October 1992
Gerontology Research Center, National Institutes of Health, 4940 Eastern Avenue
Baltimore, MD 21224

Awards:
Quality Step Increase Award, NIH 2011; for innovative biomarker development using HPLC/ tandem mass spectrometry used in several human clinical trials.

On the Spot Award, NIA 2010; for mentoring postbac pre-medical track students.

Special Act Award, NIA 2010; for developing an exercise endurance testing lab and protocol for testing transgenic mouse models of aging and age-related neurological diseases.

Quality Grade Increase Award, NIH 2005; for setting up, and managing the lab, and mentoring students.

Special Act Award, NIA 2004; for setting up and managing an Analytical Chemistry lab for the Laboratory of Neurosciences.

Outstanding Achievement Award, NIH 2004; for personal commitment in training students in the NIH Summer Research Program.

NIA Employee of the Month August 2004.

On the Spot Award NIA, 2003; for invaluable contributions to the Cellular and Molecular Neuroscience program.

On the Spot Award, NIA 2002; for setting up 8OHdG LC/MS/MS assay.

On the Spot Award, NIA 2002; for setting up an sphingolipid research program.

On the Spot Award, NIA 2001; for setting up folic acid assay & research study.

Hirotomo Ochi, Young Scholar Award from the Japan Institute for the Control of Aging, 1995.

Paul Glenn Summer Fellowship from the Paul Glenn Foundation for Medical Research, 1992.

Patents:
Title: The Use of compounds to prevent the Build-up of Sphingolipids in Cells
Filed: September 20, 2000
Inventor: Roy G. Cutler
U.S. Patent Application No. 09/648,828

Title: A Method of Assay for Detecting Modified Mitochondrial Nucleotides
Filed: March 12, 1993
Inventor: Roy G. Cutler
U.S. Patent Application No. 08/030,692

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Book Chapters:

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