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The Effects of XanthigenTM Supplementation on Body Composition, Serum Markers of the Metabolic Syndrome, and Hepaptic Enzyme Levels in an Obese Population

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THE EFFECTS OF XANTHIGEN™ SUPPLEMENTATION ON BODY COMPOSITION, SERUM MARKERS OF THE METABOLIC SYNDROME, AND HEPAPTIC ENZYME LEVELS IN AN OBESE POPULATION

By

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THE EFFECTS OF XANTHIGEN™ SUPPLEMENTATION ON BODY COMPOSITION, SERUM MARKERS OF THE METABOLIC SYNDROME, AND HEPAPTIC ENZYME LEVELS IN AN OBESE POPULATION

Xanthigen™ [100 mg brown seaweed extract (0.8 % fucoxanthin) and 100 mg pomegranate seed oil (70 % punicic acid)] has been shown to significantly reduce body fat, liver fat, and improve serum markers of liver function in obese females. Twenty-nine participants were matched for age, gender, and body fat percentage and randomized into either a Xanthigen™ group or a placebo group. For 16-weeks, participants were asked to consume a reduced calorie diet while supplementing their diet with their respective pills three times per day. Data were analyzed using multivariate ANOVA with repeated measures and presented as mean ± standard deviation. While most participants saw weight loss, body fat percentage did not significantly change, indicating that weight loss was from both fat mass and lean mass. Significant changes were seen in serum levels of alkaline phosphatase (ALP), alanine transaminase (ALT), total protein, and albumin. The changes in both the placebo and Xanthigen groups were similar, however. No significant changes were seen in other serum markers, but there was a trend for a reduction in triglycerides with a statistical trend for a greater decrease in the Xanthigen group. While both placebo and Xanthigen groups experienced weight loss and reduced liver enzyme levels, the Xanthigen supplementation did not produce significant results.

Keywords: Brown seaweed; pomegranate seed oil; obesity; lipids; liver enzymes

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Chapter 1: Problem Statement

Over the past two decades in the United States, the incidence of overweight and obesity has been a rising trend (Center for Disease Control and Prevention [CDC], 2011a). Although the dramatic increase in these numbers has begun to diminish, more than half of the states in the U.S. had a twenty-five percent or greater obesity rate as of 2009 (CDC, 2011a). Furthermore, nine states, mostly in the south, have obesity rates of greater than thirty percent (CDC, 2011a). In addition to the large number of Americans that are obese, thirty-four percent of all Americans are overweight (CDC, 2011a). The problem created by this great prevalence of overweight and obesity is that these individuals are at a greater risk for the development of other diseases. According to the National Heart, Lung, and Blood Institutes, overweight and obesity lead to an increase in the morbidity rate of diseases such as hypertension, Type II diabetes, coronary heart disease, stroke, various forms of cancer, and many other dangerous diseases (National Health Institutes, 1998; Pi-Sunyer, 2002). Along with the increasing morbidity rates, individuals with a body-mass index (BMI) greater than thirty have a 50 to 100 times greater risk of all-cause mortality, especially due to cardiovascular disease (National Health Institutes, 1998; Pi-Sunyer, 2002).

Because overweight and obesity are such common problems, many studies have attempted to determine appropriate ways to combat them. A proper diet and physical activity are two of the main factors in the cure for obesity, but often times they are not enough. Since healthy diet and regular physical activity may not be practical options for

all Americans, many nutritional supplements have entered the market that promise to promote weight loss. In recent years, a nutritional supplement called Xanthigen has been produced and testing has begun on its anti-obesity effects. Abidov and colleagues (Abidov, Ramazanov, Seifulla, & Grachev, 2010) conducted studies on obese women using Xanthigen supplementation to determine the physiological changes elicited by the supplement. This study reported that Xanthigen was well tolerated, reduced body weight, body fat, and waist circumference. Additionally, all groups had a reduction in serum triglycerides and in liver enzymes. Xanthigen has proven to be safe and effective thus far, quite possibly providing Americans with a "quick fix" to their weight problems.

Because Xanthigen is new to the market, very little testing has been completed on this supplement. Abidov and his fellow researchers (2010) used only obese women in their study, and thus present results are very exclusive to a certain population. To gain more knowledge about the Xanthigen supplement, a broader study must be done including men and women of broader age categories. In the study at hand, the effects of Xanthigen supplementation on obese men and women was assessed by examining body composition, lipid levels [triglycerides, high-density lipoproteins (HDL), low-density lipoproteins (LDL), and total cholesterol levels], liver enzymes [alanine aminotransferase (ALT), aspartate aminotransferase (AST), and γ-glutamyltransferase (GGT)], and glucose and insulin concentrations in the blood. In this study, obesity was defined as individuals with a BMI of greater than 30 kg/m^2. This particular study was a 16-week double blind experiment. It was hypothesized that with Xanthigen supplementation, body composition would improve (body fat percentage decrease), lipid levels improve (triglyceride and LDL decrease, but HDL increase), liver enzymes levels improve (decrease), and glucose

concentrations would improve (decrease). Many of the results of this study are contrary to Abidov's study (2010), thus leading to new potential studies to further elucidate the mechanism and effectiveness of Xanthigen supplementation.

Chapter 2: Literature Review

Obesity

Over the past two decades, obesity has become a major health concern worldwide. According to the CDC, adult obesity has been defined as a body-mass index (BMI) of greater than 30 kg/m^2 (CDC, 2011b). Since the 1980s, obesity has reached epidemic proportions worldwide. In the United States alone, almost one hundred million people suffer from the effects of overweight and obesity (National Health Institutes, 1998; Pi-Sunyer, 2002). As of 2005, nearly one-third of all US adults were classified as obese (Baskin, Ard, Franklin, and Allison, 2005). The CDC reports that in 2009, 33 states reported 25% or greater obesity rates (CDC, 2011a). Additionally, nine states had obesity rates of 30% or greater (CDC, 2011a). Of these nine states, Mississippi held the highest obesity rate, with 34.4 percent of all Mississippians being obese (CDC, 2011a). It is predicted that by the year 2020, greater than 40% of men and greater than 43% of women will be considered obese (Ruhm, 2007). This great prevalence of obesity poses concerns for all.

Obesity is closely linked with many other diseases and higher mortality rates than what is seen in a normal, healthy population. Excessive body weight alone is linked with hypertension, type II diabetes mellitus, coronary heart disease, gallbladder disease, osteoarthritis, sleep apnea, respiratory problems, and some types of cancer (National Health Institutes, 1998; Pi-Sunyer, 2002). As BMI increases, blood pressure increases

proportionally (Must et al., 1999). According to the National Health Institutes (1998), between thirty and forty percent of all obese people have hypertension. These increases in blood pressure are also correlated with increases for the likelihood of stroke and coronary heart disease (National Health Institutes, 1998; Pi-Sunyer, 2002). Along with hypertension, obesity is also related to dyslipidemia. As is the case with blood pressure, serum lipid levels rise with weight gains. Obese persons see rises in total cholesterol, triglycerides, and LDL (National Health Institutes, 1998; Pi-Sunyer, 2002). Increases in obesity are often also linked to an increased death rate (Flegal, Graubard, Williamson, and Gail, 2005).

Another issue raised by the prevalence of obesity is the cost to care for those who are obese. Previously it was estimated that on an annual basis, obese individuals' healthcare costs about $395 more than normal adults (Finklestein, Fiebelkom, and Wang, 2003). This was an increase of about 36%. More recent numbers indicate that obesity costs approximately $732, or 37.4% more than adults of a healthy body weight. About 5.3% of all medical spending is put towards obesity-related expenses. With these statistics, the monetary costs of overweight and obesity are quickly becoming rivals to the monetary costs of smoking (Finklestein et al, 2003).

Lipids and Obesity

Like many other issues, obesity is strongly linked with dyslipidemia, including hypertriglyceridemia and hyperlipidemia. According to the American Heart Association (AHA, 2011), triglycerides are "the chemical form in which most fats exist in food as well as in the body." While normal triglyceride levels are considered less than 150 mg/dl, someone with elevated triglycerides, or hypertriglyceridemia, will have over 200

mg/dl (AHA, 2011). Although there are five major families of lipoproteins, dyslipidemia primarily focuses on HDL, LDL, and total cholesterol. HDL is considered "good" cholesterol. To be protected against heart disease, one should have an HDL level of at least 60 mg/dl (AHA, 2011). A person whose HDL levels fall below 50 mg/dl (for women) or 40 mg/dl (for men), are considered to be at greater risk for heart disease (AHA, 2011). LDL is considered "bad" cholesterol. Optimal LDL levels are less than 100 mg/dl, while high LDL levels reach above 130 mg/dl (AHA, 2011). For healthy individuals, total cholesterol should be less than 200 mg/dl (AHA, 2011). Any person with total cholesterol of greater than 200 mg/dl more than doubles their risk for heart disease (AHA, 2011).

It has been shown that among the obese, hypertriglyceridemia is almost two times more likely to occur than in the non-obese (Kaplan, 1989). "Obesity is probably the metabolic stressor most frequently associated with hypertriglyceridemia…" (Yuan, Al-Shali, and Hegele, 2007). The combination of obesity and non-alcoholic fatty liver disease (NAFLD) also correlates with elevated triglycerides and low HDL levels (Yuan et al., 2007). Similarly, obesity is related to the metabolic syndrome. Features of the metabolic syndrome include elevated triglycerides and lowered HDL levels (Hardman, 1999). The connecting factor here is lipoprotein lipase (LPL), which is the enzyme responsible for breaking down triglycerides (Hardman, 1999). With a dysfunction in LPL, triglycerides are not appropriately removed, and are rather dissociated (Hardman, 1999). Parts of this dissociation, including free cholesterol, may be transferred to HDLs, thus changing their make-up (Hardman, 1999). According to Kannel and colleagues (1979), HDL seems to be the lipoprotein most strongly related to obesity (Kannel,

Gordon, and Castelli, 1979). As HDL levels have an inverse relationship with obesity, total cholesterol levels often have a positive correlation with obesity. As BMI increases, so does serum cholesterol levels (Brown et al., 2000).

Liver Enzymes and Obesity

Elevated transaminase levels are often of concern when looking at obesity. Liver enzyme levels can be elevated for multiple reasons. Although alcoholic liver disease is often a cause of elevated transaminases, NAFLD can also be a major underlying cause of this problem (Palmer, 2004a; Palmer, 2004b). Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are the known markers for hepatocellular injury (Kundle, Lazenby, Clements, and Abrams, 2005). In obese youth populations, NAFLD is considered the primary reason for elevated ALT (Burgert et al., 2006). It has been found that with NAFLD, elevated ALT is the most common liver abnormality (Kerner, et. al., 2005). Elevated ALT levels often indicate liver damage and may be predictors of type 2 diabetes mellitus (Burgert et al., 2006). Burgert and colleagues hypothesized that elevated ALT may be a cause of defective glucose and insulin metabolism, and increases in free-fatty acids and triglycerides.

NAFLD is defined as "a condition of fat accumulation in the liver in the absence of excessive alcohol consumption and any other specific causes of hepatic steatosis," (Bellentani and Marino, 2008). Although not completely understood, NAFLD is clearly linked with insulin resistance, and may often accompany obesity, as well as type 2 diabetes (Bellentani and Marino, 2008). According to publications in *Arteriosclerosis, Thrombosis, and Vascular Biology* , NAFLD affects an estimated 25% of the American

population (Kerner et al., 2005). Complications with NAFLD include a link to further, more damaging, and often fatal liver disease, as well as cardiovascular disease (Bellentani and Marino, 2008).

Fucoxanthin

Edible seaweeds are very nutritious and are often full of nutritional benefits such as fiber, nutrients, and some minerals (Yan, Chuda, Suzuki, and Nagata, 1999). Fucoxanthin is a carotenoid found in *Undaria pinnatifida* that has been researched for its anti-obesity effects. In a study done by Maeda and colleagues (2005), edible seaweed (fucoxanthin) was fed to rats and mice and this study anti-obesity effects of fucoxanthin. Obese mice given a diet supplemented with 2% fucoxanthin saw a great reduction in the weight of their white adipose tissue. Through the results of these studies, it was determined that an increase in heat production from oxidation of fats will produce less incidence of diabetes (Maeda etal, 2005).

Maeda and colleagues (2008) also found that fucoxanthin can produce effects that have yet to be found in other dietary seaweed carotenoids, including, anti-obesity and anti-diabetic effects. Through multiple studies in rodents and cell cultures, Maeda et al. (2005, 2008) found fucoxanthin to be an upregulator of uncoupling protein-1 (UCP-1) (Maeda et al, 2005; Maeda et al, 2008). This being said, upregulating UCP-1 is the alteration that produces the desired anti-obesity results. Furthermore, the presence of UCP-1allows for uncoupling of cellular respiration, which promotes the usage of fatty acids, and therefore promotes resistance to obesity (Ricquier, 2005). Fucoxanthin supplementation also led to an increase in docoshexaenoic acid (DHA) production,

which, in turn, led to an increase in the fat burning process of beta-oxidation (Maeda et al, 2008).

Pomegranate Seed Oil

Pomegranate seed oil (PSO) contains a type of conjugated linolenic acid (CLN) called punicic acid (Yamasaki et al., 2006). In this study from Yamasaki and colleagues, mice were fed different dosages of PSO to determine its immunological effects. Many changes in immunoglobin production were observed; and contrary to what might be expected, PSO supplementation resulted in increased triglycerides, but did not affect the weight of adipose tissue (Yamasaki, et. al., 2006). The conclusions of this study were that PSO supplementation modulates lipid metabolism and promotes immunoglobin production, possibly producing desirable effects (Yamasaki, et. al., 2006).

Similarly, Arao, Yotsumoto, Han, Nagao, and Yanagita (2004b) discovered other effects of CLN. Rather than investigating immunoglobins and triglycerides, Arao and colleagues examined the relationship between apolipoprotein B100 (ApoB100) secretion and human liver derived cells, known as HepG2 cells. The findings of this study indicate that: CLN has the ability to suppress apoB100 secretion in the liver through the suppression of triglyceride synthesis in HepG2 cells. In conclusion, Arao's study stated the possibility of using 9c,11t,13c-CLN as a hypolipidemic component (Arao, Yotsumoto et al., 2004b).

In a study similar to the Yamasaki study (2006), rats were used to investigate the dietary effects of CLNA on lipid metabolism (Arao, Wang et al., 2004a). In one group of rats, obesity-like effects were induced. When comparing the obese rats to another

"control" group, it was determined that triglyceride concentration in the liver was significantly lowered by a diet including CLN (Arao, Wang et al., 2004a). It was decided that this was due to the suppression of delta-9 desaturation (Arao, Wang et al., 2004a). These previously mentioned studies have shown that through many mechanisms, pomegranate seed oil may produce desirable results.

Xanthigen

Although proper diet and regular exercise are simple solutions for obesity, lack of compliance often impairs their results. In recent years, researchers have combined the anti-obesity effects of fucoxanthin and the positive effects of pomegranate seed oil to create a dietary supplement that can safely and effectively treat obesity. Through this combination, Xanthigen was born.

Abidov and his colleagues (2010) studied Xanthigen and its effects during a 16-week, double-blind study on obese, premenopausal women. To do so, the Abidov research team recruited obese, premenopausal, non-diabetic women presenting with or without NAFLD (Abidov et al., 2010). A group of 72 women with NAFLD participated, while a group of 38 with normal liver fat also participated. The NAFLD group was split into two groups of 36, one control group and one Xanthigen group. NLF participants were also split into two equal groups (n=19), with one control group and one Xanthigen group. Another group of 41 women diagnosed with NAFLD was used specifically to measure changes in resting energy expenditure (REE). REE groups were further subdivided into: a placebo group, a Xanthigen-200/0.8 mg group, a Xanthigen-400/1.6 mg group, a Xanthigen-600/2.4 mg group, a Xanthigen-1000/4 mg group, a fucoxanthin-1.6 mg group, a fucoxanthin-2.4 mg group, a fucoxanthin-4.0 mg group, a fucoxanthin-

8.0 mg group, a PSO-1500 mg group, and a PSO-2000 mg group. All food was provided to the participants throughout the duration of the study and their diets were restricted to 1800 calories. Respective supplements were to be taken three times a day 15-30 minutes before each meal. A visit was required three times per week for measures to be taken.

Total weight and body fat were measured upon beginning the trial. Dual-energy X-ray absorptiometry was used to analyze body fat content. Liver fat content was quantified using proton magnetic resonance spectroscopy. Resting energy expenditure was measured using indirect calorimetry. Blood samples were taken and analyzed for ALT, AST, GGT, c-reactive protein (CRP), and triglycerides.

In the end, it was determined that Xanthigen and both of its components were well tolerated by all participants with no adverse effects reported. In this population, a 10 pound or greater reduction in body weight was seen in groups supplemented with Xanthigen (Abidov et al, 2010). Although the placebo groups also lost weight, the Xanthigen supplemented group lost significantly more weight than did those participants who were given the placebo. Along with body weight improvements, body fat and, incidentally, liver fat percentages were also decreased. When looking at body fat and liver fat percentages, the placebo group saw no changes from the beginning of the study to the end. As might be expected due to the decrease in body weight, waist circumference also decreased in the Xanthigen supplemented group. The placebo group saw little to no changes in their waist circumference measures. Although serum triglyceride levels were reduced, they were not reduced significantly versus the control group except in the NAFLD group, where significant reduction was seen. Liver enzyme levels improved, as did C-reactive protein levels. These levels were significant compared to the placebo

groups. At certain concentrations, REE was significantly increased. Ultimately, Abidov and his colleagues (2010) determined that Xanthigen-600/2.4 was the best combination that safely produced many desired results to help combat overweight and obesity.

The study at hand sought to determine the effects of Xanthigen supplementation on an obese population. Through a 16-week, double-blind study, many changes were examined. It was expected that body composition, specifically body fat percentage, blood lipids, including triglycerides, HDL levels, LDL levels, and total cholesterol, certain liver enzymes, and glucose concentrations would all improve. Although Xanthigen supplementation was the most important factor in weight loss, participants were also placed on slightly restricted calorie diets to combat this confounding variable as much as possible. This being said, exercise still played a small role as another confounding variable that was monitored.

Chapter 3: Methodology

This study was a modified replication of Abidov and his colleagues' original study on Xanthigen supplementation (2010). The methods used in the study at hand followed closely with the previous study's methodology. After recruiting 58 participants, 31 obese, non-diabetic participants between the ages of 18 and 50 completed a 16-week double-blind study assessing the effects of Xanthigen supplementation on body composition, serum lipoprotein levels, and liver enzymes. Participation was open to males and females in the Hattiesburg area who met the following criteria: between ages 18 and 50, pre-menopausal, qualify as being obese (BMI of greater than 30 kg/m^2), must not be pregnant, must not have any known metabolic disorders, must not have taken any

thyroid, hyperlipidemic, hypoglycemic, anti-hypertensive, androgenic medications, or nutritional supplements known to influence fat metabolism within the past six month, must not have had a history of excessive alcoholic consumption. Participants were recruited from USM and the Hattiesburg area via word-of-mouth, email announcements, and online postings. All data collection was completed by the lead investigator or research assistants in the USM Laboratory of Applied Physiology.

After recruiting participants, they were separated into two experimental groups: a placebo group and a Xanthigen supplement group. Participants were grouped according to gender, age, and body fat percentage before being placed into either the placebo or Xanthigen groups using a randomized, double-blind procedure. To uphold the integrity of the double-blind aspect of this study, placebo capsules were packaged identically to the Xanthigen capsule, but were instead filled with 200 mg extra virgin olive oil. Xanthigen capsules were comprised of a combination of 100 mg of pomegranate seed oil and 100 mg of brown seaweed extract with 0.8 mg of fucoxanthin.

Participants were first familiarized with the purpose, design, risks, and benefits associated with the study. After any questions were answered by the lead investigator or research assistants, an Informed Consent form and medical history questionnaire were completed by the participants. At the initial testing session (T1) research assistants assessed baseline body mass, height, blood pressure, waist circumference, total body water (via bioelectrical impedance analysis), REE, and body composition (via Dual Energy X-ray Absorptiometry). The first samples of blood were also drawn by the lead investigator at this time. Blood was drawn the morning after an overnight fast using standard phlebotomy techniques. Serum samples were left standing at room temperature

for 10 minutes before being centrifuged. At the conclusion of T1, participants were randomly divided into placebo or supplement groups according to gender, age and body fat percentage.

Upon the conclusion of T1, participants were given dietary counseling concerning supplementation and the required standardized diet by a dietician. Two weeks of supplements were handed out at this time with directions instructing the participant to take their particular pills three times per day at 15-30 minutes prior to mealtime. Participants were placed on standardized diets throughout the 16-week study. A diet based on the Dietary Exchange Program was used to allow participants some freedom in their dietary choices, but still maintain a constant caloric intake. Caloric intake was determined according to each participant's resting energy expenditure. Because of this freedom, participants were required to turn in four-day food logs every two weeks. Additional dietary counseling was provided if participants were found to be struggling with compliance or have additional questions concerning their diets. To encourage participants' continued participation in the study, participants were only given two weeks' worth of supplement upon each visit.

Testing session 2-5 (T2-5) occurred during weeks four through 16. Data collecting sessions occurred every four weeks throughout the study, although participants were still required to pick-up their next set of supplements and drop off food journals every two weeks. During testing sessions, participants reported to the Laboratory of Applied Physiology to have body mass, blood pressure, waist circumference, total body water, body composition, and REE assessed by research assistants. Another blood sample was also taken by the lead investigator upon each of these visits. Serum levels of

triglycerides, HDL, LDL, total cholesterol, glucose, AST, and ALT were assessed by medical technologists at the USM Health Services Clinic.

During each testing session, multiple tests were conducted. Each of these tests had a very specific procedure. First, the anthropometric measurements such as total body mass and waist circumference were performed using a standard digital scale (Ohaus Champ II) and standard anthropometric measurements, respectively (Pollock, Jackson, 1984). These measures were collected and recorded by research assistants. Total body water was measured using bioelectrical impedance analysis (BIA; Tanita TBF-310GS). Percent body fat, fat mass, and fat-free mass were determined using dual energy X-ray absorptiometry (DEXA; GE Lunar Prodigy), and conducted by research assistants. Resting energy expenditure (REE) was assessed using the Vyasis Vmax Encore metabolic cart. Through the use of a transparent canopy connected to a gas mixing chamber, metabolic measurements were taken. To obtain these measurements, participants remained in a supine position for approximately 25 minutes without falling asleep. During these 25 minutes, respired gases were analyzed and oxygen uptake and carbon dioxide production was measured. These measurements were then used to calculate the participants' REE and respiratory quotient (RQ). To assure that the combination of diet, exercise, and supplementation were safe, hemodynamic measures were taken and recorded during each session. Both blood pressure and heart rate were taken twice while resting with an automated blood pressure monitor (Omron HEM 907XL) and the average of the two results recorded by the research assistant.

As previously mentioned, participants were required to keep four-day food journals every two weeks. Dietary records were collected and entered into dietary

assessment software by research assistants. Finally, records were analyzed using the Food Processor dietary assessment software program (ESHA Research, Inc., Salem, OR).

Blood samples taken by the lead investigator during T1-T5 were drawn using standard sterile venipuncture technique. Samples were taken from the antecubital vein using standard phlebotomy procedures. Samples were taken only by the primary researcher or phlebotomists at the USM Health Services Center. Gloves and other safety equipment were always used when taking and handling blood samples. To obtain samples, participants were seated in a chair and had their arms cleaned with a sterile alcohol wipe and sterile gauze. A standard rubber tourniquet was placed on the upper arm and was tightened enough to slightly indent the arm, but not cause discomfort to the participant. After palpating the vein, a twenty-two gauge sterile needle attached to a vacutainer holder was inserted into the vein using standard procedure. Two serum separator tubes were subsequently inserted into the vacutainer holder. After collecting both samples, the needle and holder were removed. Needles were disposed of as hazardous waste in a plastic sharps container. The site of needle entry was promptly cleaned and pressure was applied using gauze to ensure proper clotting. All blood collection tubes were labeled and placed in their respective racks. Properly trained and clothed technicians, including research assistants, centrifuged the serum and plasma samples, transferred the samples into storage containers, and stored them at -80^{o}C for later analysis. The USM Health Services Clinic ran a standard chemistry panel.

To determine the side effects of Xanthigen supplementation, participants were given surveys to complete during each testing session. Questionnaires were confidentially administered. Participants were asked to report whether their placebo or Xanthigen

supplement was tolerated, whether supplementation protocol was followed, and whether any medical problems or symptoms were encountered throughout the study.

Finally, the data were analyzed by the lead investigator using SPSS for Windows Version 17 software. Multivariate, repeated measures analyses of variance (MANOVAs) were used to assess BMI, body composition, REE, lipid markers, and liver function markers in a time versus supplement fashion. For further breakdown, separate, univariate ANOVAs were performed to assess the main effects of the experiment. To be considered significantly different, the probability of Type I error must be 0.05 or less. If a significant difference was found between variables, Tukey's honestly significant differences post-hoc procedures was performed.

Chapter 4: Results

Demographic Data

Demographic data for Xanthigen and placebo groups are presented in Table 1 below. Upon baseline measures, there were no significant differences in age, body mass, BMI, or body fat percentage. This indicates that the matching and randomization based on these measures was successful.

Table 1: Demographic data collected at the beginning of the 16-week intervention[†]

Variables	Xanthigen	Placebo
Age (years)	29 ± 8	27 ± 8
Body mass (kg)	102.62 ± 12.95	105.16 ± 14.89
BMI (kg/m^2)	35.71 ± 3.72	37.24 ± 6.64
Body fat percentage	42.6 ± 8.7	44.4 ± 7.5

[†]Data presented as mean ± standard deviation.

Body Composition

Table 2 summarizes the changes seen in body composition, measured by DEXA, after the 16-week protocol was completed. Multivariate ANOVA indicated no significant main effects for time ($p = 0.396$), between group ($p = 0.858$), or group*time interaction effect ($p = 0.897$). Although most participants successfully lost weight, body fat percentage did not decrease in a similar fashion. Because body fat percentage did not decrease as weight was lost, it was assumed that weight loss was due to a combination of the loss of fat mass as well as lean mass. These findings are not consistent with the findings of Abidov and colleagues.

Table 2: Body composition changes after the 16 week intervention[†]

	Xanthigen			Placebo		
Variables	**Week 0**	**Week 16**	**16 wk Change**	**Week 0**	**Week 16**	**16 wk Change**
Body mass (kg)	41.74 ± 9.81	40.71 ± 10.42	-1.025 (-2.5%)	45.19 ± 11.78	43.43 ± 13.95	-1.765 (-3.9%) *
Bone mineral density (g/cm^2)	1.292 ± 0.116	1.298 ± 0.115	0.006 (0.5%)	1.318 ± 0.088	1.323 ± 0.092	0.005 (0.4%)
Body fat percentage	42.6 ± 8.7	42.2 ± 8.7	-0.4 (-0.9%)	44.4 ± 7.5	43.7 ± 8.7	-0.7 (-1.6%)
Body fat mass (kg)	41.738 ± 9.813	40.713 ± 10.420	-1.025 (-2.5%)	45.193 ± 11.782	43.428 ± 13.948	-1.765 (-3.9%)
Lean mass (kg)	56.522 ± 12.023	55.870 ± 11.326	-0.652 (-1.2%)	55.684 ± 8.554	54.370 ± 8.434	-1.314 (-2.4%)

[†]Data presented as mean ± standard deviation.

*Significant change over time ($p < 0.05$).

Serum Lipids

Table 3 summarizes the observed changes in serum lipids over 16 weeks. Multivariate ANOVA revealed a significant main effect for time ($p = 0.043$), but no between group ($p = 0.816$) or group*time interaction effect ($p = 0.201$). After the completion of a univariate analysis, it was determined that no significant changes were found. This being said, there was a trend for decreased triglycerides (132 ±90 mg/dL to

101 ± 58 in Xanthigen versus 138 ± 86 mg/dL to 133 ± 79 in placebo). HDL levels did increase by 9.5% (from 42±9 to 46±8) in the Xanthigen group and by 6.8% in the placebo group (44±12 mg/dL to 47±12 mg/dL). On the other hand, LDL levels decreased 8.1% (111±29 mg/dL to 102±25 mg/dL) in the placebo group, but actually increased 8.3% (109±37 mg/dL to 118±36 mg/dL) in the Xanthigen group. Total cholesterol also decreased in the placebo group (183±30 mg/dL to 175±30 mg/dL) by 4.4%, but increased in the Xanthigen group (177±34 mg/dL to 178±40 mg/dL) by 0.6%.

Table 3*:* Changes in serum lipids after the 16 week intervention†

	Xanthigen			Placebo			
Variables	**Week 0**	**Week 16**	**16 wk Change**	**Week 0**	**Week 16**	**16 wk Change**	
Triglycerides (mg/dL)	132 ± 90	101 ± 58	-31 (-23.5%)	138 ± 86	133 ± 79	-5 (-3.6%)	□Ξ
Total Cholesterol (mg/dL)	177 ± 34	178 ± 40	1 (0.6%)	183 ± 30	175 ± 30	-8 (-4.4%)	
HDL (mg/dL)	42 ± 9	46 ± 8	4 (9.5%)	44 ± 12	47 ± 12	3 (6.8%)	
LDL (mg/dL)	109 ± 37	118 ± 36	9 (8.3%)	111 ± 29	102 ± 25	-9 (-8.1%)	
Glucose (mg/dL)	99 ± 9	98 ± 10	-1 (-1.0%)	99 ± 12	95 ± 9	-4 (-4.0%)	□

†Data presented as mean ± standard deviation.

Statistical trend for a change over time.

Statistical trend for an interaction.

Serum Markers of the Metabolic Syndrome and Hepatic Enzyme Levels

Table 4 summarizes the observed changes in markers of liver and kidney function. Multivariate ANOVA revealed a significant main effect for time ($p < 0.001$), but no between group ($p = 0.327$) or group*time interaction effect ($p = 0.136$). Univariate analysis revealed a significant decrease in ALP ($p = 0.001$), ALT ($p = 0.021$) and a trend for a decrease in AST ($p = 0.062$). Both groups showed a small decrease in ALP levels over 16-weeks, although the Xanthigen group showed larger decreases (-7.8% versus -5.5%, respectively). This indicated a significant change over time. AST levels decreased

in both groups as well. The Xanthigen group decreased from 26 ± 16 U/L to 22 ± 13 U/L (-15.4% change), while the placebo group decreased from 19 ± 5 U/L to 16 ± 7 U/L (-15.8% change). While decreases were seen in both, these decreases were not considered significant. Like AST, ALT levels significantly decreased over 16 weeks. Xanthigen group decreased from 31 ± 27 U/L to 20 ± 8 U/L, indicating a -35.5% change. The placebo group decreased from 21 ± 7 U/L to 18 ± 12 U/L, indicating a -14.3% change.

Multivariate ANOVA revealed no significant main effects for time ($p = 0.066$), between group ($p = 0.249$), or group*time interaction effect ($p = 0.966$) for total bilirubin, total protein, or albumin. Baseline levels of creatinine and blood urea nitrogen (BUN) were significantly higher in the Xanthigen group than placebo at baseline, so these variables were analyzed as delta changes from baseline. This analysis revealed a significant main effect for time ($p = 0.001$), but no between group ($p = 0.878$) or group*time interaction effect ($p = 0.881$). In the Xanthigen group, there was no change in total bilirubin content (0.7 ± 0.4 to 0.7 ± 0.4 mg/dL), but the placebo group saw a -14.3% change (0.7 ± 0.2 mg/dL to 0.6 ± 0.3 mg/dL). Both groups saw a decrease in creatinine – -10.0% in the Xanthigen group (1.0 ± 0.1 mg/dL to 0.9 ± 0.2 mg/dL) and -11.1% in the placebo group (0.9 ± 0.1 mg/dL to 0.8 ± 0.2 mg/dL). Blood urea nitrogen (BUN) did not change in the Xanthigen group (14 ± 4 mg/dL to 14 ± 3 mg/dL), but increased in the placebo group (11 ± 2 mg/dL to 12 ± 3 mg/dL). Total protein decreased in both groups as well, -4.2% in Xanthigen (7.1 ± 0.4 g/dL to 6.8 ±0.4 g/dL) and -2.9% in the placebo group (7.0 ± 0.3 g/dL to 6.8 ± 0.3 g/dL). While albumin did not change in the Xanthigen group (4.5 ± 0.4 g/dL to 4.5 ± 0.4 g/dL), albumin levels increased in the placebo group by 2.3% (4.3 ± 0.4 g/dL to 4.4 ± 0.3 g/dL).

Table 4: Changes in serum markers of liver and kidney function after 16 weeks

Variables	Xanthigen			Placebo			
	Week 0	Week 16	16 wk Change	Week 0	Week 16	16 wk Change	
ALP (U/L)	64 ± 18	59 ± 16	-5 (-7.8%)	73 ± 25	69 ± 25	-4 (-5.5%)	*
AST (U/L)	26 ± 16	22 ± 13	-4 (-15.4%)	19 ± 5	16 ± 7	-3 (-15.8%)	□
ALT (U/L)	31 ± 27	20 ± 8	-11 (-35.5%)	21 ± 7	18 ± 12	-3 (-14.3%)	*
Total bilirubin (mg/dL)	0.7 ± 0.4	0.7 ± 0.4	0.0 (0.0%)	0.7 ± 0.2	0.6 ± 0.3	-0.1 (-14.3%)	
Creatinine (mg/dL)	1.0 ± 0.1	0.9 ± 0.2	-0.1 (-10.0%)	0.9 ± 0.1	0.8 ± 0.2	-0.1 (-11.1%)	‡*
Blood urea nitrogen (mg/dL)	14 ± 4	14 ± 3	0 (0.0%)	11 ± 2	12 ± 3	1 (9.1%)	‡
Total protein (g/dL)	7.1 ± 0.4	6.8 ± 0.4	-0.3 (-4.2%)	7.0 ± 0.3	6.8 ± 0.3	-0.2 (-2.9%)	*
Albumin (g/dL)	4.5 ± 0.4	4.5 ± 0.4	0.0 (0.0%)	4.3 ± 0.4	4.4 ± 0.3	0.1 (2.3%)	

†Data presented as mean ± standard deviation.

*Significant change over time ($p < 0.05$).

□Statistical trend for a change over time.

‡Significant difference at baseline ($p < 0.05$). Data analyzed as changes over time.

Chapter 5: Discussion

When comparing the current study's results to results from the Abidov study (2010), there are some obvious differences. The Abidov study saw significant changes in many areas that the current study did not. These differences are the likely result of the small sample size of the current study and the large standard deviations presented with these variables. Other variations in study design that could have attributed to these different results involve the inclusion of obese men in the participant pool and not using a standardized 1800 kcal diet, but instead individualizing participants' caloric intake based on their specific REE.

One of the most obvious differences between the current study and the Abidov study (2010) is the change in body mass. Specifically, the Abidov study participants saw a significant reduction in body mass after completing their 16-week intervention. In the

current study, as a whole, participants significantly reduced their body mass, but this reduction was not significantly greater in the Xanthigen group as it was with the Abidov study. It is probable that this inconsistency is due to the small sample size of the current study and the different diet criteria. Abidov strictly controlled the caloric intake of the participants to 1800 kcals; since the current study included both male and female participants, their diet was individualized based on their specific REE. Moreover, these participants were only provided with dietary guidelines and biweekly monitoring, while the previous study provided them with every meal.

Similar to body composition, there were inconsistencies between the Abidov study (2010) and current study when examining changes in liver enzymes. Abidov and colleagues saw significant reductions in ALT and AST with Xanthigen, but did not investigate ALP. The current study showed significant declines in ALT and ALP overall, but not as a result of Xanthigen. The current study also did not demonstrate a significant change regarding AST either. Again, these differences are apparently due to the small sample size of the current study and the large standard deviations.

The Abidov et al. (2010) study did not report cholesterol levels, but other lipids were reported. Much like the Abidov study, triglyceride levels in the current study decreased. This being said, the triglyceride levels in the current study dropped much more than levels in the Abidov study. It is common to see HDL levels increase, while LDL levels and triglycerides decrease with weight loss. It is probable that weight loss produced the lipid improvements, rather than Xanthigen supplementation.

While the Abidov study did not take into account kidney function markers, the current study did. Although there were no significant changes between groups, there were significant changes from baseline levels. The most important information gathered from these measures was that these markers remained within safe and normal limits and were unaffected by the Xanthigen supplementation.

Conclusion

Overall, Xanthigen supplementation did not produce the desired effects. Although the Xanthigen group did see weight loss and slight improvements in serum markers, the placebo group saw many of the same results, indicating that it was not necessarily the Xanthigen supplementation that created the results seen. While this study did cover more bases than previous studies regarding this supplement, there are some improvements that could have been made. One key aspect for improvement would be to more carefully monitor the diets of participants. Another way to improve the study would be to expand the sample size of the study. Future studies on Xanthigen supplementation may consider these options to have a more comprehensive study.

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