THE EFFECTS OF A STRUCTURED LIFESTYLE INTERVENTION PROGRAM IN CONJUNCTION WITH DIETARY SUPPLEMENTATION ON WEIGHT LOSS AND RISK FACTORS FOR THE METABOLIC SYNDROME

by

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ABSTRACT

The objective of this study was to determine the effects of a structured weight loss program that included hypocaloric diet, exercise and dietary supplementation, on weight loss, metabolic syndrome risk factors and antioxidant levels in healthy overweight and obese females.

Thirty-seven healthy overweight and obese women (BMI 29.5 ± 2.3 kg/m^2, 41.1 ± 7.1 yrs) participated in this study. The subjects were randomized into one of two groups: an exercise, hypocaloric diet and antioxidant supplement (LifePak®; LSANT group, n=20) or an exercise, hypocaloric diet and appetite suppression supplement (HTP Complex® and TēGreen®; LSAS group, n=17).

A significant weight loss occurred in both groups after 12 weeks (LSANT: -2.8 ± 2.8 kg and LSAS: -4.3 ± 2.7 kg, $p<0.001$). Body fat mass, percent body fat, and waist circumference significantly improved in both groups ($p<0.05$). No significant difference was found between the groups for weight loss ($p>0.05$). However, a significant difference was found between the groups for body fat mass (LSANT: -1.8 ± 2.6 kg; LSAS: -3.4 ± 2.4 kg, $p \leq 0.05$). Glucose, insulin and insulin resistance (HOMA-IR) were significantly decreased in the LSAS group (glucose: -5.0 ± 6.8 mg/dl, p=0.008; insulin: -2.6 ± 3.8 uIU/dl, p=0.013; and HOMA-IR: -0.7 ± 1.0, p=0.012) but not in the LSANT group ($p>0.05$). There were no significant differences ($p>0.05$) observed within or between the groups for cholesterol, triglycerides or LDL-c. HDL-c decreased significantly in the LSANT group (-2.9 ± 5.3 mg/dl, p=0.024) but not in the LSAS group ($p>0.05$). Skin carotenoid scores (SCS) increased significantly within the LSANT group (LSANT: 10950 ± 8395 SCS, $p<0.001$) but not the LSAS group ($p>0.05$).

Lifestyle intervention that involves a structured hypocaloric diet and increased physical activity results in weight loss and improvements in body composition. However, supplementation with an appetite suppressant (HTP Complex®) did not enhance weight loss beyond what was achieved with a structured lifestyle intervention. Antioxidant supplementation may be of benefit during a weight loss program that incorporates physical activity and a low energy diet.

This effort is dedicated to my parents John and Dolores Zukley for helping me become the person I am today. I would also like to dedicate this to my husband Robert Lowe for his love, support, and understanding.

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LIST OF ABBREVIATIONS/ACRONYMS

ACSM	American College of Sports Medicine
BMI	Body Mass Index
CV	Cardiovascular
CVD	Cardiovascular Disease
DPP	Diabetes Prevention Program
DXA	Dual Energy X-Ray Absorptiometry
FDPS	Finnish Diabetes Prevention Study
FRAS	Free Radical Analytical System
ECG	Electrocardiogram
EGCG	Epigallocatechin Gallate
HDL-c	High Density Lipoprotein Cholesterol
HOMA	Homeostasis Model Assessment
Hs-CRP	High Sensitivity C-Reactive Protein
5-HTP	5-Hydroxytryptophan
KCAL	Kilocalorie
KG	Kilogram
LDL-c	Low Density Lipoprotein Cholesterol
MS	Metabolic Syndorme
NDS-R	Nutrient Data System for Research
NHANES III	Third National Health and Nutrition Examination Survey
RMR	Resting Metabolic Rate

RPE	Rating of Perceived Exertion
SCS	Skin Carotenoid Score
SPSS	Statistical Package for the Social Sciences
VO_{2max}	Maximal Oxygen Consumption

INTRODUCTION

The prevalence and incidence of overweight and obesity continues to rise. Data from NHANES III 2003-2004 indicate that over 97 million Americans or an estimated 66% of adults nineteen years and older have a body mass index (BMI) greater than 25 kg/m^2 (Ogden et al., 2006). Children are showing similar trends with the incidence of childhood obesity having tripled (5% to 15%) over the past 30 years (Hedley et al., 2004). This trend in childhood obesity rates, coupled with the adult obesity epidemic, will undoubtedly have enormous impact on future health care economics and practices. Furthermore, life expectancy is anticipated to be lower in young obese individuals and mortality rates higher in adults with higher BMI when compared to their counterparts with normal BMI (Fontaine, Redden, Wang, Westfall, & Allison, 2003; Olshansky et al., 2005).

It is well accepted that obesity and its co-morbidities present an enormous public health challenge. Prospective cohort studies as well as national surveys show that obese are at increased risk for hypertension, stroke, diabetes, cardiovascular disease, arthritis, some forms of cancer, and disability. ("Clinical Guidelines on the Identification, Evaluation, and Treatment of Overweight and Obesity in Adults--The Evidence Report. National Institutes of Health," 1998; Mokdad et al., 2003; Must et al., 1999). In addition, obese individuals are more likely to exhibit a cluster of risk factors commonly known as the metabolic syndrome. These risk factors include insulin resistance, increased levels of central adiposity, low levels of high density lipoprotein cholesterol, elevated triglycerides, impaired fasting glucose, and hypertension. The clustering of these risk factors has been shown to enhance the likelihood of developing cardiovascular disease

(CVD) (Isomaa et al., 2001; Lakka et al., 2002; Wilson, Kannel, Silbershatz, & D'Agostino, 1999).

Obesity and obesity related metabolic and CV abnormalities have been linked to, and may be partially the result of high oxidative stress levels (Bartsch & Nair, 2006; Kaneto et al., 2007; Vaziri, 2004; R. Wolfram, Oguogho, Palumbo, & Sinzinger, 2005). Oxidative stress, the result of chronically elevated levels of highly reactive molecular species (chiefly oxygen and nitrogen) has been shown to cause cellular injury and tissue damage, especially in the presence of low cellular antioxidant levels. Of particular importance, BMI and fat accumulation have been shown to correlate with markers of systemic oxidative stress (Furukawa et al., 2004; Keaney et al., 2003). Several physiological mechanisms have been proposed that link oxidative stress with adiposity. In the obese, increased lipid peroxidation, protein carbonylation, and oxidative damage to amino acids results in increased free radical production and oxidative stress (Dandona et al., 2001). Furthermore, increases in adipose tissue-specific oxidative stress may result in increases in the oxidative catabolism of lipid soluble nutrients. Dandona et al., found that many of the factors associated with increased oxidative stress in obese subjects fell significantly (>50%) with dietary restriction and weight loss. These data suggest that reducing oxidative stress through weight loss, caloric restriction and consuming diets rich in antioxidants may potentially further reduce the morbidity and mortality associated with obesity.

Several studies have shown that weight loss can be an effective means to reduce CV and metabolic abnormalities associated with excess adiposity (Yamaoka & Tango, 2005). For example, results from the U.S. Diabetes Prevention Program (DPP) showed that a diet and exercise program resulting in a 5-7% weight loss reduced diabetes by 58% (Knowler et al., 2002). The Finnish Diabetes Prevention Study (FDPS) found that diet and exercise in

overweight men and women with impaired glucose tolerance resulted in an average weight loss of 7 pounds or less than 5% of body weight after 4 years (Lindstrom et al., 2006). However, the risk for diabetes was reduced by 58% which was directly associated with changes in lifestyle. Reducing adiposity has been shown to decrease the risk for diabetes and cardiovascular disease in addition to improving blood pressure, triglyceride, total cholesterol, LDL-c, HDL-c, and glucose levels (Dansinger, Gleason, Griffith, Selker, & Schaefer, 2005; Goldstein, 1992; Meckling, O'Sullivan, & Saari, 2004; Obarzanek et al., 2001; Pereira, Swain, Goldfine, Rifai, & Ludwig, 2004). Besides lifestyle, therapeutic approaches commonly used for weight loss include pharmacotherapy, surgery, behavioral therapy, and other interventions (acupuncture, herbal remedies, supplements, and hypnosis). In a select population, pharmacotherapy and surgery can be an effective means to weight loss. Improved surgical techniques (bariatric surgery) and the development of new long term drug therapies (sibutramine and orlistat) have made these options more readily available. However, side effects and cost continue to be significant barriers to their widespread use. Although significant advances have been made towards understanding the pathophysiology of obesity, successful long term therapeutic strategies to reduce or prevent the epidemic have proven to be more challenging.

It is well accepted that lifestyle intervention is a suitable therapeutic choice for reducing adiposity. Weight loss due to caloric restriction or regular exercise alone is generally not maintained. A comprehensive program that combines these approaches results in weight loss that is maintained over time, in addition to reducing visceral fat and improving cardiorespiratory fitness. Furthermore, increasing energy expenditure through regular physical activity and monitoring caloric intake provides for maintenance of the weight loss. Numerous studies have shown that lifestyle modification may be an effective strategy for weight loss ("Clinical

Guidelines on the Identification, Evaluation, and Treatment of Overweight and Obesity in Adults--The Evidence Report. National Institutes of Health," 1998). As noted previously, the Finnish DPS and the U.S. DPP showed that lifestyle intervention (diet and exercise) was more effective than other treatment modalities in reducing the risk of diabetes. Improvements in blood pressure, high sensitive C-reactive protein, insulin sensitivity and endothelial function have also been demonstrated (Aronson et al., 2004; Garrow & Summerbell, 1995; Hambrecht et al., 2000; Janssen, Fortier, Hudson, & Ross, 2002; Okita et al., 2004; Selvin, Paynter, & Erlinger, 2007). Furthermore, many of the type II diabetes and cardiovascular disease cases due to excess adiposity may be prevented or reversed with simple lifestyle changes such as adopting a healthy low calorie balanced diet and increasing physical activity (Tuomilehto et al., 2001; Volek, Vanheest, & Forsythe, 2005).

Over the past decades eating habits and food choices have changed dramatically. Significant changes in portion size and the type of foods consumed (energy dense versus nutrient dense) have occurred. Lean individuals tend to consume nutrient dense diets that include foods such as fruits and vegetables that provide substantial amounts of vitamins, minerals and phytochemicals (Rolls, Ello-Martin, & Tohill, 2004). Energy dense diets on the other hand, lack essential nutrients necessary for good health and are typically low in fiber and high in fat and sugar. Several studies have found an association between energy dense diets and obesity (Howarth, Murphy, Wilkens, Hankin, & Kolonel, 2006; Ledikwe et al., 2006). The recent popularization of many hypocaloric diets that offer very low or very high amounts of protein, carbohydrates, or fat tend to be poor in micronutrient content and are often nutritionally unbalanced. However, even well planned hypocaloric diets often fail to meet recommended daily allowances for many nutrients. As the intake of macronutrients fall above or below the

Acceptable Macronutrient Distribution Range, the risk for chronic diseases increases (Kant, 2000). Therefore a diet containing a variety of foods is considered the best approach to ensure sufficient intake of all nutrients.

Fruits and vegetables contain thousands of biologically active phytochemicals and antioxidants that interact in a number of ways to prevent disease and promote health. Antioxidants work synergistically to sustain elaborate defense systems to prevent or lessen the damage caused by oxidative stress. Furthermore, antioxidants have been shown to enhance immune function, increase longevity and prevent many chronic disease states (Fairfield & Fletcher, 2002; Tapiero, Townsend, & Tew, 2004).

Several studies have found an inverse relationship between carotenoid levels (diet and/or serum) and BMI (Andersen et al., 2006; Brady, Mares-Perlman, Bowen, & Stacewicz-Sapuntzakis, 1996; Moor de Burgos, Wartanowicz, & Ziemlanski, 1992). The decreased consumption of dietary antioxidants, in addition to alterations in absorption, distribution, metabolism and/or excretion may be responsible for the lower levels of antioxidants found in individuals with higher BMI. Furthermore, because many carotenoids are fat soluble (increased storage in adipose tissue) and adipose tissue generates higher levels of oxidative stress, the circulating (serum) levels of antioxidants may be lower in overweight and obese individuals. Results from our lab and by others found a strong inverse relationship to exist between indices of adiposity and antioxidant levels (Fiutem J, 2004; Kimmons, Blanck, Tohill, Zhang, & Khan, 2006).

The measurement of antioxidants in serum, tissue and urine is cost and time prohibitive when applying conventional methods. New technology however, has allowed for the measurement of antioxidants to be quick, affordable and noninvasive. Carotenoids, which are fat

soluble antioxidants, can now be measured in the stratum corneum layer of the skin using Raman Spectroscopy (Ermakov, Sharifzadeh, Ermakova, & Gellermann, 2005). Raman Spectroscopy uses a powerful laser that detects characteristic vibrations/rotational energy levels of a molecule and has been validated and shown to be as effective as blood and tissue sampling (Ermakov et al., 2005; Rerksuppaphol & Rerksuppaphol, 2006; Stahl et al., 1998). Recent work in our lab using Raman Spectroscopy demonstrated that overweight and obese individuals on a well designed program of mild caloric restriction and exercise significantly reduced skin catotenoid scores and presumably overall antioxidant status despite the fact that dietary carotenoid consumption did not change (Zukley L, 2004). Therefore, dietary supplementation may be necessary in order to maintain acceptable levels of micronutrients, especially during times of caloric restriction.

Food intake (eating) is determined by complex interactions between biological mechanisms of appetite control and responses to challenges from the physical environment (Figure 1).

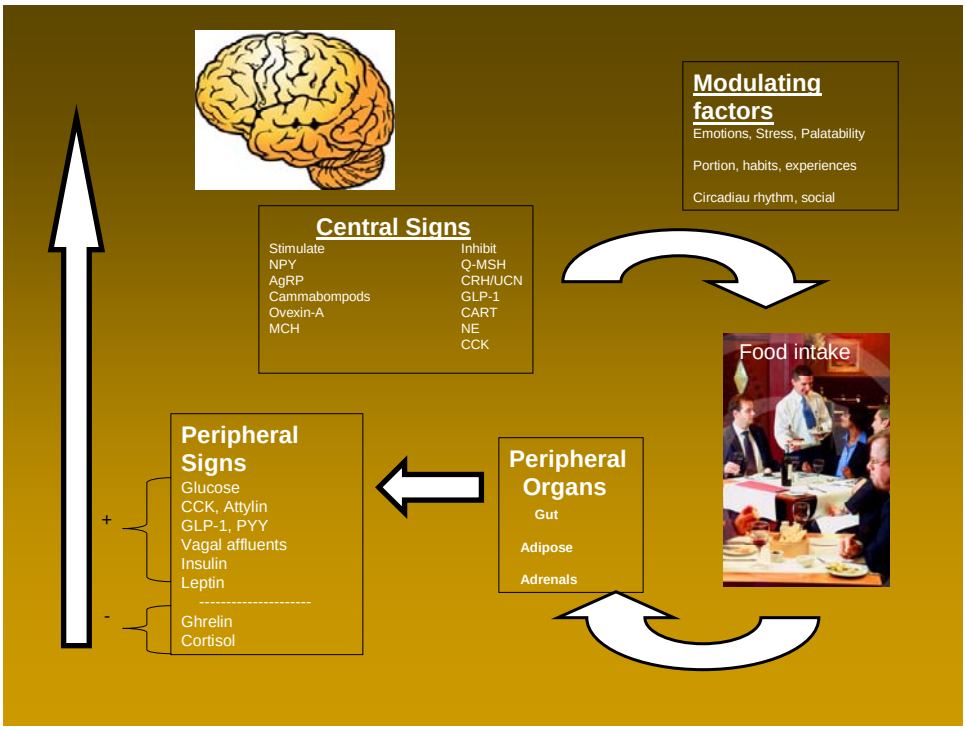


Figure 1. Factors Regulating Appetite, Energy and Metabolism

A number of hormones participate in appetite control and are currently under investigation (leptin and ghrelin). Serotonin is a neurotransmitter that controls appetite. Taken orally, serotonin cannot pass the blood brain barrier but relies on the amino acid tryptophan and its metabolite 5-hydroxytryptophan (5-HTP). Overweight and obese individuals have been found to have lower blood levels of serotonin (Ceci et al., 1989). In studies involving obese individuals taking daily dietary supplements containing 5-HTP, significant weight loss was observed (Cangiano et al., 1992; Cangiano et al., 1998; Ceci et al., 1989). Recent research has also investigated the role of plant ingredients in weight control. Currently under investigation is green tea. Catechins found in green tea may activate thermogenesis and/or fat oxidation through

the sympathetic nervous system generating favorable changes in body composition (Dulloo et al., 1999; Dulloo, Seydoux, Girardier, Chantre, & Vandermander, 2000).

Furthermore, green tea contains polyphenols which have strong antioxidant effects. Numerous studies have reported significant increases in plasma antioxidant capacity and reductions in the biomarkers for oxidative stress after one to four weeks of green tea consumption (Higdon & Frei, 2003; Rietveld & Wiseman, 2003). Research suggests that adding green tea to a balanced controlled diet may improve overall antioxidant status and protect against oxidative damage. Therefore, combining a weight loss program involving hypocaloric diet and exercise with a dietary supplement may help individuals maintain or improve their antioxidant status in addition to promoting weight loss.

Given the health promoting effects of weight loss and the importance of antioxidants in maintaining health, the purpose of this study was three-fold: 1) To determine the effect of a lifestyle modification program (diet and exercise) on weight and risk factors for the metabolic syndrome and CVD; 2) To determine the effect of a dietary appetite control supplement on weight; and 3) To determine the effect of a dietary antioxidant supplement and weight loss on skin antioxidant levels.

METHODS

Study Design

This study was a single-site, randomized, controlled 12-week study. Subjects were randomized into one of two groups: an exercise, hypocaloric diet and antioxidant supplement (LifePak®; LSANT group) or an exercise, hypocaloric diet and appetite suppression supplement (HTP Complex® and TēGreen®; LSAS group). The protocol was approved by the University of Central Florida Institutional Review Board, Orlando, Florida and written informed consent was obtained on all subjects.

Subjects

One hundred and twenty-three healthy, sedentary, overweight males and females with a BMI of 27-35 kg/m^2 and 25 to 50 years old were enrolled. Characteristics of all subjects at baseline are shown in Table 1.

Table 1.

Baseline Characteristics of All Subjects Enrolled

Characteristics	All (N=123)
Age (yr)	40.3 ± 8.2
Height (cm)	163.6 ± 7.4
Weight (kg)	80.7 ± 11.0
BMI (kg/m^2)	30.92 ± 2.5
Skin carotenoid (SCS)	19246 ± 6184
Oxidative stress (U.Carr)	326.4 ± 82.7

Note. BMI, body mass index. SCS, skin carotenoid score

The subjects (N=123) enrolled in the study were part of a major interventional study examining the effects of a structured weight loss program on risk factors for metabolic syndrome and oxidative stress. A sub-cohort (n=37) was used in the present investigation. Based on the premise that gender, cigarette smoking, and birth control are known to adversely affect oxidative stress and antioxidant levels, female subjects that did not smoke and were not on birth control were included (Alberg, 2002; Berg, Kohlmeier, & Brenner, 1997). Therefore, analyses were conducted and reported on 37 Caucasian females that met these criteria and for which complete data were available at week 12. Flow of subjects based on screening and selection criteria is depicted in Figure 2.

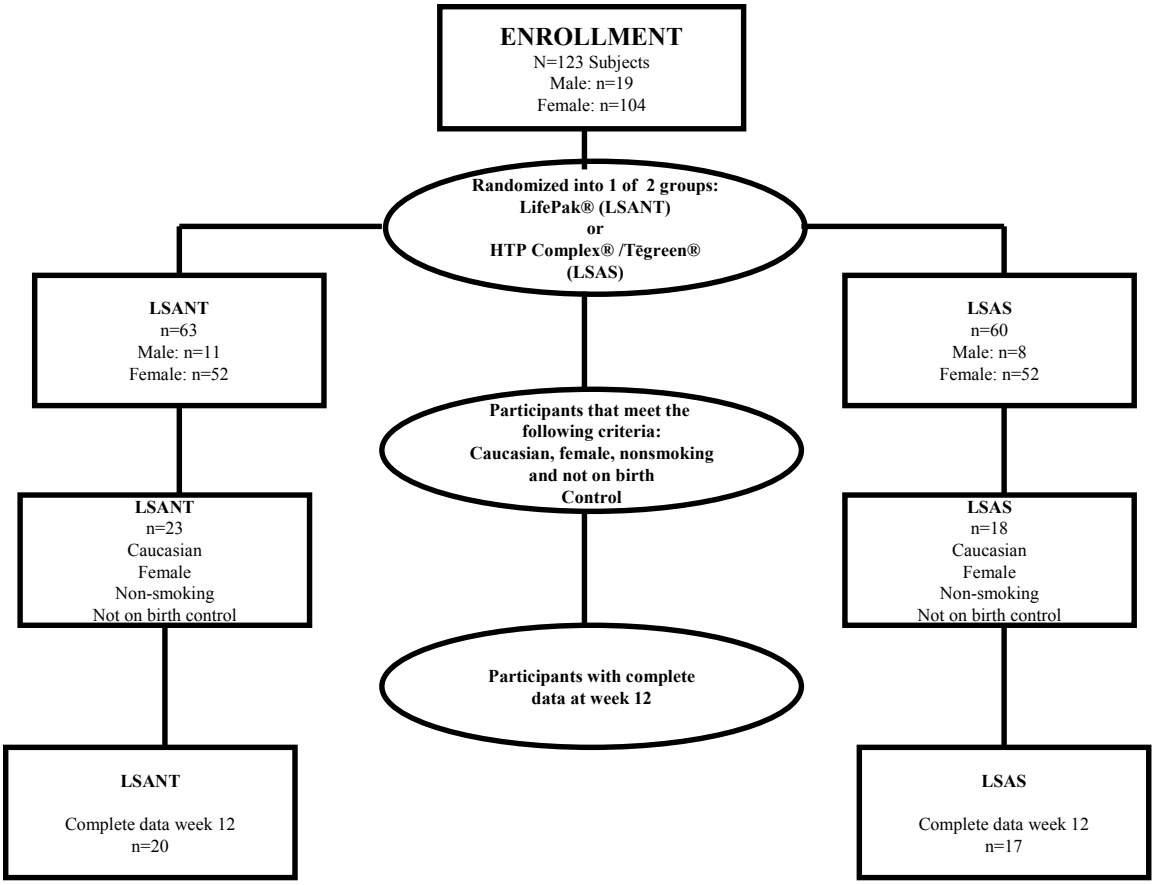


Figure 2. Flow of Subjects Based on Screening and Selected Criteria

The subjects responded to newspaper advertisements and fliers in their communities. Subjects were weight stable (± 5 pounds) and sedentary (less than 150 minutes a week of moderate intensity physical activity) for at least 30 days prior to enrollment. A history of cancer, diabetes, glucose intolerance, limitations to exercise (orthopedic or cardiac), recent surgery (within 3 months), diagnosed psychological illness, or previous significant cardiovascular events precluded subjects from study participation. In addition, all vitamin, herbal, or mineral supplements that had any known affect on antioxidant levels were discontinued prior to screening visit 1.

Intervention

Subjects were randomly assigned to an exercise, hypocaloric diet and antioxidant supplement (LSANT) group or an exercise, hypocaloric diet and appetite suppression supplement (LSAS) group. See Table 2 for the study procedure chart.

Table 2.

Study Procedure Chart

Evaluation	Baseline	Week 2	Week 12
Informed Consent	X		
Anthropometric: Weight, Height, BMI calculated, Waist circumference, DXA	X		X
Blood Analysis: Glucose, Insulin, hs-CRP, Lipid panel, Oxidative stress	X		X
Skin Carotenoid Score	X	X	X
Exercise Tolerance Test with VO_{2max}	X		X
3-Day Food Record	X		X
Resting Metabolic Rate	X		X
Evaluate Compliance	X	X	X
Dispense Product❶	X	X	

Note. BMI, body mass index. DXA, dual energy x-ray absorptiometry.
HS-CRP, high sensitivity C-reactive protein.
VO_{2max}, maximal oxygen consumption.
X=All subjects
❶ LifePak® or HTP Complex®/Tēgreen®

Exercise Prescription

An exercise physiologist instructed subjects on an individualized aerobic exercise program that gradually increased in duration, intensity and frequency ("American College of Sports Medicine Position Stand. The recommended quantity and quality of exercise for developing and maintaining cardiorespiratory and muscular fitness, and flexibility in healthy adults," 1998). Participants were instructed on how to fill out an exercise diary and then were instructed to exercise at 60-80% of their heart rate based on their maximal exercise test (to be described below). Table 3 provides a description of the exercise prescription given to the subjects.

Table 3.

Exercise Prescription

Week	1	2	3	4	5	6	7
Day 1	30 min	30 min	45 min	45 min	45 min	45 min	45 min
Day 2	30 min	30 min	45 min	45 min	45 min	45 min	45 min
Day 3	30 min	30 min	45 min	45 min	45 min	45 min	45 min
HR %	50-60%	50-60%	60-80%	60-80%	60-80%	60-80%	60-80%

Week	8	9	10	11	12
Day 1	45 min	45 min	45 min	45 min	45 min
Day 2	45 min	45 min	45 min	45 min	45 min
Day 3	45 min	45 min	45 min	45 min	45 min
Day 4	45 min	45 min	45 min	45 min	45 min
HR %	60-80%	60-80%	60-80%	60-80%	60-80%

Note. HR, heart rate

Walking was encouraged. However, any aerobic activity that allowed the participant to exercise at 60-80% of their heart rate was acceptable.

Nutrition Counseling

The nutrition plan for the hypocaloric diet in both groups was based on the *Exchange Lists for Meal Planning* from the American Diabetes Association and the American Dietetic Association (American Diabetes Association, 2003). A registered dietitian determined energy intake for each subject and a plan was developed that resulted in a 1.0 -1.5 lb (0.5-0.7 kg) weight loss per week. This weight loss amount corresponded to a 500 kcal deficit per day for each participant. Energy intake was determined based on their resting metabolic rate (RMR) measured by fasting oxygen consumption (MedGem, Healthetech, Golden, CO USA). Resting metabolic rate was then multiplied by the activity factor (1.3) to estimate total energy expenditure and then 500 was subtracted to provide the calorie level necessary to achieve this goal.

(RMR x 1.3) – 500 kcal=calorie level recommended for weight loss

All subjects were instructed to consume at least 1000 kcals a day. Macronutrient content of both groups was based on percent of ingested calories and was approximately 55% carbohydrate, 20% protein, and 25% fat. Subjects were provided with and were instructed on following and keeping daily calorie specific, nutrition checklists. The checklists included goals for fruit, vegetable, grain, dairy, lean protein sources and water consumption.

Supplements

LifePak® or HTP Complex®/Tēgreen® (Pharmanex, Provo, Utah) were dispensed at baseline and at week 2. Refer to Appendix B for dietary supplement information. Subjects taking LifePak® (LSANT group) were instructed to take 1 packet consisting of 4 capsules twice a day with food. Those assigned to the LSAS group were instructed to take 2 HTP Complex® capsules and 1 Tēgreen® capsule three times a day with meals. A 2-week supply of supplements was given at baseline. An additional 10-weeks supply of supplement was provided at week 2. Compliance

was observed by unused supplement being returned and adverse affects reported as they occurred. In addition, skin carotenoid scores (SCS) were measured at week 2 (SCS would be expected to increase above baseline in the LSANT group after 2 weeks of taking the supplement). All subjects were instructed to refrain from taking any additional vitamin, mineral or herbal supplements for the duration of the study.

Physiological Measurements

Anthropometric

Weight was measured while in a fasting state on a balance beam scale with attached stadiometer (Seca, Hanover, MD USA). Height was measured with shoes off. Body mass index (BMI) was calculated (mass (kg) ÷ height (m^2)). A tape measure with a calibrated spring loaded 6 oz tension was used to measure waist circumference (Gulick tape measure). The average of three measurements was used. The smallest area between the umbilicus and the ziphoid process was measured during a normal exhalation. Body fat mass and fat free mass was determined using whole-body dual energy x-ray absorptiometry (DXA), (DPX-MD+, GE Lunar).

Blood Sampling and Analysis

A 10-12 hour over night fast preceded each blood draw. A 3 ml additive free vacutainer tube and a 9.5 ml SST gel and clot activator vacutainer tube (Becton Dickinson and Company, Franklin Lake NJ, USA) were drawn with a 21g butterfly needle inserted into the antecubital fossa of either arm. Whole blood for serum analyses was coagulated for 30 minutes at room temperature (20°C) before centrifugation for 30 minutes. The SST tube was refrigerated at 4°C until analysis completed. Serum was analyzed for glucose, insulin, high sensitivity C-reactive protein, and lipid levels (Cognoscenti, Orlando, FL). Reactive oxygen metabolites were

measured immediately upon collection using a sample of blood from the 3 ml additive free vacutainer tube. The Free Radical Analytical System (FRAS) was utilized (IRAM, Parma Italy FRAS-3 version 1.3). The FRAS system consists of a photometer, a mini-centrifuge and a reagent called d-ROMs test Diacron. The FRAS measures the products of oxidation that the free radicals generate in the cell, oxidating lipids, proteins, and amino acids. These products (hydroperoxides) are expelled from the cell, enter the blood flow, are stable and are proportional to the number of free radicals that generated them. The results from the blood test reveal the level of oxidative stress which is measured in Carr units. One Carr unit corresponds to a concentration of hydrogen peroxide of 0.08 mg. Oxidative stress levels are normal at 250 U.Carr, borderline at 250 to 300 U.Carr, light to strong at over 300 U.Carr, and very strong at over 400 U.Carr. Recent physical activity, smoking, birth control, pathological conditions and inflammation increase oxidative stress levels.

Skin Carotenoids

Skin carotenoids were measured using the Biophotonic scanner (Pharmanex, Provo, Utah USA). Subjects were instructed to place the palm of their right hand in front of the scanners low-energy blue light laser for 3 minutes. Upon completion, the skin carotenoid score (SCS) was reported on a lap-top computer screen. Raman spectroscopy is based on the principal that molecules in the body can reflect a different set of colors when stimulated with a light source of a known frequency. This color spectrum is a unique optical fingerprint. The scanner operates with two light-emitting diodes (471.3 nm and 473 nm; blue light). When either diode hits a carotenoid molecule in the hand, a green light (507.8 nm or 509.8 nm) is produced and detected by a photomultiplier tube. The resulting value is the skin carotenoid score (SCS). Each 1000 units of a skin carotenoid score represents approximately 0.04 - 0.06 mcg of carotenoid per milliliter of

blood. The correlation of serum carotenoid concentrations to scanner scores is a linear equation. A skin carotenoid score of 25,000 represents the daily recommended intake of 5-6 servings of fruits and vegetables a day and/or the use of a dietary supplement containing carotenoids.

Exercise Tolerance Testing/Maximal Oxygen Consumption (VO_{2max})

Subjects were instructed not to eat for at least 3 hours prior to testing and to be dressed for exercise. A brief history and physical examination were obtained by an exercise physiologist or registered nurse. The skin on the chest, abdomen and or back was prepped (hair was shaved if required for good skin contact) with alcohol prep pads in order to remove lotions and/or oils for lead placement (10 leads, 6 precordial and 4 limb) for continuous electrocardiogram (ECG) monitoring during the test. A supine 12-lead ECG and blood pressure were obtained to rule out abnormalities. Blood pressure was repeated in a standing position. An explanation on procedures and expectations of the test in accordance with the American College of Sports Medicine (ACSM) guidelines was given to each subject (Johnson, 2000). Subjects were further instructed to rate their perceived exertion (Borg's RPE scale, 6-20) every 3 minutes during the test and immediately at the end by pointing at a scale provided (Borg, 1998). Calibrations were performed prior to each test on the metabolic cart (PhysioDyne, Quague NY). Appropriate sized head gear, mouth piece, and nose clips were fitted on each subject. All tests were performed on a treadmill (Marquette 2000) using a ramp protocol. In brief, an exercise physiologist evaluated the subject's capabilities and assigned one of two protocols (walking or athletic/running). Regardless of the protocol chosen, ramp speed and grade increased every 30 seconds. All tests included a 2-3 minute warm up and a 5 minute cool down period. Blood pressure was taken every 3 minutes during the test, immediately at recovery and at cool down. Subjects were instructed to continue the test until a maximal test was attained (no change in oxygen uptake

despite increasing workload), the operator terminated the test due to participant condition, or the participant terminated the test.

Diet and Nutrient Intake

Detailed instruction by registered dietitians using visual tools such as food models and common household measuring utensils was done in order for participants to accurately complete the 3-day food records at baseline and week 12. Participants were instructed to maintain normal dietary habits and record diet during 2 work days and 1 day off. The diet records were reviewed with the participants the following week by the dietitian to ensure accuracy and then entered by a trained dietitian into the Nutrient Data System for Research (NDS-R), software (University of Minnesota Minneapolis, Minnesota USA). In addition to the above, nutrient information was obtained through food labels, recipes, online resources or NDS support staff.

Resting Metabolic Rate

Following a 10-12 hour fast and prior to exercise, direct measure of oxygen consumption was measured by indirect calorimetry using the MedGem (Healthetech, Golden, CO USA). Subjects were directed to a quiet room where they sat for 5 minutes prior to starting the test. Subjects were then given a hand held device with a mouthpiece and nose plugs and they were instructed to breathe normally and to sit as quietly as possible during the measurement which took 5-10 minutes.

Statistical Analyses

Values are presented in the figures and tables as means ± standard deviation. All analyses were conducted at the two-tailed 0.05 α-level. Paired sample *t*-tests were used to examine differences

over time within the groups. Independent *t*-tests were used to compare the delta scores over time between the groups. Significance level was set at $p \leq 0.05$. Statistical analyses was performed using SPSS windows software version 11.0 (2001, SPSS Inc).

RESULTS

Subjects

Characteristics of the subjects that completed the study are shown in Table 4.

Table 4.

Characteristics at Baseline for Criteria-Based Subjects that Completed the Study

Characteristics	LSANT (n=20)	LSAS (n=17)	p[a]
Age (yr) 42.0 ± 8.1	42.0 ± 5.8	40.0 ± 5.8	0.388
Height (cm)	160.8 ± 5.3	162.3 ± 5.8	0.428
Weight (kg)	75.9 ± 9.0	79.1 ± 9.0	0.298
BMI (kg/m^2)	29.2 ± 2.3	29.9 ± 2.3	0.385
Systolic blood pressure (mm/Hg)	111.4 ± 13.2	115.4 ± 11.3	0.333
Diastolic blood pressure (mm/Hg)	67.0 ± 18.2	71.6 ± 11.4	0.369
Cholesterol (mg/dl)	205.3 ± 40.6	182.9 ± 28.5	0.065
Triglycerides (mg/dl)	127.7 ± 66.1	115.6 ± 60.2	0.569
HDL-c (mg/dl)	54.1 ± 10.1	57.3 ± 15.3	0.436
LDL-c (mg/dl)	125.5 ± 31.9	105.3 ± 24.7	0.041
Insulin (uIU/dl)	9.7 ± 5.6	9.5 ± 5.6	0.930
Glucose (mg/dl)	91.6 ± 6.0	94.7 ± 9.5	0.243
HOMA-IR	2.2 ± 1.3	2.3 ± 1.5	0.903
Skin carotenoid (SCS)	19850 ± 6115	18882 ± 7381	0.665
Oxidative stress (U.Carr)	310.8 ± 52.3	325.4 ± 79.0	0.507

Note. BMI, body mass index. HDL-c, high density lipoprotein cholesterol. LDL-c, low density lipoprotein cholesterol. HOMA-IR, homeostasis model assessment insulin resistance.
[a]p-values for between group differences compared by independent samples *t*-test
Treatment group hypocaloric diet, exercise and dietary antioxidant supplement (LSANT) or hypocaloric diet, exercise and appetite suppression supplement (LSAS)

No differences were observed in body weight at baseline between the two groups (LSANT: 75.9 ± 9.0 kg; LSAS: 79.1 ± 9.0 kg). Among CV and metabolic risk factors, there were no differences at baseline between the groups for glucose, insulin, HOMA-IR, systolic and diastolic

blood pressure and total cholesterol, HDL-c, and triglycerides. There was a significant difference ($p=0.41$) at baseline between the groups for LDL-c (LSANT: 125.5 ± 31.9 mg/dl; LSAS: 105.3 ± 24.7 mg/dl). As per the protocol, subjects were instructed to discontinue use of dietary or herbal supplements known to increase antioxidant levels at least 3 weeks prior to enrollment. There was no difference in skin carotenoid scores at baseline between the two groups (LSANT: 19850 ± 6115 SCS; LSAS: 18882 ± 7381 SCS). Oxidative stress levels also were not significantly different at baseline (LSANT: 310.8 ± 52.3 U.Carr; LSAS: 325.4 ± 79.0 U.Carr).

Intervention Effects

Body Weight and Composition

As shown in Table 5, significant ($p \leq 0.05$) within group changes were observed for BMI, body weight, fat mass, percent body fat, and waist circumference in both the LSANT and the LSAS group following 12 weeks of lifestyle intervention (hypocaloric diet and exercise).

Table 5.

Body Weight and Composition at Baseline and Week 12

Measure	Study Period	LSANT ± s.d. (P-value)[a] (n=20)	LSAS ± s.d. (P-value)[a] (n=17)	p[b]
BMI (kg/m^2)	Baseline	29.2 ± 2.3	29.9 ± 2.3	
	Week 12	28.2 ± 2.1	28.3 ± 2.1	
	Change	-1.0 ± 1.0 (<0.001)	-1.6 ± 1.0 (<0.001)	0.089
Body weight (kg)	Baseline	75.9 ± 9.0	79.1 ± 9.0	
	Week 12	73.2 ± 8.1	74.7 ± 8.6	
	Change	-2.8 ± 2.8 (<0.001)	-4.3 ± 2.7 (<0.001)	0.092
Body fat mass (kg)	Baseline	34.1 ± 3.9	36.3 ± 6.2	
	Week 12	32.3 ± 3.9	32.8 ± 6.6	
	Change	-1.8 ± 2.6 (0.006)	-3.4 ± 2.4 (<0.001)	0.052
Body fat (%)	Baseline	46.7 ± 3.0	47.3 ± 4.2	
	Week 12	45.7 ± 3.2	45.2 ± 5.2	
	Change	-1.0 ± 2.1 (0.052)	-2.0 ± 2.2 (0.002)	0.138
Body lean mass (kg)	Baseline	39.2 ± 5.8	40.2 ± 4.5	
	Week 12	38.3 ± 5.1	39.2 ± 4.0	
	Change	-0.8 ± 1.6 (0.028)	-1.0 ± 1.5 (0.010)	0.808
Waist circumference (cm)	Baseline	88.0 ± 9.4	88.1 ± 7.7	
	Week 12	84.7 ± 8.7	84.3 ± 7.1	
	Change	-3.3 ± 2.4 (<0.001)	-3.8 ± 2.2 (<0.001)	0.516

Note. BMI, body mass index
Treatment group hypocaloric diet, exercise and dietary antioxidant supplement (LSANT) or hypocaloric diet, exercise and appetite suppression supplement (LSAS)
[a]p-values for within-group change from baseline compared by paired samples *t*-test
[b]p-values for between group mean changes compared by independent samples *t*-test

Average weight loss from baseline to week 12 was -2.8 ± 2.8 kg (3.7%) for the LSANT group and -4.3 ± 2.7 kg (5.4%) for the LSAS group. Significant decreases in lean body mass were found in both groups after 12 weeks (LSANT: -0.8 ±1.6 kg, p=0.028; LSAS:-1.0 ± 1.5 kg, p=0.010). A significant mean difference in body fat mass was found between the groups at week

12 (p=0.052). No between group differences in weight were found following 12 weeks of dietary supplementation (p=0.092).

Cardiovascular Fitness and Blood Pressure

Significant improvements in relative VO_{2max} occurred within the groups (LSANT: 2.2 ± 2.6 ml/kg/min, p=0.008; LSAS: 2.1 ± 2.4 ml/kg/min, p=0.006) after 12 weeks of increasing physical activity (Table 6).

Table 6.

Blood Pressure and Aerobic Capacity at Baseline and Week 12

Measure	Study Period	LSANT ± s.d. (P-value)[a] (n=20)	LSAS ± s.d. (P-value)[a] (n=17)	p[b]
Systolic blood pressure (mm/Hg)	Baseline	111.4 ± 13.2	115.4 ± 11.3	
	Week 12	107.8 ± 12.3	112.5 ± 10.0	
	Change	-3.6 ± 11.6 (0.183)	-2.9 ± 9.9 (0.237)	0.855
Diastolic blood pressure (mm/Hg)	Baseline	67.0 ± 18.2	71.6 ± 11.4	
	Week 12	69.5 ± 12.7	75.3 ± 7.4	
	Change	2.5 ± 15.9 (0.490)	3.6 ± 11.4 (0.208)	0.806
Relative VO_{2max} (ml/kg/min)	Baseline	23.7 ± 4.9	25.5 ± 6.3	
	Week 12	25.9 ± 4.8	27.6 ± 6.5	
	Change	2.2 ± 2.6 (0.008)	2.1 ± 2.4 (0.006)	0.966
Absolute VO_{2max} (L/min)	Baseline	1.9 ± 0.4	2.0 ± 0.4	
	Week 12	2.0 ± 0.4	2.2 ± 0.4	
	Change	0.1 ± 0.2 (0.154)	0.2 ± 0.3 (0.028)	0.285

Treatment group hypocaloric diet, exercise and dietary antioxidant supplement (LSANT) or hypocaloric diet, exercise and appetite suppression supplement (LSAS)
[a]p-values for within-group change from baseline compared by paired samples *t*-test
[b]p-values for between group mean changes compared by independent samples *t*-test

Absolute VO_{2max} improved significantly in the LSAS group (0.2 ± 0.3 L/min, p=0.028) but not the LSANT group (p>0.05). Systolic and diastolic blood pressure did not change significantly in either group. There were no between group differences noted for blood pressure or aerobic capacity.

Blood Chemistry

As shown in Table 7, there were no significant within group or between group differences found in cholesterol, triglycerides, LDL-c, and hs-CRP in either group (p>0.05).

Table 7.

Serum Values at Baseline and Week 12

Measure	Study Period	LSANT ± s.d. (P-value)[a] (n=20)	LSAS ± s.d. (P-value)[a] (n=17)	p[b]
Cholesterol (mg/dl)	Baseline	205.3 ± 40.6	182.9 ± 28.5	
	Week 12	204.5 ± 45.6	174.8 ± 38.2	
	Change	-0.7 ± 18.3 (0.857)	-8.2 ± 22.2 (0.149)	0.273
Triglycerides (mg/dl)	Baseline	127.7 ± 66.1	115.6 ± 60.2	
	Week 12	142.2 ± 76.0	102.3 ± 51.2	
	Change	14.5 ± 46.0 (0.175)	-13.3 ± 57.8 (0.357)	0.112
HDL-Cholesterol (mg/dl)	Baseline	54.0 ± 10.1	57.3 ± 15.3	
	Week 12	51.1 ± 9.4	54.9 ± 15.6	
	Change	-2.9 ± 5.3 (0.024)	-2.5 ± 9.9 (0.318)	0.867
LDL-Cholesterol (mg/dl)	Baseline	125.5 ± 31.9	105.3 ± 24.7	
	Week 12	124.7 ± 35.4	101.8 ± 31.1	
	Change	-0.8 ± 15.3 (0.817)	-3.6 ± 19.1 (0.450)	0.625
Glucose (mg/dl)	Baseline	91.7 ± 6.0	94.7 ± 9.5	
	Week 12	91.6 ± 7.4	89.7 ± 10.0	
	Change	-0.05 ± 6.1 (0.971)	-5.0 ± 6.8 (0.008)	0.026
Insulin (uIU/dl)	Baseline	9.74 ± 5.6	9.6 ± 5.6	
	Week 12	8.28 ± 5.7	7.0 ± 3.9	
	Change	-1.4 ± 4.6 (0.175)	-2.6 ± 3.8 (0.013)	0.437
HOMA-IR	Baseline	2.2 ± 1.3	2.3 ± 1.5	
	Week 12	1.9 ± 1.3	1.6 ± 0.9	
	Change	-0.3 ± 1.1 (0.187)	-0.7 ± 1.0 (0.012)	0.301
hs-CRP (mg/L)	Baseline	5.6 ± 5.0	4.5 ± 4.7	
	Week 12	4.9 ± 4.0	3.7 ± 3.2	
	Change	0.7 ± 3.0 (0.315)	-0.9 ± 2.7 (0.208)	0.841

Note. HOMA, homeostasis model assessment insulin resistance. hs-CRP. high sensitivity C-reactive protein
Treatment group hypocaloric diet, exercise and dietary antioxidant supplement (LSANT) or hypocaloric diet, exercise and appetite suppression supplement (LSAS)
[a]p-values for within-group change from baseline compared by paired samples *t*-test
[b]p-values for between group mean changes compared by independent samples *t*-test

A significant difference in HDL-c was found in the LSANT group but not the LSAS group (LSANT: 54.0 ± 10.1 mg/dl to 51.1 ± 9.4 mg/dl, p=0.024; LSAS: 57.3 ± 15.3 mg/dl to 54.9 ± 15.6 mg/dl, p=0.318). In the LSAS group, glucose (94.7 ± 9.5 mg/dl to 89.7 ± 10.0, p=0.008), insulin (9.6 ± 5.6 uIU/dl to 7.0 ± 3.9 uIU/dl, p=0.013) and insulin resistance (HOMA-IR) (2.3 ± 1.5 to 1.6 ± 0.9, p=0.012) were significantly decreased but not in the LSANT group (p>0.05). A significant mean difference was found between the groups for glucose (LSANT: -0.05 ± 6.1 mg/dl; LSAS -5.0 ± 6.8 mg/dl, p=0.026) but not for insulin or for HOMA-IR after 12 weeks (p>0.05).

Skin Carotenoid Score

There was a significant increase in skin carotenoid scores in the LSANT group (19850 ± 6115 to 30800 ± 8745 SCS, p<0.001), but not the LSAS group (18882 ± 7381 to 20176 ± 6757 SCS, p=0.071) following 12 weeks of dietary supplementation (Table 8).

Table 8.

Skin Carotenoid Scores at Baseline and Week 12

Measure	Study Period	LSANT ± s.d. (P-value)[a] (n=20)	LSAS ± s.d. (P-value)[a] (n=17)	p[b]
Skin carotenoid (SCS)	Baseline	19850 ± 6115	18882 ± 7381	
	Week 12	30800 ± 8745	20176 ± 6757	
	Change	10950 ± 8395 (<0.001)	1294 ± 2756 (0.071)	<0.001

Note. SCS, skin carotenoid score
Treatment group hypocaloric diet, exercise and dietary antioxidant supplement (LSANT) or hypocaloric diet, exercise and appetite suppression supplement (LSAS)
[a]p-values for within-group change from baseline compared by paired samples *t*-test
[b]p-values for between group mean changes compared by independent samples *t*-test

A significant mean difference was found between the groups after 12 weeks ($p<0.001$).

Diet Intake

Changes in dietary intake are depicted in Table 9**.**

Table 9.

Diet Characteristics at Baseline and Week 12

Measure	Study Period	LSANT ± s.d. (P-value)[a] (n=20)	LSAS ± s.d. (P-value)[a] (n=17)	p[b]
Energy (kcal)	Baseline	2065.0 ± 521.7	2018.6 ± 639.5	
	Week 12	1399.4 ± 441.3	1327.1 ± 336.0	
	Change	-665.6 ± 682.7 (<0.001)	-691.5 ± 614.9 (<0.001)	0.905
Fat calories (%)	Baseline	34.8 ± 6.7	39.8 ± 6.4	
	Week 12	29.9 ± 6.4	28.9 ± 7.1	
	Change	-4.9 ± 8.1 (0.013)	-10.9 ± 9.3 (<0.001)	0.044
Carbohydrate calories (%)	Baseline	47.6 ± 8.1	43.1 ± 8.0	
	Week 12	52.8 ± 8.1	53.8 ± 9.4	
	Change	5.2 ± 11.5 (0.056)	10.7 ± 10.9 (0.001)	0.147
Protein calories (%)	Baseline	17.02 ± 3.4	17.8 ± 3.0	
	Week 12	19.8 ± 3.5	19.3 ± 3.2	
	Change	2.8 ± 4.1 (0.006)	1.5 ± 4.7 (0.221)	0.351
Total Fiber (mg/dl)	Baseline	17.3 ± 8.0	15.6 ± 6.7	
	Week 12	18.7 ± 8.6	18.4 ± 8.7	
	Change	1.3 ± 8.0 (0.466)	2.8 ± 6.7 (0.106)	0.560

Note.
Treatment group hypocaloric diet, exercise and dietary antioxidant supplement (LSANT) or hypocaloric diet, exercise and appetite suppression supplement (LSAS)
[a]p-values for within-group change from baseline compared by paired samples *t*-test
[b]p-values for between group mean changes compared by independent samples *t*-test

Total energy consumed decreased significantly ($p<0.001$) at 12 weeks within both groups (LSANT: 2065 ± 522 kcal to 1399 ± 441 kcal; LSAS: 2018 ± 639 kcal to 1327 ± 336 kcal).

Percent calories from carbohydrate (LSANT: 53%; LSAS: 54%), fat (LSANT: 30%; LSAS: 29%) and protein (LSANT: 20%; LS: 19%) were close to the amounts planned by week 12 in both groups (55%, 25% and 20% respectively). There was a significant mean difference found between the groups for percent calories from fat (LSANT: -4.9 ± 8.1%; LSAS: -10.9 ± 9.3%, $p=0.044$).

Dietary Antioxidant Intake

As shown in Table 10, there were no significant changes ($p>0.05$) after 12 weeks in the intake of total fiber, vitamin E, total vitamin A, beta-carotene, alpha-carotene, beta-crypoxanthin, or lycopene within or between the groups.

Table 10.

Dietary Antioxidants at Baseline and Week 12

Measure	Study Period	LSANT ± s.d. (P-value)[a] (n=20)	LSAS ± s.d. (P-value)[a] (n=17)	p[b]
Vitamin E (mg)	Baseline	9.4 ± 7.9	9.6 ± 8.1	
	Week 12	7.6 ± 7.8	7.4 ± 5.4	
	Change	-1.7 ± 11.0 (0.496)	-2.1 ± 9.7 (0.382)	0.906
Selenium (mcg)	Baseline	114.1 ± 32.7	118.2 ± 44.4	
	Week 12	91.8 ± 42.6	85.3 ± 31.9	
	Change	-22.3 ± 53.8 (0.080)	-32.9 ± 40.6 (0.004)	0.509
Total Vitamin A (mcg)	Baseline	902.6 ± 823.4	1103.9 ± 789.3	
	Week 12	967.0 ± 488.5	1178.2 ± 561.2	
	Change	64.3 ± 997.1 (0.776)	74.3 ± 605.9 (0.620)	0.972
Beta-Carotene (mcg)	Baseline	2649.6 ± 4859.2	3051.2 ± 3400.6	
	Week 12	3446.4 ± 2416.4	4229.6 ± 2526.2	
	Change	796.8 ± 5275.8 (0.508)	1178.3 ± 2625.2 (0.083)	0.788
Alpha-Carotene (mcg)	Baseline	230.6 ± 410.9	690.3 ± 1077.2	
	Week 12	674.9 ± 1028.4	1056.8 ± 979.2	
	Change	444.3 ± 1163.2 (0.104)	366.6 ± 926.4 (0.122)	0.826
Beta-Cryptoxanthin (mcg)	Baseline	271.9 ± 640.4	84.4 ± 85.3	
	Week 12	309.4 ± 773.5	255.1 ± 593.3	
	Change	37.6 ± 566.8 (0.770)	170.7 ± 593.5 (0.253)	0.490
Lutein/zeaxanthin (mcg)	Baseline	1960.2 ± 1551.1	1882.4 ± 1082.0	
	Week 12	3088.4 ± 2322.8	2420.9 ± 1873.4	
	Change	1128.1 ± 2222.0 (0.035)	538.5 ± 2199.4 (0.328)	0.424
Lycopene (mcg)	Baseline	4629.9 ± 5691.2	5482.8 ± 5574.5	
	Week 12	3903.2 ± 2703.1	3956.0 ± 4245.8	
	Change	-726.7 ± 5698.9 (0.575)	-1526.8 ± 6519.3 (0.349)	0.693

Note.
Treatment group hypocaloric diet, exercise and dietary antioxidant supplement (LSANT) or hypocaloric diet, exercise and appetite suppression supplement (LSAS)
[a]p-values for within-group change from baseline compared by paired samples *t*-test
[b]p-values for between group mean changes compared by independent samples *t*-test

A significant decrease in selenium intake occurred in the LSAS group at week 12 (118.2 ± 44.4 mcg and 85.3 ± 31.9 mcg p=0.004). Lutein/zeaxanthin consumption was significantly increased

from baseline at week 12 for the LSANT group (1960.2 ± 1551.1 mcg and 3088.4 ± 2322.8 mcg, $p=0.035$) but not the LSAS group ($p>0.05$).

Adverse Effects

The most frequently reported symptom from the administration of HTP Complex®/Tēgreen® was nausea (18%, n=3). Nausea was attributed to the administration of HTP Complex® and not to the administration of Tēgreen®. Previous studies have reported nausea to be a side effect of 5-HTP administration (Cangiano et al., 1992). The dose of HTP Complex® was reduced to once a day until the nausea subsided. Gradual increments in dose were achieved until in 2-3 weeks, the prescribed dose of three times a day was again taken. Subjects taking LifePak® reported no adverse effects.

Compliance

Exercise

Exercise compliance was determined by the exercise physiologist using clinic visit notes and the subject's exercise logs. Missing one exercise day a week was considered noncompliant. Overall, 86.5% (n=32) of the subjects were compliant with the exercise prescription. Compliance was 85% (n=17) in the LSANT group and 88% (n=15) in the LSAS group. Noncompliance was due to missing a day of the week as opposed to the amount of time needed to perform the physical activity. Missing days were primarily due to illness, work, and travel in that order. Based on improvements in the relative and absolute VO_{2max} after 12 weeks, the data would indicate good compliance to this exercise prescription.

Diet

Dietary compliance was determined by the dietitian using clinic visit notes and the subject's diet records. Subjects that failed to record or track > 25% of their diet entries every week were considered noncompliant. Overall, 89% (n=33) of the subject's were compliant in completing dietary study documents. Compliance was 90% (n=18) in the LSANT group and 88% (n=15) in the LSAS group. It should be noted that overall compliance should also be evaluated on the basis of the 3-day food record at week 12. The percent calorie amounts for this study for subjects on the Exchange diet were set at 55% calories from carbohydrate, 25% calories from fat and 20% percent calories from protein. At 12 weeks, the average percent calories for each group were (LSANT: 53%; LSAS: 54%) from carbohydrate, (LSANT: 30%; LSAS: 29%) from fat and (LSANT: 20%; LS: 19%) from protein. This data would indicate that dietary compliance was good.

Supplements

Compliance in taking LifePak® was determined by the number of packets taken every week. Missing more than 2 packets a week was considered noncompliant. Compliance was 90% (n=18) in the LSANT group. The morning packet was the most often missed dose and the reason for missing it was forgetting to take it and then not having it available until later in the day. Compliance in taking HTP Complex®/Tēgreen® was determined by taking at least 2 of the 3 doses of both HTP Complex® and Tēgreen® on a daily basis. Compliance was 65% (n=11) in the LSAS group. However, three of these subjects had an adverse affect as was noted above. The afternoon dose was frequently the dose missed in this group. This was also due to not having the supplement with them when they ate lunch.

DISCUSSION

The main purpose of this study was to determine the effects of a lifestyle modification program (hypocaloric diet and exercise) on weight and risk factors for the metabolic syndrome and CVD. The secondary purpose was to determine the effect of dietary supplementation on weight loss and skin carotenoid levels. Thirty-seven subjects were randomized into one of two groups (LSANT, n=20; LSAS, n=17). At baseline, the only significant difference found between the groups was LDL-c (p=0.041). This difference was attributed to the differences in total cholesterol found at baseline (LSANT: 205.3 ± 40.6 mg/dl and LSAS: 182.9 ± 28.5 mg/dl, p=0.06).

The main findings of the present investigation are: 1) Lifestyle intervention that involves a structured hypocaloric diet and increased physical activity results in weight loss and improvements in body fat mass and waist circumference; 2) Supplementation with an appetite suppressant (HTP Complex®) does not enhance weight loss beyond what is achieved with a structured lifestyle intervention; 3) Antioxidant supplementation may be of benefit during a weight loss program that incorporates physical activity and a low energy diet; and 4) Overall compliance was high to the lifestyle intervention (exercise and hypocaloric diet).

The decrease in morbidity and mortality associated with weight loss in overweight and obese individuals following lifestyle intervention programs has been well documented. Results from the U.S. DPP and the FDPS as well as numerous other studies have reported that modest reductions in weight (5%) results in favorable changes in lipid concentrations, insulin resistance, blood pressure, waist circumference, percent body fat, and visceral adiposity (Anderson & Konz, 2001; Dattilo & Kris-Etherton, 1992; Knowler et al., 2002; Lindstrom et al., 2006; MacMahon,

Cutler, Brittain, & Higgins, 1987; Selvin et al., 2007; Stewart et al., 2005; Tuomilehto et al., 2001). In the present investigation, a weight loss of 3.7% (LSANT) and 5.4% (LSAS) produced favorable changes in total body fat mass, percent body fat and waist circumference (Figure 3-4).

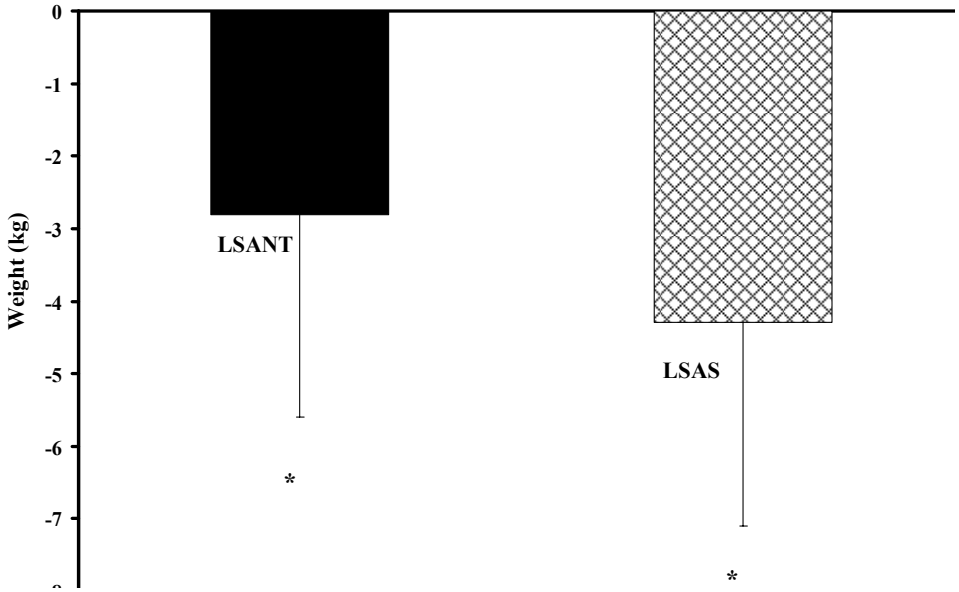
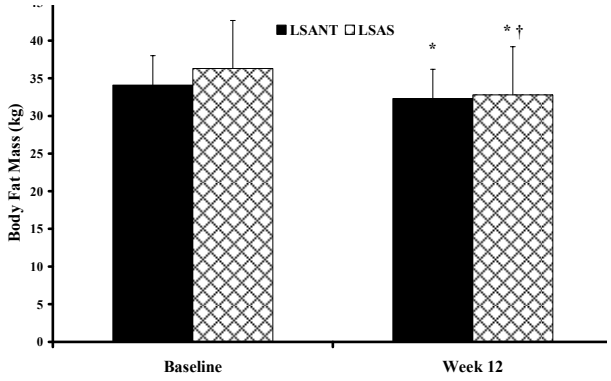


Figure 3.

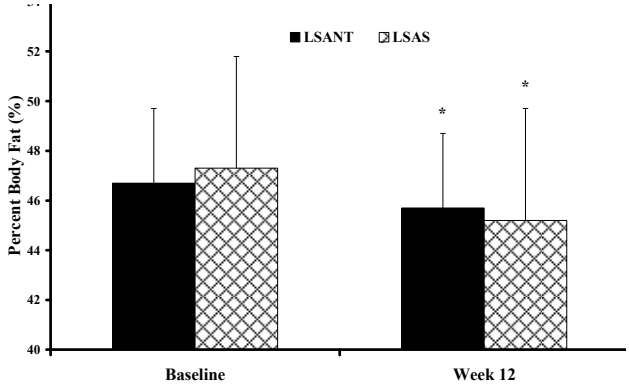
Mean Change in Weight

*Different from baseline within the group $p<0.05$

Body Fat Mass Baseline to Week 12



Percent Body Fat Baseline to Week 12



Waist Circumference Baseline to Week 12

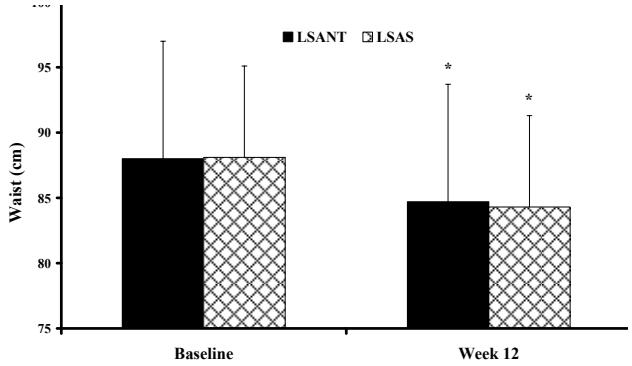


Figure 4.

Total Body Fat, Percent Body Fat, and Waist Circumference Baseline to Week 12

* Different from baseline within the group $p<0.05$
† Different between the groups $p<0.05$

In addition, a significant mean difference was found between the groups for body fat mass (Figure 5).

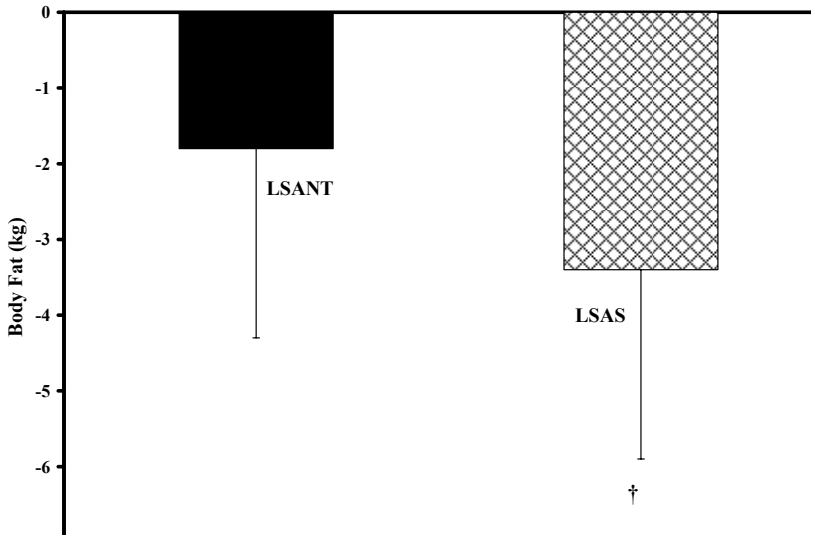


Figure 5.

Mean Change in Body Fat Mass

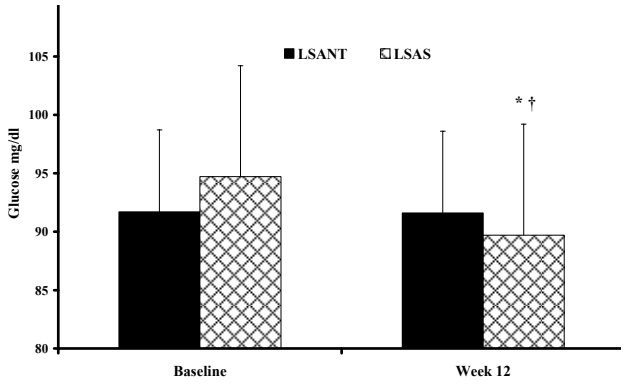
† Different between the groups $p<0.05$

The difference found between the groups for body fat mass (LSANT: -1.8 kg vs. LSAS -3.4 kg) can be attributed to the difference in weight loss found in the groups (LSANT: -2.8 kg vs. LSAS -4.3 kg). However, the dietary supplement Tēgreen® should also be considered. Tēgreen® contains 97% polyphenols of which 65% are catechins. Catechins have been found to play a role in the control of body composition via sympathetic activation of thermogenesis and/or fat oxidation (Dulloo et al., 1999; Dulloo et al., 2000). Green tea extract has been found to increase 24-hour energy expenditure by 4% due to its affect on stimulating brown adipose tissue thermogenesis (Dulloo et al., 1999). Furthermore, green tea extract (containing catechins) taken for 3 months without dietary restriction decreased weight (4.6 %) and waist circumference

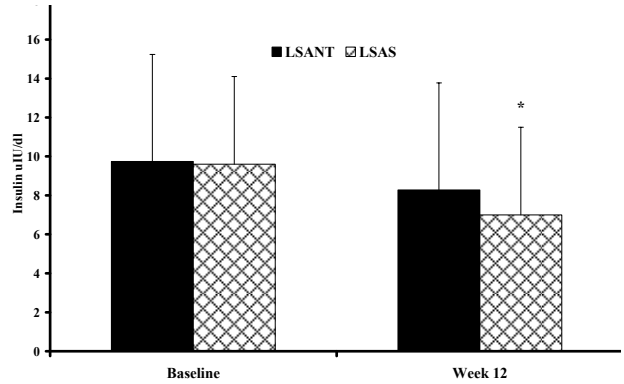
(4.5%) in overweight and obese adults (Chantre & Lairon, 2002). Moreover, decreases in body fat were found in healthy young men that consumed green tea (690 mg catechins) for 12 weeks (Nagao et al., 2005). Therefore, although the difference found in body fat mass is most likely due to the amount of weight lost in each group, the effect of Tēgreen® cannot be ruled out.

Green tea has also been found to improve insulin sensitivity and regulate blood glucose (McKay & Blumberg, 2002; S. Wolfram, Wang, & Thielecke, 2006; Wu et al., 2003). We found decreased glucose and insulin levels and improvements in insulin resistance in the LSAS group but not the LSANT group (Figure 6).

Glucose Baseline to Week 12



Insulin Baseline to Week 12



HOMA-IR Baseline to Week 12

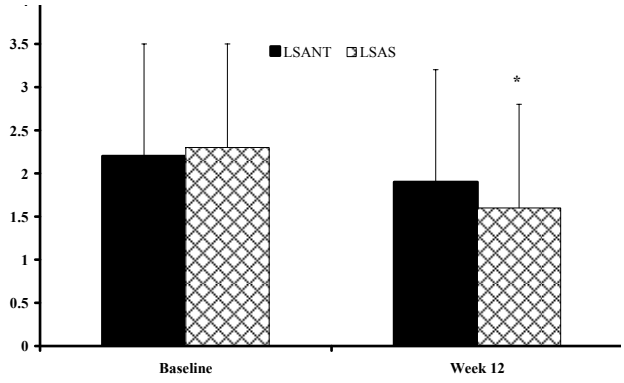


Figure 6.

Glucose, Insulin and HOMA-IR Baseline to Week 12

* Different from baseline $p<0.05$
† Different between the groups $p<0.05$

Furthermore, a significant mean difference was found between the groups for glucose (Figure 7).

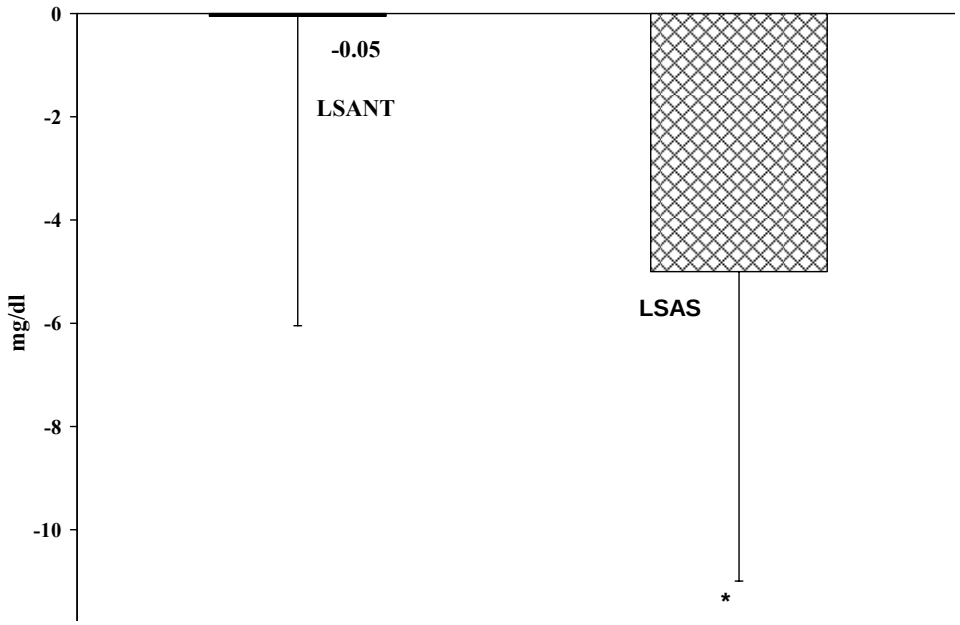


Figure 7.

Mean Change in Glucose

* Different between the groups $p<0.05$

Weight loss and decreases in body fat mass, specifically visceral fat, have been shown to improve insulin resistance (Knowler et al., 2002; Lindstrom et al., 2006). The improvements found in glucose, insulin and insulin resistance in the LSAS group were most likely due to the differences in body fat mass (LSAS: 9.4%; LSANT: 5.3%) and weight loss (LSAS: >5%; LSANT: <5%). However, factors other than weight loss may be considered. As first noted above, green tea has been shown to improve glucose tolerance and insulin sensitivity (S. Wolfram et al., 2006). This is thought to be due to the action of the catechins in the green tea. Currently under investigation, it is thought that the catechins increase the basal and insulin

stimulated glucose uptake in the adipocytes in addition to the rehabilitation of damaged beta-cells. In addition, epigallocatechin gallate (EGCG), a bioactive polyphenol in green tea, has been shown to mimic the metabolic actions of insulin to inhibit gluconeogenesis in hepatocytes (Kao, Chang, Lee, & Chen, 2006). Until recently, the majority of research on green teas effects has been done in the laboratory on animals and most human studies on green tea consumption have been epidemiological in nature. Further research is needed to determine what role green tea has in reducing obesity and the risk factors associated with the metabolic syndrome in humans.

Dyslipidemias are a common characteristic of the MS and CVD. Results from weight loss lifestyle studies show varying levels of change in total cholesterol, HDL-c, LDL-c, and triglyceride concentrations depending on the lifestyle intervention used (diet, exercise, or combination of diet and exercise) and the extent of the dyslipidemia present in the population studied. The mean values found for cholesterol, triglycerides, HDL-c, and LDL-c at baseline in our subjects were normal. There were no significant effects on total cholesterol, triglycerides, or LDL-c following 12 weeks of lifestyle intervention (low energy diet and physical activity). A significant, but modest decrease was found in HDL-c in the LSANT group. Lifestyle intervention studies involving women have shown decreases in HDL-c following weight loss (Anderson, Brinkman-Kaplan, Lee, & Wood, 1994; Dattilo & Kris-Etherton, 1992; Thompson, Jeffery, Wing, & Wood, 1979). Furthermore, data from other studies suggest that the decrease in HDL-c that occur with active weight loss is reversed during subsequent periods of weight maintenance (Dattilo & Kris-Etherton, 1992).

Lifestyle intervention (hypocaloric diet and exercise) is considered the first line of treatment to reduce the risk factors for the MS and CVD. As previously shown (Figure 1), a number of factors are involved in regulating appetite. A number of experimental data support

the role played by serotonin in the regulation of feeding behavior. Decreased levels of serotonin have been found in the central nervous system of dieters which has been associated with carbohydrate cravings and binge eating in obese individuals (Ceci et al., 1989). 5-Hydroxytryptophan (5-HTP) is the immediate precursor to serotonin. HTP Complex® contains 150 mg of 5-HTP per capsule. Subjects were instructed to take 300 mg of 5-HTP three times a day. This dose is equivalent to the dosage used in previous weight loss studies using 5-HTP. In the present study, HTP Complex® in combination with a calorie restricted diet and physical activity did not significantly increase weight loss more than low energy diet and physical activity alone (Figure 8).

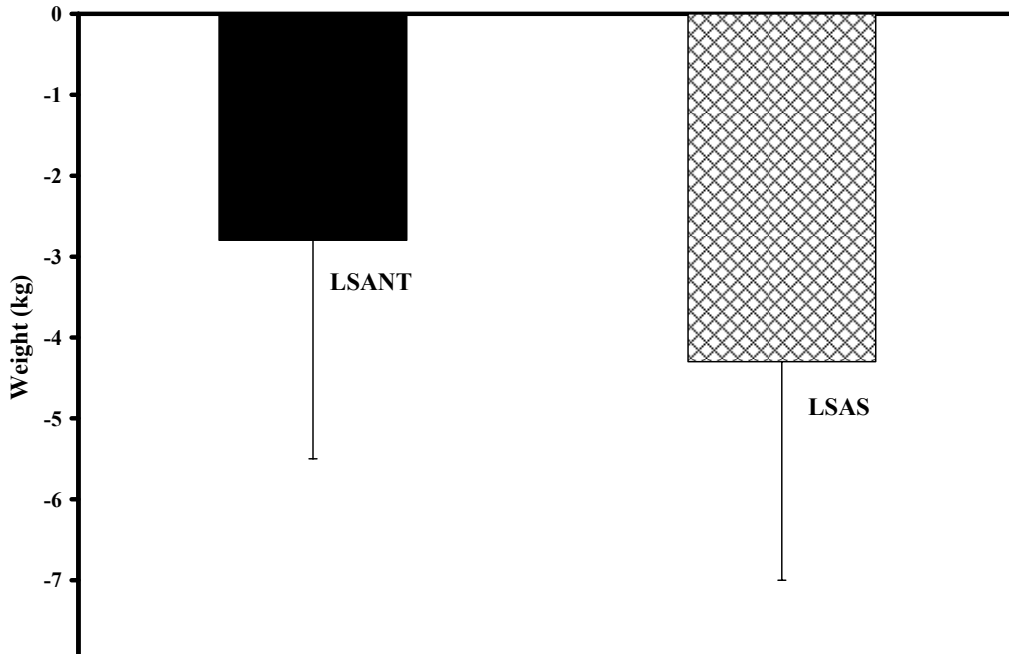


Figure 8.
Mean Change in Weight
$p>0.05$

However, although statistical significance was not found between the groups, we did find a trend toward more weight loss in the LSAS group. Sample size in addition to poor compliance (65%) in taking the dietary appetite supplement may account for the lack of statistical significance found. Several studies have found weight loss following administration of 5-HTP in combination with and without diet restriction in overweight an obese women (Cangiano et al., 1991; Cangiano et al., 1992; Ceci et al., 1989). In a double-blind, placebo-controlled study with subjects taking 300 mg 5-HTP three times a day or placebo, weight loss in addition to a spontaneous reduction in energy intake (3220 to 1879 kcal) and carbohydrate intake (50%) was noted in the 5-HTP group (Cangiano et al., 1992). These results occurred during the first six weeks of the study during which no caloric restriction was prescribed. It was suggested that the results were due to the administration of 5-HTP which decreased the subjects carbohydrate cravings and binge eating. However, as many as 80% of the subjects initially reported nausea and/or vomiting. This suggests that the reductions in energy intake found during the first 6 weeks of the study may have been due to the adverse effects (nausea and vomiting) associated with 5-HTP administration. The results of this study also indicate that nausea may be a problem with 5-HTP at this dose. It is possible that lower doses of 5-HTP may be effective in reducing body weight. However, more time may be needed to achieve this result. The administration of lower doses of 5-HTP in combination with and without caloric restriction have not been studied to our knowledge.

Weight loss programs typically reduce dietary energy consumption and vary the percentages of carbohydrates, fats and proteins which can create nutritionally unbalanced diets. In addition, lower fruit and vegetable consumption (and presumably antioxidant intake) has been found in overweight and obese individuals as compared to their leaner counterparts (Bialostosky,

2002; Fairfield & Fletcher, 2002; Tapiero et al., 2004). These diets are often poor in micronutrients thereby increasing the risk for chronic disease. Furthermore, carotenoid levels in serum and skin have been found to be lower in individuals with higher BMI and adiposity (Andersen et al., 2006; Brady et al., 1996; Fiutem J, 2004; Moor de Burgos et al., 1992). In the present study, skin carotenoid scores at baseline were 18,000 to 20,000 (Figure 9).

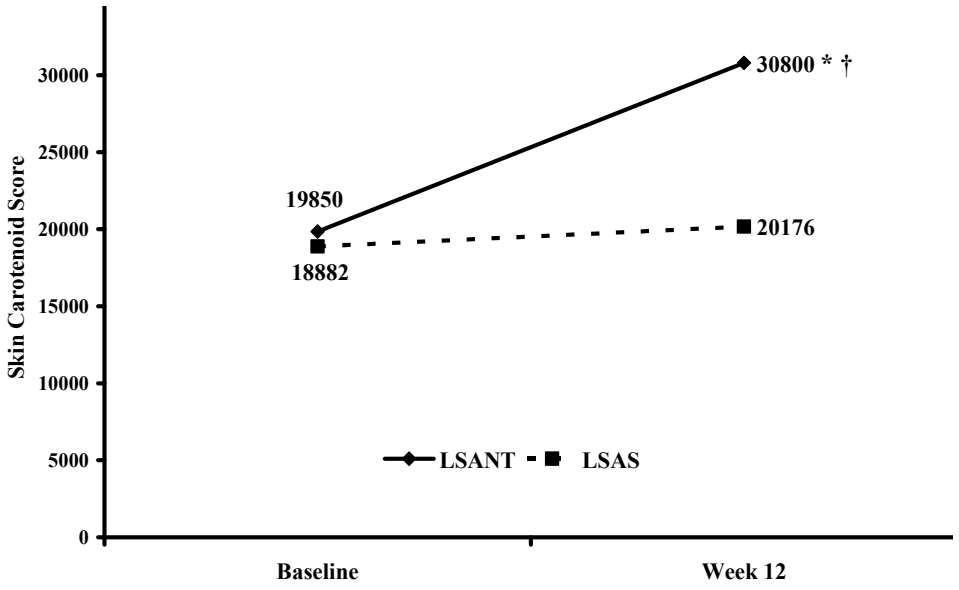


Figure 9.

Skin Carotenid Scores Baseline to Week 12

* Different from baseline within the group $p<0.05$
† Different between the groups $p<0.05$

Twelve weeks of supplementation with a dietary antioxidant containing significant amounts of antioxidants, vitamins, and minerals (LSANT group) resulted in a significant increase in skin carotenoid scores (Figure 9). Previous research in our lab found that following 24 weeks of a well designed program of caloric restriction and physical activity, skin carotenoid scores

decreased significantly with weight loss (Zukley L, 2004). Subjects were instructed not to take any dietary supplements (over-the-counter or prescribed) for the duration of the study. In the current study however, the LSAS group was instructed to take Tēgreen®. Tēgreen® contains large amounts of polyphenols, and to a lesser extent carotenoids, tocopherols, vitamin C, as well as the minerals manganese, chromium, selenium, and zinc and other phytochemicals which have strong antioxidant activity. These additional compounds could account for the slight increase we found in the skin carotenoid score. Limited research is available on the effects of weight loss on antioxidant status in overweight and obese individuals. Studies have investigated antioxidant status following weight loss associated with bariatric surgery. However, the applicability of this data to the general population is limited.

Dietary compliance was found to be good in our subjects based on the completion of diet records. In addition, the fact that both groups closely obtained the recommended percent levels of carbohydrates, fats and proteins (55%, 25% and 20% respectively) at week 12 suggests good dietary compliance in these subjects. Furthermore, mean average intake as determined by the 3-day food records at week 12 indicate an energy reduction of 650-700 kcal/day. However, this reduction in energy intake would indicate that on average, a weight loss of 12 pounds (5.4 kg) would occur. Subjects in the LSANT group lost on average 6.2 pounds (-2.8 kg) and the LSAS group lost on average 9.5 pounds (-4.3 kg) after 12 weeks. Our results suggest that subjects may have under reported nutritional intake. Research from numerous studies indicates that women and higher BMI status is representative of under reporters compared to those individuals that accurately report their dietary intake (Bailey, Mitchell, Miller, & Smiciklas-Wright, 2007; Huang, Roberts, Howarth, & McCrory, 2005; Voss, Kroke, Klipstein-Grobusch, & Boeing, 1998). Furthermore, foods that are deemed unhealthy are usually underreported by subjects and

may account for as much as a 10-50% difference in energy intake a day (Huang et al., 2005; McCrory, McCrory, Hajduk, & Roberts, 2002). Therefore, although compliance in completing diet records was high, the data suggest that underreporting of nutritional intake on the 3-day food record occurred.

Micronutrient intake was found to be similar between the groups. However, the dietary intake of selenium was significantly lower in the LSAS group at week 12. Selenium deficiency is rare in the U. S. due to high selenium soil levels and the fact that food is distributed from geographic regions around the country. Furthermore, despite the decrease in dietary selenium intake, the levels were still above the recommended intake of 55 μg. The decrease in intake is most likely a result of transient dietary changes (decreased consumption of meat and seafood). The increase in lutein/zeaxanthin that was found in the LSANT group at 12 weeks may be due to the increased consumption of fruits and especially dark green vegetables and the concomitant use of dietary supplements containing beta-carotene. Currently, there is no recommended dietary intake for this carotenoid and there are no reported cases of over dosage.

A limitation of this study was sample size. This cohort of subjects taken from the larger intervention study was not powered to find differences between the groups. In addition, compliance to the dietary appetite suppressant was low. This suggests that significant differences may have been found between the groups had compliance been higher. Recommendations for future research involve the evaluation of how chronic dieting and weight loss affect overall antioxidant status. In addition, due to the increasing prevalence of obesity and obesity related complications and the conflicting reports found with weight loss on skin carotenoid levels, further research is necessary to determine the effect of weight loss on antioxidant levels.

CONCLUSION

In conclusion, we examined the effects of a lifestyle intervention program (low energy diet and physical activity) on weight loss and risk factors for the MS and CVD. We also looked at the effect of dietary supplementation on weight loss and skin carotenoid levels. A hypocaloric diet in combination with physical activity resulted in weight loss and improvements in waist circumference, body fat mass, and percent body fat. Furthermore, weight loss in combination with losses in body fat mass resulted in additional improvements in glucose, insulin and insulin resistance. This suggests that fat mass loss in combination with weight loss is an important factor in risk reduction for the MS and CVD. Additionally, due to the health benefits associated with green tea, green tea consumption in conjunction with lifestyle intervention changes may provide additional health benefit. The addition of an appetite suppressant (HTP Complex®) did not enhance weight loss beyond that of the lifestyle intervention alone, although there was a trend toward increasing weight loss. Furthermore, weight loss was not shown to adversely affect skin carotenoid scores. The addition of green tea however, which contains small amounts of carotenoid antioxidants may be responsible for these results.

APPENDIX A
IRB APPROVAL LETTER

University of Central Florida

January 20, 2005

Theodore Angelopoulos, Ph.D.
University of Central Florida Child,
Family & Community Sciences
Orlando, FL 32816-1250

Dear Dr.Angelopoulos:

With reference to your protocol entitled, "The Effects of Antioxidant Versus Dietary Supplementation Human Skin Carotenoids and Antioxidant Status during a Structured Weight Loss Program". I am enclosing for your records the approved, expedited document of the UCFIRB Form you had submitted to our office.

This study was reviewed by the full board at the 12/17/04 IRB meeting at which there was a quorum present. The Board granted contingent approval at that meeting. Please be advised that this approval is given for one year from the date of contingent approval with An expiration date of 12116105, Should there be any addendums or administrative changes to the already approved protocol they must also be submitted w the Board. Changes should not be initiated until written MB approval is received. Adverse events should be reported to the IRB as they occur. Further should there be a need to extend this protocol, a renewal form must be submitted for approval at least one month prior to the anniversary date of the most recent approval and is the responsibility of the investigator (UCF).

Should you have any questions, please do not hesitate to call me at 407-823-2901

Please accept our best wishes for the success of your

endeavors. Cordially,

Barbara Ward

Barbara Ward, CIM
IRB Coordinator

Copies: IRB File

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THE UNIVERSITY OF CENTRAL FLORIDA
INSTITUTIONAL REVIEW BOARD (IRB)

IRB Committee Approval Form

PRINCIPAL INVESTIGATOR(S): Theodore Angelopoulos, Ph.D. IRB #: 04-2100

PROJECT TITLE: The Effects of Antioxidant Versus Dietary Supplementation on Human Skin Carotenoids and Antioxidant Status during a Structured Weight Loss Program

[X] New project submission [ ] Continuing review of #

Full Board Approval **IRB Co-Chairs:**
[X] Contingent Approval Dated; 12/17/04
Dated: 12/17/04 Signed:

Dr. Sophia Dziegielewski

[X] Final Approval
Dated: 1/20/05 Signed:

Jacqueline Byers

Dr. Jacqueline Byers

[X] Expiration [ ] Waiver of documentation of consent approved

Date: 12/16/05 [ ] Waiver of consent approved

NOTES FROM IRB CHAIR (IF APPLICABLE):

cc change letter and emails for Contingencies. DW

APPENDIX B
DIETARY SUPPLEMENTS

LifePak®

Supplement Facts

Serving Size One Packet

Amount Per Packet		%Daily Value*
Vitamin A (83% as Beta-Carotene from Palm Fruit and Blakeslea trispora Extracts, Vitamin A Palmitate)	7500 IU	150%
Vitamin C (as Calcium Ascorbate)	250 mg	417%
Vitamin D (as Cholecalciferol)	200 IU	50%
Vitamin E (as d-Alpha Tocopheryl	150 IU	500%
Vitamin K (as Phytonadione)	20 mcg	25%
Thiamin (as Thiamine Mononitrate)	3.75 mg	250%
Riboflavin (as Riboflavin)	4.25 mg	250%
Niacin (as Niacin, Niacinamide)	20 mg	100%
Vitamin B6 (as Pyridoxine Hydrochloride)	5 mg	250%
Folate (as Folic Acid)	300 mcg	75%
Vitamin B12 (as Cyanocobalamin)	15 mcg	250%
Biotin (as Biotin)	150 mcg	50%
Pantothenic Acid	15 mg	150%
Calcium (as Tricalcium Phosphate, Calcium Carbonate, Calcium Propionate, Calcium Ascorbate)	250 mg	25%
Iodine (as Potassium Iodide)	50 mcg	33%
Magnesium	125 mg	31%
Zinc (as Zinc Chelate)	7.5 mg	50%
Selenium	70 mcg	100%
Copper (as Copper Chelate)	0.5 mg	50%
Manganese (as Manganese Chelate)	1.0 mg	100%
Chromium (as Chromium Chelate)	100 mcg	83%
Molybdenum (as Molybdenum Chelate)	37.5 mcg	50%
Polyphenol and Flavonoid Blend	97.5 mg	*
Catechins (from Camellia sinensis)	(45 mg)	*
Quercetin	(25 mg)	*
Grape Seed Extract	(12.5 mg)	*
Citrus Bioflavonoids	(12.5 mg)	*
Isoflavones (from Soy Extract)	(2.5 mg)	*
Alpha-Lipoic Acid	15 mg	*
Inositol (as Inositol)	5 mg	*
Carotenoid Blend (other than Beta-Carotene)	4.5 mg	*
Lycopene (as Lycopene)	(2.5 mg)	*
Alpha-Carotene (from Palm Fruit Extract)	(1 mg)	*
Lutein (from Marigold Flower Extract)	(1 mg)	*
Boron (as Boron Citrate)	1.5 mg	*
Silicon (as Sodium Metasilicate)	1.5 mg	*
Vanadium (as Vanadyl Sulfate)	10 mcg	*

* Daily Value not established.

HTP Complex®

Supplement Facts

Serving Size One Capsule

Amount Per Serving		%Daily Value*
Vitamin B_6	3.3 mg	165%
(as Pyridoxine Hydrochloride)		
5-Hydroxy-L-Tryptophan	150 mg	**
(from Griffonia simplicifolia) Seed Extract (40 :1)		

* Percent Daily Values are based on a 2,000 Calorie Diet.
** Daily Value not established

Other Ingredients: Gelatin, Microcrystalline Cellulose, Magnesium Stearate.

TēGreen®

Supplement Facts

Serving Size One Capsule

Amount Per Serving		%Daily Value*
Green Tea Leaf Extract (20:1)	250 mg	*
(Camellia sinensis)		

*Daily Value not established.

Other Ingredients: Millet, Gelatin, Magnesium Stearate, Magnesium Silicate, Silicon Dioxide.

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