DIET, BODY FAT DISTRIBUTION, AND SERUM LEPTIN IN YOUNG MEN WITH UNDIAGNOSED OBSTRUCTIVE SLEEP APNEA SYNDROME

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DIET, BODY FAT DISTRIBUTION, AND SERUM LEPTIN IN YOUNG MEN WITH UNDIAGNOSED OBSTRUCTIVE SLEEP APNEA SYNDROME

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ABSTRACT

Background and Purpose: Little is known about influences of obstructive sleep apnea syndrome (OSAS) on dietary intake and body composition. The purpose of this study was to evaluate dietary status, body fat distribution and leptin in overweight young men with and without OSAS in comparison to published values for normal weight counterparts. **Methods:** Groups were comprised of 24 sedentary overweight young men with and without OSAS, who had a body mass index (BMI) greater than 25 kg/m^2. Serum leptin concentration was measured in the 24 subjects using radioimmunoassay, while OSAS assessment was done using nighttime home somnography. Analysis of 4-day diet recalls was performed using Nutritionist Pro (First DataBank, Inc., San Bruno, CA). A Healthy Eating Index (HEI) score was calculated for the 24 overweight subjects. **Results:** There were no differences between the two overweight groups for total fat mass, central abdominal fat, BMI, waist circumference, leptin, or the HEI. The HEI was not predictive of overall OSAS severity; however, BMI was moderately related to OSAS severity ($r = 0.39$; $p=0.05$). The normal weight group did have a 50% higher report of carbohydrate intake, and consumed on average, 500 more kilocalories per day. The normal weight group consumed 50% less sodium, and 50% more Vitamin's C and E including a 13% increase in the HEI. **Conclusions:** Regulation of eating behavior and related influences on diet composition may be affected by a number of neurohormonal disturbances associated with OSAS and/or obesity, itself. Further research is needed to quantify these possible differences on dietary status and the underlying mechanism involved.

DEDICATION

I dedicate this thesis to my mom and dad for all their support and love throughout my five years in Blacksburg. I also dedicate this thesis to my husband, Nate for all his love, support, admiration, and encouragement since we have been together.

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CHAPTER I
INTRODUCTION

Background

As society has evolved during the past decade, there have been significant increases in body weight in the United States, along with other developed countries. The National Health and Nutrition Examination Survey shows that 127 million people are overweight, which is described as having a body mass index (BMI) between 25 kg/m^2 to 30 kg/m^2, 60 million individuals are obese (BMI > 30 kg/m^2), and 10 million people are morbidly obese (BMI > 40 kg/m^2). About 325,000 people die annually due to obesity related disorders, such as cardiovascular disease (CVD). Body weight is increasing due to many factors, particularly a lack of physical activity and unhealthy diets, which are producing increased incidence of Metabolic Syndrome, insulin resistance, diabetes and CVD (Esposito et al., 2006). Abdominal obesity can cause increases in cytokine release by fat tissue, leading to the previously mentioned disorders. Not only are adults suffering from obesity, but obesity in children is trending in the same direction. This has become quite a public health problem not only in the United States, but also in most European countries, with 20% of Southern, Central and Eastern European adults being obese. The Middle East has remarkably high obesity rates and is also the region, with the highest national prevalence of insulin resistance and type II diabetes in the world. Asian and African populations have also shown dramatic reductions in physical activity levels, as well as alterations in dietary intake adding to the already increasing obesity prevalence in developed countries (James, 2008).

Obesity and Obstructive Sleep Apnea Syndrome (OSAS)

Sleep loss and restriction have been associated with changes in energy balance and can result in overall weight gain, as well as the development of obstructive sleep apnea syndrome (OSAS). OSAS is a disorder defined as a repeated collapse of the upper airway, resulting in cessation of breathing even though respiratory effort continues. OSAS is also characterized by an apnea hypopnea index (AHI) of greater than 5, representing the average amount of times an individuals breathing ceases in an hour of sleep. This leads to arousal from sleep resulting in sleep fragmentation, and sleep deprivation (Keller at al., 2007). Sleep deprivation may cause changes in appetite regulation due to

disturbances in glucose metabolism, as well as reducing motivation to exercise and increase dietary intake due to elevated leptin levels (Knutson et al., 2007). Van Cauter and associates (2007) found that healthy young subjects who are sleep deprived, have alterations in their neuroendocrine control of appetite. This then can lead to increased hunger and changes in glucose tolerance that result in an increased risk for the development of diabetes.

Obesity is one of the strongest risk factors for OSAS, which includes an increase in body weight traits such as body mass index (BMI), waist/hip ratio, neck circumference, percent body fat, and skin fold thickness (Sharma et al., 2006). These characteristics have been studied in the middle-aged male population, with little attention being concentrated on younger adults. Weight loss is recommended for those who are overweight with OSAS to help improve CVD risk (Esposito et al., 2006). However, the relationship between obesity and OSAS is still unclear; this may be attributed to the complexity of feeding regulations that are controlled by the central nervous system (Cherinack, 2005).

Disturbances in Leptin in Obesity and Implications for OSAS

Adipose tissue is an endocrine organ that produces many adipokines, including leptin, which is released during inflammation in response to dysregulation of many systems. Leptin is a peptide hormone produced by fat tissue that helps regulate body weight, metabolism, food intake, and reproductive function (Esposito et al., 2006). Serum leptin levels are elevated in those who are obese, and are positively correlated with BMI, percent body fat and overall fat mass. On average, leptin expressed in obese subjects is two times higher than in lean individuals because fat cell size in the obese is 2 to 4 times the size of those who are lean. Therefore, an increased number of adipocyte cells especially in the morbidly obese can contribute to increased serum leptin (Fried et al., 2000).

Leptin reduces appetite and is one part of the dual control system of energy intake and expenditure regulation. Leptin decreases feeding by acting on the hypothalamus to reduce production of neuropeptide Y, which inhibits appetite (Cherinack, 2005). Serum leptin has a diurnal rhythm with leptin levels highest during lunch time, and declining in the early afternoon. Massive overfeeding increases plasma leptin levels by an average of 40% above baseline (Fried et al., 2000). There is a high prevalence of OSAS among those who are obese, with 50% of obese men having OSAS. Obese individuals have higher serum leptin levels, which is an appetite suppressant and a respiratory stimulant, and can lead to increased difficulties with OSAS. In a study by Ciftci et al., a positive

correlation was seen between serum leptin levels and RDI (Ciftci et al, 2005). This relationship can be explained by increased fat deposition in the neck and upper airway, which can lead to upper airway collapse in the subject. OSAS individuals also have a larger neck circumference, which represents greater fat or soft tissue depositions and is significantly associated with OSAS. Elevated serum leptin levels are reported as a better predictor than percent body fat for obesity-hypoventilation syndrome (Phipps et al., 2002). Blood leptin concentrations have also been determined to increase the presence of persistently elevated plasma carbon dioxide levels, particularly in hypercapnea. Leptin levels have improved in OSAS individuals who are receiving continuous positive airway pressure treatment (Cherinack, 2005).

Diet and OSAS

There have been few studies that have explored the relationship between OSAS and dietary intake. It is important to look at the role of nutrition in the relationship between controlling increased visceral fat accumulation that may lead to OSAS. In sleep deprived adults, Royotanni et al. reported that higher energy intake particularly of high-fat foods can lead to obesity (Royotanni et al., 2007). However, there have been inconclusive results to show the relationship between increased energy intake and short sleep duration or sleep fragmentation. Decreasing obesity in individuals with OSAS is important, and can be done not only by systematically increasing physical activity, but also addressing diet composition and reduction in caloric intake to help improve overall disease risk.

Understanding the effects of OSAS on nutritional status constitutes an important first step in determining the cause and effect relationship associated with increased caloric intake and its effects on adipocyte deposition and increased leptin levels in OSAS. OSAS is a disorder associated with hypoxia, which can destroy cellular reductants, which constitute the main line of antioxidant defense in the body. As a result, free oxygen radicals are formed and can also lead to the development of CVD in OSAS individuals. Cellular reductants help to prevent the damage done by free radicals and prevent the onset of diseases such as hypertension and other cardiovascular abnormalities (Christou et al., 2003). A diet rich in fruits and vegetables that contains Vitamins C and E can help to decrease oxidative stress and blood pressure in obese individuals with hypertension and OSAS (Baldwin et al., 2005). The usefulness of a diet high in fruits and vegetables has not been studied in young, overweight men with undiagnosed OSAS, but future research is need to help clear up misconceptions (Svendsen et al., 2007).

Statement of Problem

Dietary intake is believed to be altered in those with sleep deprivation, particularly leading to an increase in high-energy foods (Royotanni et al., 2007). However, little, if anything is known about the relationship of overall caloric intake in OSAS particularly in an obese younger male adult population. This study will be important to help start establishing a relationship between dietary intake and how it can influence the severity of OSAS.

Published evidence has shown that obesity is a major independent risk factor for OSAS as well as a co-morbidity associated with the development of CVD. The causal relationship between obesity and OSAS is still unclear, but it is believed to be associated with a disruption in energy intake and expenditure (Cherinak, 2005). This disruption between energy intake and expenditure can lead to increased visceral fat accumulation. Visceral fat accumulation in the neck can lead to upper airway collapse in OSAS, as well as increased overall neck circumference, which can negatively influence the RDI score. It is important to further understand the relationship among obesity and OSAS, and looking at dietary intake can help advance discussion on the issue.

To date, there is no evidence to support the relationship of leptin and OSAS in overweight, young adult men (Cherinack, 2005). Serum leptin levels are elevated in the obese, and can increase the likelihood of developing hyperventilation issues. It is important to understand leptin and its influence on OSAS, as well as its causal relationship with overall dietary intake and quality.

Significance of the Study

This is an exploratory study to determine the relationship among obesity and its effects on leptin, dietary intake and body composition in young adults with obstructive sleep apnea. The presence of OSAS may have unfavorable effects on leptin, or a combination of leptin and dietary intake on body composition markers such as neck circumference, and fat deposition located around the upper airway. Focusing on this particular target population of young men, is important not only due to the lack of research in this particular group, but due to the increasing prevalence of obesity in younger age ranges and the associated medical costs associated with therapeutic prevention and treatment of obesity, OSAS, CVD and other related disorders. The evaluation of dietary intake in this younger age group can help to determine ways to influence healthier food choices at a young age to help prevent premature onset of chronic disease, and control the worsening of already presented diseases such as OSAS. Evaluating changes in leptin in relation to dietary quality and intake, as well as

markers of body composition including neck circumference may help to better understand OSAS as a disorder of the obese.

Research Aims

This was a cross sectional study, with a primary aim of determining whether young overweight men with and without OSAS differed in their diet composition and caloric intake, when assessed against published data from normal weight, aged matched counterparts. A secondary aim was to evaluate overall caloric intake between men with and without OSAS. A tertiary aim was to evaluate serum leptin and body composition markers in men with and without OSAS, based on their dietary and caloric intake.

Specific Research Hypotheses

The specific hypotheses were to determine if overweight young adults with undiagnosed OSAS had:

(1) higher energy and fat consumption and more energy storage, based on total kilocalories consumed, compared to overweight young men without OSAS and aged matched, normal weight counterparts;

(2) higher total body fat and central abdominal fat than overweight young adults without OSAS;

(3) inferior HEI scores that were positively related to the severity of OSAS, as assessed by RDI score from home somnography testing.

Delimitations

The primary delimitations of the study were: (1) All subjects were volunteers between the ages of 18-23 years attending Virginia Polytechnic Institute and State University or members of the local community; (2) Subjects were on no prescribed medications and had no medical problems known to affect respiratory or metabolic function; (3) Subjects had not been exercising regularly during the 6 months preceding the study; (4) All dual x-ray absorptiometry measurements were analyzed by the same technician to minimize inter-tester variation; (5) Blood collection and analysis after overnight fasting was performed by the same technician to minimize inter-tester variation; (6) Diet entry and analysis was performed by the same technician for the same reason as blood collection

Limitations

Limitations of this study included small sample sizes in all groups, due to the fixed subject numbers available from the parent project. There are numerous variables of interest that have been measured for this study, and there are a number of uncontrolled effects that were observed by previous investigators, including RIA procedures. Subject error could have occurred during completion of diet records, with food intake mis-represented.

Definition of Terms

1. Obstructive Sleep Apnea Syndrome (OSAS): a condition characterized by repetitive obstruction of the upper airway often resulting in oxygen desaturation and arousal from sleep.
2. Respiratory disturbance index (RDI) (events/hour): The RDI is similar to the RDI, but it includes respiratory events that do not qualify as apneas or hypopneas but do influence sleep. The RDI score is taken from physician's adjusted a home somnography assessment.
3. Healthy Eating Index (HEI): A measure of dietary intake quality that is in accordance with the 2005 Dietary Guidelines for Americans, with the food groups inspired by the recommendations in MyPyramid. Broad ranges of points are awarded to consumption of grains, vegetables, fruit, milk, total fat, saturated fat, meats, beans, oils, and calories from alcohol consumption. The maximum amount of points awarded is 100.
5. Dual-energy X-ray absorptiometry (DXA): A two-dimensional x-ray that measures bone mineral density, bone mineral content, body composition, central abdominal fat, and fat free mass. It is the current gold standard measure for bone mineral density.
6. Body Mass Index (BMI): An individual's body weight in kilograms (kg) divided by their height in meters squared (m^2), a BMI of greater than 30 kg/m^2 is considered obese.
7. Serum Leptin (ng/mL): polypeptide hormone of 16 KDa released by white-adipose cells; important component in the long-term regulation of body weight and appetite.

Summary

As obesity has risen to epidemic proportions over the last decade, there has also been an increase in obesity related disorders like CVD and OSAS. OSAS is associated with hypoxemia that can lead to dysregulation of appetite regulation due to elevated blood leptin levels. OSAS

can affect leptin levels, but can also change dietary habits and energy expenditure of obese, OSAS individuals. Few studies have examined nutrient and caloric content of the diet in OSAS and how any such differences may relate to metabolic rate, body fat distribution or serum leptin, a protein produced by adipose cells and known to suppress appetite in healthy individuals of normal weight.

CHAPTER II
REVIEW OF LITERATURE

Introduction

Body weight is increasing due to many factors, particularly a lack of physical activity and unhealthy diets, which are producing increased incidence of Metabolic Syndrome, insulin resistance, diabetes and cardiovascular disease (CVD) (Esposito et al., 2006). Sleep loss and restriction have been associated with changes in energy balance and can result in overall weight gain. Sleep deprivation may cause changes in appetite regulation due to disturbances in glucose metabolism, as well as reducing motivation to exercise and increase dietary energy due to elevated leptin levels (Knutson et al., 2007). Obstructive Sleep Apnea Syndrome (OSAS) is associated with comorbidites, including obesity and CVD. Weight loss is recommended for those who are overweight with OSAS to help improve overall disease risk. Adipose tissue is an endocrine organ that produces many adipokines, particularly leptin which is released during inflammation in response to dysregulation of many systems. Leptin is a hormone produced by fat tissue that helps regulate body weight, metabolism, food intake, and reproductive function (Esposito et al., 2006). It is important to look at all previously mentioned issues to determine the relationships among diet, overweight and obesity, neuroendocrine changes and OSAS.

Status of Overweight and Obesity

Defining the Disease

According to the Center for Disease Control, medical expenses that were attributed to obesity accounted for 9.1% of total U.S. medical expenditures in 1998. Obesity may have contributed to $78.5 billion spent, with half of these costs being paid by Medicaid and Medicare (Esposito et al., 2006). These increase costs are due to the remarkably increasing prevalence of obesity. Since the mid-seventies, two National Health and Nutrition Examination (NHANES II, 2003-2004) surveys show that among adults 20-74 years old, the prevalence of obesity has increased from 15.0 to 32.9% in the 2003-2004 survey (Esposito et al., 2006). Obesity is defined as a body mass index (BMI) of greater than 30 kg/m^2 (Esposito et al., 2006). These surveys also show the increasing rate of childhood obesity, with the prevalence increasing in aged 2-5 years from 5.0 to 13.9%; for those aged 6-11 years from 6.5 to 18.8%; and for those aged 12-19 years,

5.0 to 17.4% (Esposito et al., 2006). Although the national health objective for the year 2010 is to help reduce the prevalence of obesity in the United States to less than 15%, the current data shows that the situation is worsening instead of improving (Esposito et al., 2006).

Epidemiology

Many population-based studies have tracked the increasing prevalence of OSAS throughout the world. It is said that approximately 1 in 5 adults has mild OSAS (RDI $\geq$ 5) and 1 in 15 adults has moderate to severe OSAS (RDI$\geq$15) (Somers et al., 2008). In the Wisconsin Sleep Cohort, the RDI score progressively increased over an 8-year period, and was greatest in habitual snorers with a BMI of greater than 30 kg/m^2 and between 45 and 60 years of age. Over 85% of those with significant and treatable OSAS have never been diagnosed (Somers at al., 2008). A large portion of the United States population is overweight or obese. Over the past two decades, the prevalence of obesity has steadily risen and in 2002 about 65.7% of individuals were overweight or obese (Pearson et al., 2007). In a study by Pearson, 10 years of data from 1995 to 2004 were taken from the Behavioral Risk Factor Surveillance System and the National Ambulatory Medical Care Survey. This data was used to compare trends in the prevalence of obesity and overweight over the past decade (Pearson et al., 2007). The number of individuals with a BMI of $\geq$ 25 kg/m^2 increased from more than 102 million to more than 127 million representing a 23.7% increase. Doctor visits that included the primary cause of the visit being obesity related increased from 12.9 million to 27 million visits, representing a 106% increase. The average age for females with a BMI of $\geq$ 25 kg/m^2 was 48.6 years over the 10 year period, with the average age of males with a BMI of $\geq$ 25 kg/m^2 was 59.8 years (Pearson et al., 2007).

Clinical Manifestations

Kissebah et al. (1982) identified central adiposity as a key predictor of diabetes and other metabolic disorders in the Bogalusa Hearty Study. Those who were presented with central adiposity, also reported high insulin levels, and were overweight based on their BMI. These individuals were also more likely to develop the metabolic syndrome later in life (Kissebah et al., 1982). In a study by Morrison et al, a 30 year follow up of students in the Princeton Lipid Research Clinics Study assesed the likelihood of the association of overweight and metabolic syndrome components in childhood and adulthood, and it was hypothesized that metabolic syndrome in childhood would independently predict the disorder in adulthood (Morrison et al., 2008). Initial measurements were taken, and 30 years later, follow up measurements were taken.

The mean age of individuals at the initial meeting was 12.8 years, and at follow up was 38.4 years. The mean BMI for the individuals was 19.8 kg/m^2 with 18.2% being above the CDC age specific 85th percentile, and being at risk for overweight, and 7% were at the 95th percentile of being overweight. Not only did BMI increase from an average of 19.8 to 38.4 kg/m^2 but glucose increased from 85.5 to 90.5 mg/dL, triglycerides from 74.3 increasing to 134.3 mg/dL and HDL cholesterol from 54.6 decreasing to 45.8 mg/dL (Morrison et al., 2008). The results conclude that pediatric metabolic syndrome and a positive parental history of type II diabetes were major independent predictors of adult diabetes in this group (Morrison et al., 2008).

The American Heart Association identifies obesity as one of the major risk factors for CVD, even if there are no other risk factors presented. Obesity alone can increase risk for heart disease, and can hurt or worsen not only the blood vessels of the heart, but can cause degenerative joint disease as well as gallbladder disease (Alam et al., 2007). Obesity causes an increase in CVD due to raising blood cholesterol and triglyceride levels. It can also lower HDL-C, which with high levels can help to prevent heart disease, and lower stroke risk. Obesity can raise blood pressure levels, and as already stated can increase risk for developing type II diabetes and the metabolic syndrome (Alam et al., 2007). As one of the five criteria for identifying the metabolic syndrome, waist circumference for men and women of greater than 102 cm and 88 cm, respectively are at an increased risk of developing CVD. The waist is measured at the narrowest part of the torso above the umbilicus and at the mid-point between the ribcage and the iliac crest (Alam et al., 2007). A study done by Koh-Banerjee et al. (2004) showed that an increase in waist girth of 2.6 cm had a 0.8 times greater chance of developing type II diabetes, but those who showed the maximal amount of waist girth gain of 14.6 cm had a 2.4 times greater risk of developing type II diabetes (Koh-Banerjee et al., 2004).

Associations with Diet

There are many associations related to weight gain and many factors influence the energy balance equation. With energy intake exceeding energy expenditure being influence by age, gender, race, and physical activity, eating habits and lifestyle are strongly associated with obesity and its related chronic disease. In a study done by Lee and associates (2008), the Shanghai Men's Health Study was developed to focus on investigating the long term effects of diet, occupational and other lifestyle factors on risks for cancers and other chronic disease. Over 61,000 men between the ages of 40-74 years participated in the study. Each subject was

interviewed based on questions that were surrounded by their demographic, disease and surgery history, lifestyle factors including alcohol and dietary consumption as well as physical activity and occupational history. All anthropometrics were taken and obesity was defined using the World Health Organizations criteria of obesity being a BMI of ≥ 30 kg/m^2. The average BMI for the subject group was 23.7 kg/m^2, with the prevalence of obesity being 10.5%. The average caloric intake for the group was 1,909 kcal/day with a standard deviation of 485 kcal/day. Men with centralized obesity tend to have a lower total dietary energy intake and carbohydrate intake, but have a higher intake of protein and fat, particularly in the previously mentioned study (Lee et al., 2008).

In the United States, food consumption has increased steadily since the 1960's from about 3,100 kcal/day in 1965 to a high of 3,900 kcal/day in 2000. The National Health and Nutrition Examination Survey revealed data to show that an increase in daily energy intake between 1971 and 2000 showed that women increased their average intake by 335 kcal/day and men by 168 kcal/day (Gaesser et al., 2007). During this same time period, the amount of total carbohydrate intake increased 60-70 grams/day. In comparison, fat intake actually remained the same. This may be contributed to low fat eating being encouraged in Western countries during the past decade. Carbohydrate quality and quantity is important to assess when looking at weight control. Refined carbohydrates, those with a high glycemic index will increase the risk of obesity and associated diseases. Diets that are indeed higher in glycemic index or glycemic load foods are associated with CVD risk, increased plasma triglycerides, glucose, insulin, inflammatory cytokines, and reduced HDL-C. A diet that is high in carbohydrates, particularly whole grains, fruits and vegetables is associated with the consumption of many other nutrients including, fiber, antioxidants, vitamins C and E, and folate. These nutrients are also associated with the decrease in atherosclerosis, as well as a lower risk of developing type II diabetes, and overall mortality (Gaesser et al., 2007).

Endocrine Dysfunction: Leptin

Leptin is a hormone that is produced mainly by the adipose tissue and binds to receptors that are found in the hypothalamus. Obesity is characterized by high levels of leptin in the body and it has been suggested that obesity is a leptin resistant state (de Luis et al., 2008). High circulating leptin levels are commonly presented in obesity and the body fails to restore normal energy balance in a resistance state (Arora, 2008). However, in a weight maintenance state

leptin output and uptake levels are equal, and the energy regulating pathways are not influenced, as they would be in a leptin resistant state. A resistance state is usually caused by the opposition of the signaling receptors to respond, as well as a decreased ability of the blood-brain barrier to transport leptin into the brain. Increased leptin levels may also play a role in obesity caused CVD and atherosclerosis. Leptin resistance combined with changes in sympathetic activation may contribute to hypertension in patients who present signs and symptoms of the metabolic syndrome. Endothelial tone and blood pressure are altered with abnormal leptin levels, particularly in obesity, which can lead to vascular inflammation, and elevated oxidative stress (Arora, 2008).

Changes in leptin levels in obesity may lead to the central nervous system not responding to the hormone, which alters hypothalamic neurotransmitters resulting in an increase in appetite and food consumption. In a study by de Luis and associates (2008) the goal was to determine whether obese subjects who lost weight on a hypocaloric diet (> 5% of weight) experienced the same decrease in leptin levels as those who did not lose weight. Subjects consisted of two groups, group I represented those who did not lose 5% of weight, while group II did lose > 5% of their weight. Subjects were comprised of 66 obese, non-diabetic individuals (17 male, 49 female) whose anthropometric measurements (BMI, waist and hip circumferences) were taken, and subjects were then place on a hypocaloric diet. The diet consisted of 1520 kcal/day, with 52% carbohydrate, 25% fat, and 23% protein. The mean age was 46 years, with a mean BMI of 35 kg/m^2. Group II had improvement, but not significant improvements over group I in weight, BMI, fat mass and waist circumference. Group II have a significant improvement in insulin levels, as well as total cholesterol and triglyceride levels. In group I, leptin levels decreased (102 ± 86 vs. 89± 76 ng/mL). In group II, leptin levels significantly decreased (69 ± 67 vs. 53 ± 59 ng/mL), with group II having a significantly higher basal level of leptin than group I. The findings of this study show patients with weight loss that is secondary to a hypocaloric diet, and as a result had decreased leptin levels. However, patients without weight loss after dietary treatment had decreased leptin levels with a significant improvement in body composition and cardiovascular risk factors. Decreases in leptin levels are due to loss of body fat mass, and some have reported this decrease as much as 45% in serum leptin (de Luis et al., 2008).

Obstructive Sleep Apnea Syndrome (OSAS)

Defining the Disease

OSAS is a disorder that is characterized by repetitive collapse of the upper airway and results in oxygen desaturation, which leads to an arousal from sleep (Christou et al., 2003). According to the National Sleep Foundation, OSAS occurs when the muscles in the back of the throat fail to stay open, and the airway is forced to close despite efforts by the individual to breathe. OSAS can cause fragmentation in sleep patterns leading to blood oxygen desaturation. The combination of low blood oxygen and disruption in sleep can lead to hypertension, heart disease, mood and memory issues. Those with OSAS tend to have excessive daytime sleepiness (EDS) and can be predisposed to falling asleep at work, on the phone, or while driving. If OSAS is left untreated, it can cause heart attack, congestive heart failure, cardiac arrhythmia, stroke or depression (Christou et al., 2003).

Etiology

It is estimated that approximately 20% of the population as a whole displays symptoms of OSAS, particularly an AHI greater than 5. Full diagnosis of the disorder is seen in 1-5% of the male population and in 1-2% of the female population with those women being premenopausal. OSAS shows a peak in prevalence during the middle age years, and declines following age 65 years. According to Parati and colleagues (2007), the increasing rate of obesity in Western countries corresponds to the increasing prevalence of OSAS. As many as 40% of obese men have OSAS, and approximately 70% of those who are diagnosed with the disorder are obese as well. In a study by Vgontzas and associates (2003), two groups of men (obese, middle aged with OSAS; BMI matched, obese men without OSAS) were evaluated for various measurements of OSAS. Visceral fat was the focused measurement of this portion of the study, with body fat distribution being measured using computed tomographic (CT) scanning. There were no significant differences between the groups according to total body fat or subcutaneous fat. When compared to their obese counterpart, those with OSAS have more visceral fat. Also, visceral fat but not subcutaneous fat was correlated with severity of OSAS based on RDI ($r = 0.6$). These findings are consistent with others that report visceral fat is a significant risk fact for OSAS in obese individuals.

RDI is a significant correlate with visceral fat but not with subcutaneous fat in the neck or with pharyngeal fat (Vgontzas et al., 2003). The worsening of OSAS can lead to increased

amounts of visceral fat, as well as the development of the Metabolic Syndrome. This formation of the Metabolic Syndrome can lead to night time increases in hormonal levels leading to the promotion of visceral fat, as well as cardiovascular difficulties (Vgontzas et al., 2003).

Not only does OSAS present itself with many co-morbidities including obesity, but EDS is a common concern for road safety. Driving a vehicle is a task that demands optimum daytime functioning; for this reason, sleepiness contributes to an estimated 23% of traffic crashes (Lyznicki et al., 1998; Horne et al., 1995). Additionally, 17-19% of all deaths in traffic accidents have been attributed to sleepiness or driver fatigue. Excessive daytime sleepiness (EDS), a major public health concern, affects about 15% of the general population and can lead to impaired cognitive function, poor quality of life, and accidents (Hasler et al., 2005; Ohayon et al., 1997). Sleepiness compromises reaction time and psychomotor abilities crucial for safe driving (Van Dongen et al., 2003).

Clinical Manifestations

Not only does obesity pose a health threat to those with OSAS, but underlying hypertension is life threatening to those with the disorder. Hypertension can be associated with underlying organ damage and can increase ones risk for having cardiovascular difficulties (Baguet et al., 2008). Hypertension is underrated in OSAS and can be associated with a high cardiovascular risk, especially if the hypertensive state is unknown. Hypertension in OSAS is caused by nighttime repetition of desaturation and reoxygenation due to irregular respiratory patterns (Baguet et al., 2008). Changes in the sympathetic nervous system and endothelial dysfunction result in diastolic hypertension in many with OSAS. (Baguet et al., 2008). Baguet and associates (2008) determined the prevalence of unknown hypertension in OSAS, as well as the targeted damage resulting in the condition. One hundred and thirty five patients from the Grenoble University Hospital sleep lab with symptomatic OSAS participated in this study. Subjects were free of any CVD as well as not taking any vasoactive medications. Blood pressure was taken in a lying position of two separate occasions by a mercury sphygmomanometer, with clinical hypertension identified as a mean of systolic blood pressure of at least 140 mmHg and/or a mean diastolic blood pressure reading of at least 90 mmHg. Ambulatory blood pressure was used and defined as the mean blood pressure and heart rate reading over a 24 hour period, comprising both daytime and nighttime readings. Of the 130 subjects, 46 had hypertension during the regulatory blood pressure measurements and ambulatory blood pressure

measurements. Those with hypertension were older in age, and there was an increased prevalence of higher triglycerides, BMI, and metabolic syndrome components. The results of this study show that only 31.5% of the study subjects were free of hypertension, with 30% having undiagnosed hypertension (Baguet et al., 2008).

Even though there is strong evidence to support the link between OSAS and hypertension, there is more information presented about OSAS and CVD. The Sleep Heart Health Study presented evidence that OSAS is an independent risk factor for congestive heart failure and coronary artery disease (Parati et al., 2007). There is data to support that untreated, moderate to severe OSAS can lead to increased rates of nonfatal cardiovascular events. Before the introduction of nasal continuous positive airway pressure (CPAP) treatment, younger patients with OSAS were encouraged to lose weight, but they still showed a higher morbidity and mortality for CVD, particularly up to 8 years following diagnosis and the start of treatment. OSAS treatment is important to improve overall aspects of the disorder; there is no concrete evidence to suggest that those compliant with CPAP therapy have a lower mortality rate based on the general population (Parati et al., 2007). Even though OSAS is said to have independent associations with CVD, this particular population of people has many existing cardiovascular risk factors including obesity, hyperlipidemia, older age, male gender, smoking history and possibly excessive alcohol intake. As mentioned earlier, hypertension is extremely prevalent in this population, particularly hypertension that is drug resistant. According to McNicholas (2007), up to 83% of those with OSAS have uncontrollable hypertension, regardless of efforts of taking at least three antihypertensive drugs.

It is still believed that even though weight reduction, mandible devices, and perhaps surgery may help to improve the risk factors associated with CVD in OSAS, CPAP therapy has the greatest support for improving cardiovascular mortality. As reported by McNichols (2007), a study with two groups, one receiving CPAP therapy and the other receiving a table placebo, showed a significant fall in blood pressure among OSAS patients when they underwent CPAP treatment. The reduction was greatest in those with severe OSAS. OSAS has been associated with coronary artery disease, due to myocardial ischemia presented during nocturnal reductions in oxygen. As reported by McNichols (2007), CPAP therapy given to OSAS patients who had existing ischemic heart disease produced a decline in overall ischemia. The Sleep Heart Health Study showed a relationship among OSAS and nighttime cardiac arrhythmias including atrial

fibrillation and some ventricular arrhythmias. CPAP therapy can reduce cardiac arrhythmias, and those with OSAS who refuse treatment can have a higher occurrence rate of atrial fibrillation. The connection between OSAS and stroke is still unclear, due to the uncertainty about its potential cause and effect relationship. Strokes can be caused by either OSAS or central sleep apnea, and further research is needed for more precise evidence (McNichols, 2007).

The Metabolic Syndrome and insulin resistance are very prevalent in OSAS along with hypertension and CVD. Data suggests that in accordance with OSAS, hypercytokinemia is associated with obesity and insulin resistance. Cytokines such as leptin, interleukin-6 and tumor necrosis factor-alpha are related to obesity and are consistently elevated in the serum and adipose tissue of obese individuals (Alam et al., 2007). These elevated cytokine levels in OSAS can also affect insulin levels, and can influence the development of the Metabolic Syndrome due to the pro inflammatory state. In a study done by Vgontzas and associates (2005), 14 obese men with OSAS and 11 BMI and age matched obese men without OSAS were assessed for mean fasting blood glucose levels. Fasting blood glucose levels were higher in those with OSAS (106.6 ± 4.1) versus those without OSAS (85.4 ± 4.4). Mean plasma insulin levels were higher in OSAS subjects than their obese non-OSAS controls (25.7 ± 42 vs. 14.6 ± 2.5). Vgontzas et al. (2005) also reported that even non-obese individuals with OSAS had some form of insulin resistance, and even those with a mild form of OSAS presented insulin resistance. There is persuasive evidence to show that there is an independent relationship among OSAS and insulin resistance .

Associations with Diet

Higher energy intake and a preference for high fat foods is hypothesized in sleep-disrupted adults, according to Rontoyanni and associates (2007). In men who have slept on average 4 hours for two consecutive nights, there has been an increase in appetite especially for more energy dense foods. Carbohydrates typically comprise the majority of dietary intake with 40-50% of the overall diet consisting of carbohydrates. One gram of carbohydrate, produces four calories, whereas fat is more energy dense with one gram consisting of nine calories. Carbohydrates and dietary fats are a predominant energy source. Excessive carbohydrate is converted into fat and stored in the adipose tissue, with excessive fat being stored subcutaneously (Sport Nutrition, 2004). Many people with OSAS have abnormal eating habits that are associated with compensating for their excessive sleepiness throughout the day (Oki et al., 1999). These habits may include constantly eating and consuming high quantities of coffee,

soda or other caffeinated beverages. These abnormal eating habits can exacerbate their associated risk factors of OSAS, particularly obesity, and hypertension (Oki et al., 1999).

A way to assess diet quality and an individual's dietary intake is to use the Healthy Eating Index (HEI). The HEI is used to help promote health and the prevention of diseases. The HEI score can be used to examine associations between diet and OSAS. The HEI contains 10 components that replicate the recommendations associated with the Food Guide Pyramid and the 2005 Dietary Guidelines for Americans. A possible score from each component can range from 0 to 5, 0-10 and 0-20, depending on the level of dietary intake with a maximum score of 100 (Knutson et al., 2007; USDA, 2005). Few studies have looked at the relationships among OSAS and overall nutrient and caloric intake, and inconclusive results have supported the use of nutritional intervention to improve OSAS aspects (Selmi et al., 2007; Vasquez et al., 2008). It is important to evaluate OSAS patient diet to determine if specific nutrients may be beneficial in improving certain aspects of the disorder (Selmi et al., 2007).

Endocrine dysfunctions: Leptin

Adipose tissue is a site of energy storage in the form of triglycerides during periods of excessive feeding. Adipose tissue is an endocrine organ, which releases adipokines (Johannsen et al., 2008). Adipokines are proteins associated with physiological complications connected with excessive adiposity. Adipose tissue, particularly centrally located, acts specifically as a key controller in inflammation. When adipose tissue releases pro-inflammatory cytokines it can affect and deregulate many systems, such as glucose metabolism (Johannsen et al., 2008). Stimulation of adipokines, such as over eating, lack of physical activity and age can lead to an increase in cytokine secretion and can result in insulin resistance and diabetes. About 34-39% of those with diabetes have elevated cytokine levels, and these levels are not entirely explained by an increase in BMI (Johannsen et al., 2008).

Leptin works with neural signs and produces a feedback process that controls hunger and satiety (Platat et al., 2006). Sleep disruption can cause an increase in leptin levels as much as 18% compared to baseline values, as well as a 24% increase in hunger and a 23% increase in appetite (Franks et al., 2007). Leptin controls fatigue and energy expenditure. Therefore, leptin controls both sides of the energy balance equation. In obese individuals, leptin can be decreased, leading to physical inactivity as well as insulin resistance. Leptin and insulin sensitivity are crucial in glucose metabolism, and leptin appears to act as an insulin sensitizer when levels are

low or normal. When leptin is chronically elevated, it may lead to an insulin resistant state. Leptin and physical activity levels share a similar relationship, as does leptin and insulin resistance. In a cross-sectional epidemiological study, leptin levels were negatively related to physical activity, independently of obesity (Franks et al., 2007). A summary of the general neuroendocrine effects of leptin on the body can be seen in **Figure 1, Appendix C.**

Abnormal levels of adipokines, specifically leptin may be associated not only with obesity, but with OSAS (Ahmed, 2008). OSAS is associated with excessive daytime sleepiness, as well as hypoxemia and nighttime upper airway collapse and obstruction. These characteristics of the disorder increase sympathetic nervous activity. This sympathetic nervous activation may be associated with abnormal leptin levels that are related to OSAS. These abnormal leptin levels may be related to obesity, which is often presented with OSAS (Ahmed, 2008).

In obese OSAS subjects, leptin levels are indeed higher than in normal weight non-OSAS counterparts (Barcelo et al., 2005). In this study, they recruited 23 obese patients with OSAS and 24 non-obese patients with OSAS, along with 19 obese and 18 non-obese subjects without OSAS. Full diagnosis of OSAS was done by laboratory polysomnography. Blood samples were collected and leptin analysis was done thereafter. Non-obese OSAS patients had higher leptin levels compared with non-obese controls (11.5 ± 1.6 vs. 5.5 ± 0.5 ng/ml), and obese OSAS patients had similar leptin levels compared to obese controls (24.5 ± 1.7 vs. 24.7 ± 3.5 ng/ml). It is still unclear what increases in leptin levels have to do with obesity, or the disease of OSAS itself.

Increased regional body fat accumulation, such as abdominal adipose tissue leads to elevated levels of leptin secretion leading to an inflammatory state. This inflammatory state can lead to development of insulin resistance, and the metabolic syndrome (Harsch et al., 2005). OSAS has been associated in promoting storage of adipocytes in the abdominal region, and therefore, leading to the above mentioned disorders. However, a remarkable decrease in leptin and central abdominal fat is presented in those who have regular compliance with CPAP treatment (Harsch et al., 2005). These findings suggest that OSAS directly influences adipose tissue, and CPAP treatment is effective in reducing intermittent hypoxia and encouraging regular physical activity. CPAP treatment reduces systemic circulation of leptin, in accordance with reduced intermittent hypoxia; longer sleep duration and a reduction in inflammatory responses (Harsch et al., 2005). Along with CPAP therapy, nutritional intervention should be included to

help increase CPAP compliance, and to decrease abdominal adiposity and improve circulating leptin levels (Harsch et al., 2005).

Markers of Body Composition

OSAS affects approximately 4-6% of the male population, and 60-90% of those individuals are obese. Fat depositions around the neck make the upper airway more likely to collapse when lying in a supine position. Those who present with OSAS usually have respiratory problems such as reduced lung capacity, and hypoxia compared to their normal weight counterparts (Martinez-Rivera et al., 2008). Obesity in OSAS is associated with the Metabolic Syndrome, mainly due to the increased amount of central abdominal fat. Abdominal fat is a stronger prediction of OSAS comorbidites than BMI. Waist measurements in OSAS are more predictive of the disorder than BMI and can be a good estimate of central abdominal fat (Martinez-Rivera et al., 2008). A large neck circumference is a predictor of the disorder. A neck circumference of 43.8 centimeters in men has been identified as a risk factor for OSAS. Obesity and inflammation in OSAS subjects is associated with an increase in oxidative stress, as well as an excessive increase in macronutrient intake (Alam et al., 2007).

Associations of OSAS and Diet

There have been very few studies, regarding OSAS that have reviewed and discussed the use and relationship of using antioxidant supplements or dietary intake to improve oxidative stress in OSAS (Baldwin et al., 2005). There have been significant associations between nighttime hypoxemia and CVD. The cardiovascular risk factors associated with CVD are commonly connected with the Metabolic Syndrome and OSAS. Repeated upper airway constriction, induces hypoxia and sleep disturbances, results in oxidative stress, inflammation, and increased sympathetic activation. There is increased evidence that suggests endothelial dysfunction is associated with OSAS, which can exacerbate CVD risk. Some theories to explain the worsening of cardiovascular risk factors in OSAS are the release of oxygen free radicals and pro-inflammatory cytokines (Baldwin et al., 2005). Baldwin et al. (2005) suggests that increased levels of oxidative stress are in accordance with a decrease in antioxidant intake encouraging the development of CVD in OSAS. A diet that is low in antioxidants, and has increased pro-oxidant intake can aggravate oxygen free radical damage that occurs during OSAS, therefore contributing to the worsening of CVD. Free radicals are formed during episodes of abnormal metabolic processes, especially during OSAS. The free radical, nitric

oxide plays an important role in vasodilatation and in OSAS subjects, with morning nitric oxide levels being suppressed (Baldwin et al., 2005). High levels of antioxidants have been shown to lead to a lower stroke incidence, and a reduced incidence of CVD (Baldwin et al. 2005). The DASH diet, a diet rich in fruits and vegetables, whole grains, and low in dairy products is used in dietary interventions, and results in an increase in antioxidants and a decrease in oxidative stress and blood pressure in obese individuals with hypertension. There is evidence that a combination of both Vitamin C and E can have more beneficial effects than just Vitamin C alone (Baldwin et al., 2005). In a study by Grebe et al (2006) intravenous injection of Vitamin C decreased the amount of free oxygen radicals that are circulating in the body, as well as repaired nitric oxide levels resulting in an overall improvement of endothelial function. The HEI is another important tool to use to help determine the amount of fruits and vegetables consumed in the diet, as well as dairy products which can help decrease risk of inflammation and the risk of developing the metabolic syndrome (Kelishadi et al., 2008).

Instrumentation and key measures

Body composition

There has been an increase in obesity over the preceding years, particularly an increase in visceral fat and there are many ways to assess overall body composition. Computed tomography (CT) and magnetic resonance imaging (MRI) can be used to accurately measure adipose tissue and skeletal muscle mass, but these methods are impractical in a clinical setting due to the high cost of using both CT and MRI, as well as the high level of radiation exposure that can be presented with CT. On the other hand, dual energy x-ray absorptiometry (DXA) is a more cost efficient, less invasive and more readily available method for measuring body composition. As reported by Chen and associates (2007), previous studied have shown a high correlation between measurements obtained by the DXA and those acquired from both the CT and MRI (Chen et al., 2007). In a study by Snijder et al. (2002), researchers investigated the use of DXA and combined anthropometric measurements for visceral adiposity, and compared that date to the data received by CT. The scanning of 150 subjects took place, between the ages of 70-79 years, illustrated that total central abdominal fat was correlated with the same readings done by the CT ($r = 0.93$). The previous results state that DXA is as effective as CT in measuring visceral fat.

The use of using the DXA to measure body composition in the overweight and obese has increased in popularity over the years. There are some limitations when assessing ones body composition in the overweight and obese subjects, which are mainly due to incorrect positioning of the body and failure to analyze the scans properly. Brownbill et al. (2005) suggested the usage of half-body DXA scans were the most accurate procedure to use while assessing adipose tissue. To minimize error and improve accuracy, measuring both halves of the body and adding them up seems to be precise, but more research is needed in the methodology. Weekly whole body scans were performed on soft-tissue measurements to determine the coefficient of variance for a study group. The reliability tests produced a coefficient of variance of 1.07% for fat-free mass, 1.75% for overall fat mass, and 1.79% for body fat percentage (Nickols-Richardson et al., 2006).

Biomarkers

RIA for endocrine function: Leptin

Leptin is very stable when frozen for at least five cycles of freeze/thaw regardless of whether serum, plasma or cerebrospinal fluid is used. Leptin was first isolated through an immuno-precipitation/Western blot technique using antibodies that are raised against the first 20-amino terminal amino acids of leptin. Nevertheless, this technique was not very effective or efficient, and a more precise way to isolate leptin was needed. Linco Research Inc developed a more precise, and quantitative radioimmunoassay kit. This assay is widely used in research; with over 80% of current literature measuring human leptin levels uses this assay (Wallace et al., 2000). Normal leptin values for men aged 16-24 yrs are between 2.0-5.6 μg/L (Wallace et al., 2000). Mars and associates (2003) determined the coefficient of variance for a serum leptin radioimmunoassay to be between 3% to 8% for the intra-assay coefficient, and 4% to 8% for the inter-assay coefficient.

Home Somnography

Embletta at-home nighttime screening device

OSAS is a disorder that is usually diagnosed by an in laboratory overnight sleep test known as a polysomnography (PSG). There can be long waiting lists for a PSG due to limited resources such as sleep clinics and beds for patients. According to Latta et al. (2005), the coefficient of variance for an RDI score produced by a PSG is 6%, representing a high reliability. The lag time between scheduling a PSG, and diagnosis and treatment can be long and untreated

OSAS can not only be very harmful to the patient, but to the economy due to increased health care usage. Home sleep tests have been developed and there are many benefits to using these systems including decreased costs, convenience, and improved sleep quality for the patient. It is believed that those using an at home sleep device have better sleep efficiency, more time spent in rapid eye movement sleep, and significantly fewer arousals when compared to a PSG (Dingili et al., 2003). The Embletta PDS (Portable Diagnostic System) is a pocket-sized digital recording device that can be used for at home sleep testing. The device records up to 12 hours of respiratory data that can be reviewed easily and analyzed using the Somnologica software. The Embletta measures nasal airflow, chest and abdominal movements, blood oxygen levels and snoring patterns. The Embletta device has a high agreeability with the laboratory PSG (kappa coefficient: 0.54) (Dingli et al., 2003).

Dietary Evaluation

Nutritionist Pro software

Nutritionist Pro (First DataBank, Inc., San Bruno, CA) software is the latest software being used that has the most comprehensive food data set. Nutritionist Pro uses a nutrient database that originates from the Continuing Survey of Food Intake of Individuals (Spencer et al., 2005). This nutritional analysis software provides accurate nutrient data, and takes the subjects diet record and compares it to specific nutrient requirements based on their individual My Pyramid reports. Diet analysis is done by evaluating food recalls or food frequencies based on nutritional requirements, and final reports can be rapidly viewed and printed for clients based on their menu analysis. Nutritionist Pro is used by many professionals to advance nutritional assessment and knowledge (Spencer et al., 2005).

The Healthy Eating Index

The Healthy Eating Index (HEI) is an effective way to assess an individual's dietary quality. The HEI is used to help promote health and the prevention of diseases. The HEI contains ten components that replicate the recommendations associated with the Food Guide Pyramid and the Dietary Guidelines for Americans. A possible score from each component can range from 0 to 5, 0-10 and 0-20, depending on the level of dietary intake with a maximum score of 100. Components measure the degree to which the participants diet conforms to the Food Guide Pyramid based on the 5 major food groups as well as total fat, saturated fat, cholesterol

and sodium intakes. The final component measures the variety of foods consumed in the diet based on the averages of the days recorded (Kelishadi et al., 2008). A maximum score of 100 represents an ideal diet, consisting of a wide and appropriate range of nutrients to meet ones recommended daily value (USDA, 2005).

Summary

Obesity has become an epidemic in this country due to increased dietary intake and decreased physical activity, which can lead to co-morbidities such as CVD and OSAS. The typical diet of the overweight and obese includes high amounts of carbohydrates, saturated fats and refined sugars, with little emphasis on vegetable and fruit intake. Circulating leptin levels are increased during obesity, interfering with signals of satiety and sensations of being full from the hypothalamus. An inflammatory state producing increased adipokine release such as leptin can lead to a leptin resistant state. A resistance state is usually caused by the opposition of the signaling receptors to respond, as wells as a decreased ability of the blood-brain barrier to transport leptin into the brain.

OSAS is characterized by repetitive collapse of the upper airway during sleep, and is a common disorder associated with obesity. Common symptoms of OSAS include excessive daytime sleepiness and fatigue, which can decrease ones quality of life. Untreated OSAS can lead to many complications particularly hypertension, congestive heart failure, CVD, and stroke. These complications can be alleviated when treatment is introduced, including a physical activity and diet plan. Dietary patterns of those with OSAS resemble those who are overweight and obese. Increased dietary intake can result in obesity in OSAS, as well as leptin resistant state. The association between dietary intake, body composition and OSAS needs further investigation.

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CHAPTER III
JOURNAL MANUSCRIPT

DIET, BODY FAT DISTRIBUTION, AND SERUM LEPTIN IN YOUNG MEN WITH UNDIAGNOSED OBSTRUCTIVE SLEEP APNEA SYNDROME

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ABSTRACT

Background and Purpose: Little is known about influences of obstructive sleep apnea syndrome (OSAS) on dietary intake and body composition. The purpose of this study was to evaluate dietary status, body fat distribution and leptin in overweight young men with and without OSAS in comparison to published values for normal weight counterparts. **Methods:** Groups were comprised of 24 sedentary overweight young men with and without OSAS, who had a body mass index (BMI) greater than 25 kg/m^2. Serum leptin concentration was measured in the 24 subjects using radioimmunoassay, while OSAS assessment was done using nighttime home somnography. Analysis of 4-day diet recalls was performed using Nutritionist Pro (First DataBank, Inc., San Bruno, CA). A Healthy Eating Index (HEI) score was calculated for the 24 overweight subjects. **Results:** There were no differences between the two overweight groups for total fat mass, central abdominal fat, BMI, waist circumference, leptin, or the HEI. The HEI was not predictive of overall OSAS severity; however, BMI was moderately related to OSAS severity ($r = 0.39$; $p=0.05$). The normal weight group did have a 50% higher report of carbohydrate intake, and consumed on average, 500 more kilocalories per day. The normal weight group consumed 50% less sodium, and 50% more Vitamin's C and E including a 13% increase in the HEI. **Conclusions:** Regulation of eating behavior and related influences on diet composition may be affected by a number of neurohormonal disturbances associated with OSAS and/or obesity, itself. Further research is needed to quantify these possible differences on dietary status and the underlying mechanism involved.

Introduction

During the past decade, the prevalence of obesity has continued to increase, not only in the United States but also in most developed countries. The National Health and Nutrition Examination Survey reported that 127 million people are overweight, which is described as having a body mass index (BMI) between 25 kg/m^2 to 30 kg/m^2, 60 million individuals are obese (BMI greater than 30 kg/m^2), and 10 million people are morbidly obese (BMI greater than 40 kg/m^2). About 325,000 people die annually due to obesity related disorders, such as cardiovascular disease (CVD) and untreated obstructive sleep apnea syndrome (OSAS) [1]. OSAS is a disorder characterized by repetitive collapse of the upper airway during sleep and results in hypoxia, hypercapnia, disruption in sympathetic nervous system activity, and fragmented sleep [2]. It is estimated that approximately 20% of the population as a whole displays symptoms of OSAS, particularly a respiratory disturbance index (RDI) value of greater than 5 [3]. Sleep loss and restriction associated with OSAS has increased in parallel with obesity and its development is associated with declining physical activity and excessive food intake. Sleep deprivation may cause changes in appetite regulation due to disturbances in glucose metabolism, reduced vitality, lack of motivation to exercise, and increased caloric intake associated with elevated serum leptin [4].

Leptin is produced by fat tissue and helps regulate body weight, metabolism, and food intake [1]. Leptin works with neural signals and produces a feedback process that controls hunger and satiety [5]. Sleep disruption can cause an increase in leptin levels as much as 18% compared to baseline values, as well as a 24% increase in hunger and a 23% increase in appetite [6]. There is little known about dietary patterns specific to OSAS , but high energy intake and a preference for high fat foods are hypothesized as factors that contribute to obesity in sleep-disrupted adults [7]. In men who have slept on average 4 hours for two consecutive nights, there has been an increase in appetite especially for energy dense foods. Many people with OSAS have abnormal eating habits that are associated with compensating for their excessive daytimes sleepines [8]. These habits may include constantly eating and consuming high quantities of coffee, soda or other caffeinated beverages. These abnormal eating habits can exacerbate their associated risk factors of OSAS, particularly obesity and CVD [8].

The primary aim of this study was to determine whether young overweight men with and without undiagnosed OSAS, as well as young, normal weight men differed in their diet

composition and caloric intake. A secondary aim was to evaluate serum leptin and body composition markers in young, normal weight men, as well as overweight men with and without OSAS based on their dietary and caloric intake. The specific hypotheses were to determine if overweight young adults with OSAS had a higher energy consumption, and more energy storage based on total kilocalories consumed compared to overweight young men without OSAS, and normal weight, young men. Therefore, the OSAS group would have an excess of overall and central abdominal fat, and a body phenotype that predicts chronic disease compared to overweight young adults without OSAS, and normal weight young men. Finally, it has been hypothesized that BMI and HEI scores may predict the overall severity of OSAS based on the RDI.

Materials and Methods

Study Sample

This was a cross sectional study, and involved a secondary analysis of data, with additional data to be analyzed. Young, overweight men between the ages of 18-23 years of age, with a BMI of greater than 25 kg/m^2 were recruited from the Virginia Polytechnic Institute and State University and the surrounding communities. Exclusion criteria included: (1) using tobacco or nicotine products; (2) currently or within the past 6 months regularly engaging in physical activity (> 30 minutes of moderate to vigorous activity per day for 3 or more days per week); and (3) not being able to complete an at-home sleep test. Following evaluation of inclusion and exclusion criteria, if subjects qualified they completed the Epworth Sleepiness Scale (ESS), were interviewed to assess sleep and snoring history, and completed a health history to identify factors that might contribute to sleep problems. All methods and procedures were authorized by the IRB of Virginia Tech and explained to the subjects, who read and gave written informed consent. These young, overweight men and their measurements of interest were compared to normal weight, age matched counterparts from current literature. This data was used to compare body composition, serum leptin, and dietary habits between young, normal weight men and young, overweight men with and without OSAS.

Embletta PDS

Subjects were tested for OSAS according to the overnight home sleep test, using a home somnography device (Embletta ® PDS, Medcare®, Reykjavik, Iceland; city of manufacture). The Embletta is a reliable source for determining RDI scores, and has a high degree of concordance with the laboratory PSG [9]. Study personnel were trained on setting up and using the Embletta, and instructed participants on the setup and home use of the somnography device. The Embletta quantifies nasal flow, oxygen saturation, heart rate, snoring frequency and magnitude, as well as thoracic and abdominal breathing effort. Subjects returned the device following the night of testing and the data were downloaded and evaluated using proprietary software (Somnologica, Version 3.1.2, Medcare®, Reykjavik, Iceland). A trained sleep technologist interpreted these records and a sleep physician confirmed the scores. Thereafter, the physician-confirmed respiratory disturbance index (RDI), scores were used to classify subjects, with OSAS defined as an RDI of greater than 5 events/hour.

Body Composition

The dual x-ray absorptiometry (DXA) scan was used to assess total body/central adiposity and fat-free mass. Central abdominal fat was measured using total body DXA scans, and was defined by the region on scans bounded by the iliac crest and lateral lower margin of the ribcage [10]. All scans were conducted in the BONE Laboratory of Virginia Tech by one Licensed Radiologic Technologist. Technical precision in this lab has previously been demonstrated, i.e. coefficient of variation for central abdominal fat, fat-free mass, overall fat mass, and body fat percentage ranged between 1.1-1.8% [11].

Blood collection and analysis: Leptin RIA

Subjects completed an overnight fasting blood collection in which they were instructed to refrain from eating for 8 hours prior to the blood collection. Blood samples of approximately 50 mL were collected from the arm by venipuncture with a sterile needle and vacutainer by a qualified phlebotomist. All blood samples were processed by a senior lab technician and trained graduate students. Aliquots of 1.2 mL each, plasma and serum samples, were separated from

whole blood via centrifugation and frozen at -80°C for later batch analysis. Serum leptin was assayed by a radioimmunoassay (RIA) (Linco Research, St. Charles, MO; CV = 7.2%).

Nutritional Analysis

Dietary intake for normal weight subjects without OSAS was clarified using current published literature, in comparison to overweight subjects with and without OSAS. In the young overweight groups, diet analysis was performed using 4-day diet records collected from subjects. These diet records were analyzed using diet analysis software, Nutritionist Pro (First DataBank, Inc., San Bruno, CA). This nutritional analysis software provides accurate nutrient data, and takes the subject's diet record and compares it to specific nutrient requirements based on their individual My Pyramid report [12]. Overall macronutrient intake is expressed as an overall percentage of total caloric intake, while micronutrient intake is expressed in milligrams (mg) and micrograms (μg). The HEI is a measure of dietary intake quality that is in accordance with the new Dietary Guidelines for Americans in 2005 [12], with the food groups inspired by the recommendations in MyPyramid [12]. The HEI was determined in a procedure whereby points are awarded based on macronutrient intake. The HEI was calculated by hand and consists of 10 components, grains, vegetables, fruit, milk, total fat, saturated fat, meats, beans, oils, and calories from alcohol consumption [2,4]. A maximum of 10 points was awarded based on the recommended daily intake range for each component, as well an overall dietary pattern representing quality and variety. A maximum of 100 points is possible, and represents a diet with a full range of foods, as well as meeting the needs recommended by the Food Guide Pyramid [13]. According to the USDA, apparently healthy individuals represent a HEI score range from 51 to 80, while approximately 12% of persons have an HEI score of over 80, and 15% of individuals have an HEI of below 50 [14].

Statistical Analysis

All statistical analyses were performed by SPSS ® (SPSS, Inc., Chicago, IL; Version 14.0). Simple descriptive statistics, t-tests, and intercorrelations of dependent measures were computed. Independent t-tests were performed to determine group differences for all descriptive

characteristics in overweight with OSAS vs. overweight without OSAS. Overall comparison's between the overweight with and without OSAS groups, and the normal weight group was done so numerically or was based on percentage differences for dietary measures. Linear regression was used to determine if BMI and the HEI predicted overall OSAS severity based on the RDI score. Significance levels were determined throughout by $p<0.05$.

Results

Subject Characteristics

Baseline physical characteristics and sleep related measures, presented by group, are shown in **Table 1**. Body composition and morphometric measurements are presented in **Table 2**. There were no significant differences between the OSAS and non-OSAS group for total fat mass, lean body mass, percent body fat and central abdominal fat. There were also no significant differences between groups in relation to hip, waist and neck circumference. The ESS scores were also not significant between the OSAS and non-OSAS groups. RDI scores were significant between the OSAS and non-OSAS groups, with the OSAS group having an average of 15 events/hour ($p<0.05$). RDI did not correlate with any key markers of body composition, including central abdominal fat and waist circumference. Normal weight, young men have a lower BMI, neck and hip circumference, overall fat mass, percent body fat, and RDI in comparison to overweight young men with and without OSAS. These values are represented in comparison in both **Table 1 and Table 2.**

Diet Quality

Each subject was evaluated based on specific macronutrients (**Table 3**) and micronutrients (**Table 4**). There were no significant differences between the OSAS and non-OSAS groups among the macro and micronutrients measured, or for the HEI score. Young, normal weight men presented an average HEI of 63.5 which is an average of 12 points higher than the average HEI in the overweight groups [14]. The normal weight group consumed 13% more carbohydrates, and 10% more total fat than the overweight group. However, polyunsaturated fat was 50% higher in the normal weight group. Vitamin C and Vitamin E were over 50% higher in the normal weight group in comparison to the overweight groups. Finally,

the normal weight group consumed less than half the amount of sodium than the overweight groups.

Serum Leptin

Serum leptin levels were not significantly different between the OSAS and non-OSAS groups. Serum leptin levels in the OSAS group were 9.78 ng/mL vs. the non-OSAS group with leptin levels being 8.09 ng/mL. Normal weight, young men represent an average of 6.2 ng/mL for serum leptin, representing lower levels in comparison to the overweight with and without OSAS groups [15].

OSAS severity prediction by BMI and HEI

OSAS severity was predicted using two independent variables, HEI and BMI with RDI as the dependent variable as seen in **Figure 1 and Figure 2**. Prediction was not established between BMI and RDI, or HEI and RDI.

Discussion

Obesity is the most important risk factor for OSAS, and about 70% of those with diagnosed OSAS are obese [16]. In a study by Namyslowski and associates, an overall increase in BMI led to a fourfold increase in the risk of developing OSAS [16]. In fact, neck circumference was the most powerful predictor of OSAS among all other measured anthropometric data. This suggests that upper body fat in the neck region, as well as central abdominal fat may be key factors in the development of OSAS [16]. According to Martinez-Rivera et al., waist measurements in OSAS are predictive of the overall disorder, and can be a good estimate of central abdominal fat [17]. Central abdominal fat is strongly associated with OSAS, compared to other types of body fat [16]. In the current study, central abdominal fat, neck, waist or hip circumference was a predicting factor of OSAS. This does not completely agree with current literature, but a larger study sample and more research is needed in a young adult male population. In a young, normal weight population, overall body composition is lower in comparison to overweight young men with and without OSAS. A decrease in BMI, overall fat mass, neck and hip circumference in the normal weight population, may mean a lowered risk of chronic disease and OSAS development.

Even though associations between obesity and OSAS are unclear, the balance in regulation of energy intake and expenditure that can lead to weight gain is controlled by leptin. Several previous studies have reported that those with OSAS have increased plasma leptin levels [18]. Barcelo and associates also reported that leptin levels in non-obese individuals with OSAS were higher than non-obese controls. Our study showed leptin levels of the OSAS group, as well as non-OSAS group to be elevated above normal levels for 16-24 year olds by about four fold [19]. However, in a study by Spiegel and associates, healthy normal weight men who underwent sleep restriction had increased serum leptin levels, confirming that sleep disruption even in normal weight, healthy men can cause increased leptin levels [20].

Leptin is a component of this dual control system, regulating caloric intake and expenditure [21]. Changes in hormonal levels seen in OSAS and the obese can result in increased appetite and may impact dietary intake with limited fruits and vegetables, as well as excess sucrose and fat intake [7]. In the current study, there were no differences seen between overweight groups for overall macronutrient intake. However, the normal weight young men differed in their nutrient intake in comparison to the overweight groups. Normal weight men consume a higher total fat, including higher polyunsaturated fat and an overall lower carbohydrate diet. The normal weight group did consume about 500 more kilocalcories per day than the overweight groups. An increase in activity levels explains the increased fat and caloric intake seen in the normal weight, young men. The normal weight group represented a higher HEI score compared to both overweight groups, suggesting that regardless of their increased total kilocalories their diet quality is better than the overweight with and without OSAS groups. A low HEI score may predict an increase in overweight and obesity in OSAS individuals, which can lead to decreased physical activity and resting metabolism. HEI is a good predictor of overall obesity indices such as BMI and waist circumference, but there is some specificity lacking in predicting OSAS [23]. The current study did not show a significant relationship in predicting OSAS severity by the HEI, but more research and a larger sample size is needed. Vitamin C and E were also reported to be 50% higher in the normal weight group, in comparison to the overweight groups. Increased levels of vitamin C and E in the normal weight group may be attributed to their overall healthier dietary intake. Also, physical activity in normal weight individuals leads to increased reactive oxygen species formation, and supplementation with Vitamins C and E can help prevent oxidative stress in the body. Overall healthier individuals

may supplement with antioxidant vitamins, explaining the drastic difference seen between the normal weight and overweight groups [22]. Finally, the normal weight group did consume less than half the amount of sodium than what was reported in the overweight groups. In a study by He and associates, increased sodium intake is associated with an overall increased consumption of sugar, including soft drinks [23]. As previously mentioned, those with OSAS tend to consume caffeinated beverages such as coffee, and soft drinks in order to make up for their excessive daytime sleepiness [8]. This consumption of soft drinks leads to increased sugar intake and can influence sodium as well. Therefore, this may be a potential explanation of such a remarkable difference seen in the normal weight and overweight group's sodium levels.

The current study hypothesized that BMI is predictive of OSAS severity based on the RDI. However, BMI trended toward significance in predicting OSAS severity ($r=0.39$; $p=0.05$), with only 15% of RDI variance being explained by BMI. In a study by Pillar and associates there was a significant correlation between BMI and RDI, proving that BMI is a good tool to use as a predictor for OSAS in the overweight and obese [24]. Larger studies support the use of BMI to predict RDI, but the current study lacks support due to a small sample size.

In conclusion, the role of obesity, leptin and the feedback mechanism controlling appetite is still unclear. Regulation of eating behavior and related influences on diet composition may be affected by a number of neurohormonal disturbances associated with OSAS and/or obesity, itself. Further research is needed to quantify these possible differences on dietary status and the underlying mechanism involved.

Table 1. Mean (± SD) Physical and Physiological Characteristics in Overweight Subjects with (OSAS) and without Obstructive Sleep Apnea Syndrome (OWT) contrasted with published values of Normal Weight, Young Men (NWT)

Variables	OWT (n=12)	OSAS (n=12)	NWT (n>100)
Age (years)	21.4 ± 3.6	22.4 ± 2.3	20.4 ± 1.4[25]
Height (cm)	175.6 ± 3.8	177.8 ± 5.9	176.9 ± 6.5[25]
Weight (kg)	95.8 ± 14.3	96.2 ± 15.4	68.8 ± 6.7[25]
BMI (kg/m^2)	30.5 ± 4.5	30.3 ± 1.2	21.9 ± 1.5[25]
RDI (events/hr)[a]	2.2 ± 1.6[a]	14.7 ± 13.5[a]	1.8 ± 0.9[26]
Leptin (ng/mL)	8.1 ± 7.2	9.8 ± 4.2	6.2 ± 3.8[15]

Values are expressed as mean ± SD; *p*-values for t-test between groups.
BMI: Body Mass Index; RDI: Respiratory Disturbance Index; ESS scores: Epworth Sleepiness Scale scores
Numerical subscripts represent citation numbers for studies listed at the end of the chapter.
[a] Significantly different between groups, $p<0.05$

Table 2. Mean (± SD) Morphometric and Body Composition measures in Overweight Subjects with (OSAS) and without Obstructive Sleep Apnea Syndrome (OWT) contrasted with published values of Normal Weight, Young Men (NWT)

Variables	OWT (n=12)	OSAS (n=12)	NWT (n >10)
Neck circumference (cm)	40.6 ± 3.1	40.0 ± 2.3	35.5 ± 4.8[27]
Hip circumference (cm)	109.3 ± 8.7	113.5 ± 8.9	98.1 ± 6[27]
Waist circumference (cm)	94.8 ± 12.1	96.1 ± 9.2	N/A
Fat mass (kg)	24.3 ± 4.8	26.9 ± 1.7	19.0[28]
Central abdominal fat (kg)	8.7±2.4	7.0±1.9	4.5 ± 2.0[27]
Overall Body Fat (%)	24.8 ± 4.8	27.1 ± 4.2	15.8 ± 3.9[27]

Values are expressed as mean ± SD; *p*-values for t-test between groups.
Numerical subscripts represent citation numbers for studies listed at the end of the chapter.

Table 3. Mean (± SD) Overall Macronutrient Intake in Overweight Subjects with (OSAS) and without Obstructive Sleep Apnea Syndrome (OWT) contrasted with published values of Normal Weight, Young Men (NWT)

Variables	OWT (n=12)	OSAS (n=12)	NWT (n > 100)
Carbohydrate (%)	48.9 ± 8.2	52.4 ± 10.4	32.7 ± 0.3[29]
Fat (%)	33.5 ± 8.9	28.0 ± 10.9	41.2 ± 0.5[29]
Protein (%)	16.6 ± 4.5	17.3 ± 5.1	17.8 ± 0.3[29]
Saturated fat (grams)	33.9 ± 11.5	30.8 ± 15.6	45.0 ± 12.0[29]
Polyunsaturated fat (grams)	10.1 ± 3.3	10.4 ± 5.1	27 ± 1.0[29]
Total Sugar (grams)	101.4 ± 61.4	95.5 ± 68.5	95 ± 3.1[29]
Total kcals	2353 ± 625	2595 ± 530	2912 ± 54.0[29]
HEI	54.6 ± 12.5	47.8 ± 14.3	63.8 ± 5.0[29]

Values are expressed as mean ± SD; *p*-values for t-test between groups.
%: percent of total calories consumed
Numerical subscripts represent citation numbers for studies listed at the end of the chapter.

Table 4. Mean (± SD) Overall Micronutrient Intake in Overweight Subjects with (OSAS) and without Obstructive Sleep Apnea Syndrome (OWT) contrasted with published values of Normal Weight, Young Men (NWT)

Variables	OWT (n=12)	OSAS (n=12)	NWT (n >100)
Cholesterol (mg)	288.8 ± 226.2	247.3 ± 116.3	511 ± 16.4[29]
Sodium (mg)	4502± 1078	4134 ± 1315	1500[30]
Iron (mg)	16.8 ± 4.3	20.6 ± 6.4	8[30]
Chromium (μg)	0.03 ± 0.02	0.02 ± 0.02	35[30]
Copper (mg)	0.8 ± 0.3	0.9 ± 0.3	0.9[30]
Selenium (μg)	82.2 ± 30.5	93.8 ± 38.6	55[30]
Zinc (mg)	9.7 ± 4.3	10.9 ± 4.1	18 ± 0.6[29]
Magnesium (mg)	218.7 ± 89.5	234.4 ± 73.2	420[30]
Vitamin C (mg)	76.4 ± 77.4	67.1 ± 39.7	168 ± 91.0[31]
Vitamin E (mg)	3.5 ± 4.6	4.8 ± 2.8	10.1 ± 5.2[31]

Values are expressed as mean ± SD; *p*-values for t-test between groups.
Numerical subscripts represent citation numbers for studies listed at the end of the chapter.

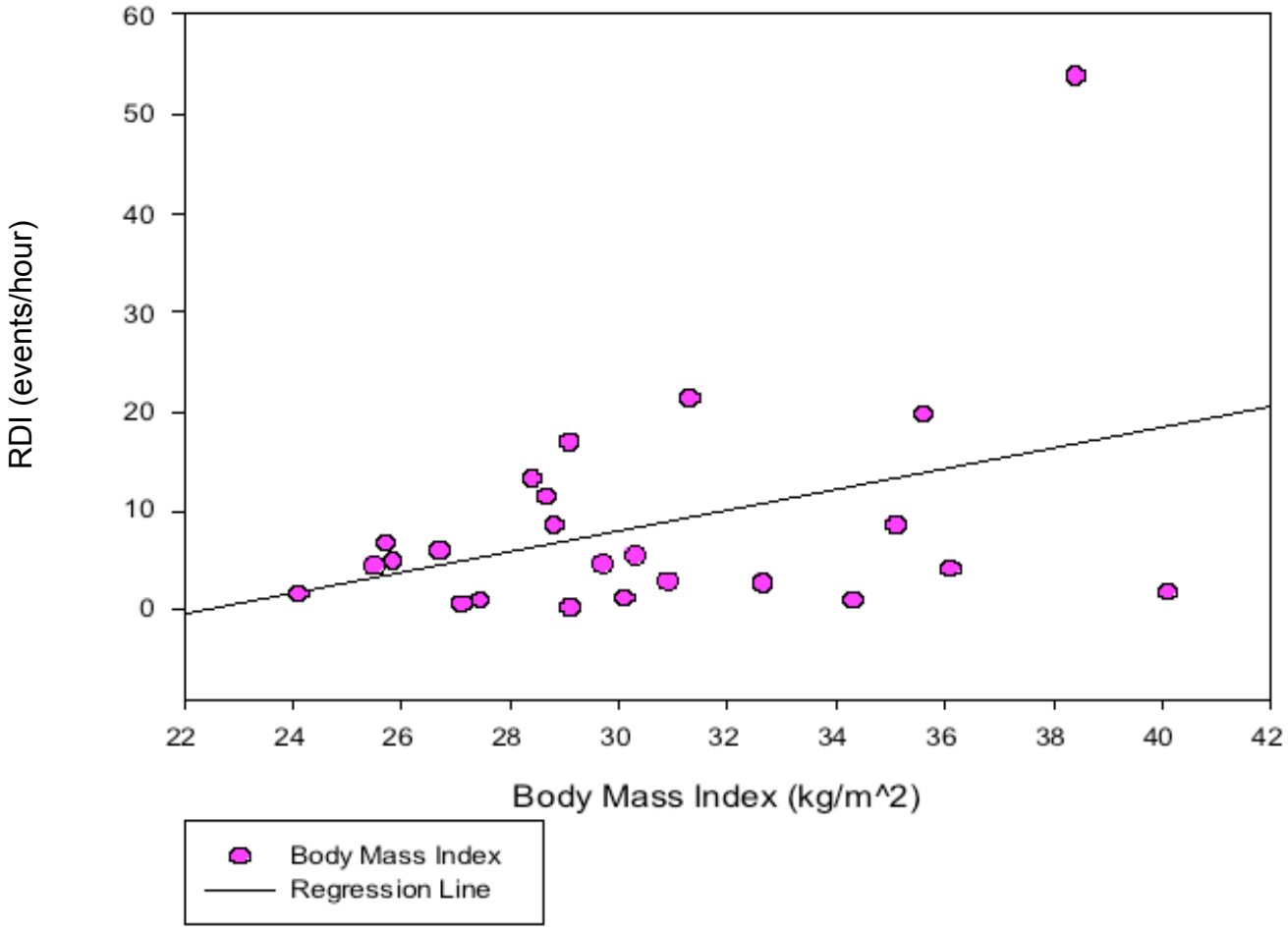

Figure 1. Prediction of OSAS severity (RDI) by BMI r = 0.39; p = 0.05

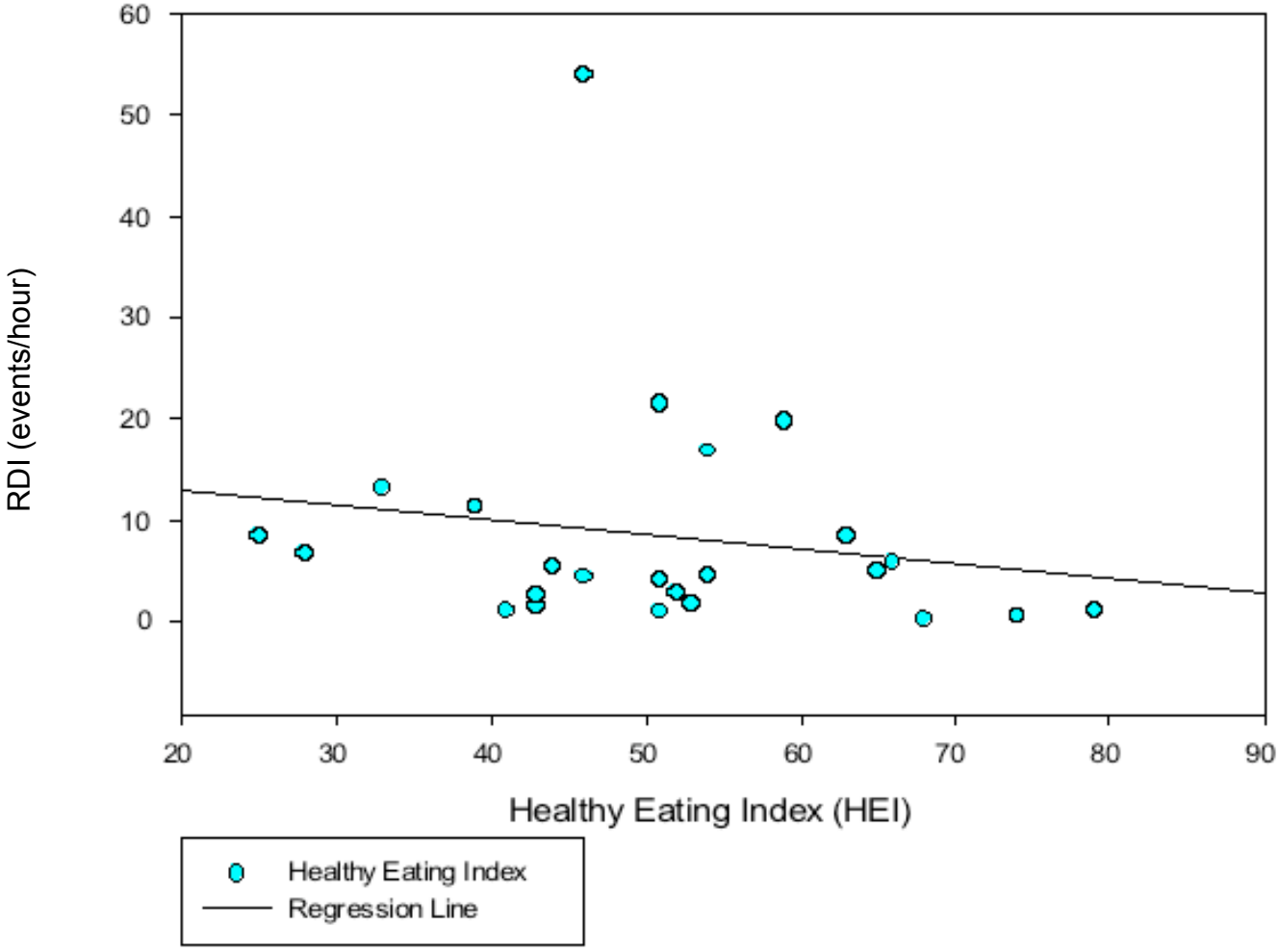

Figure 2. Prediction of OSAS severity (RDI) by HEI r = -0.18; p = 0.05

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CHAPTER IV
SUMMARY, FUTURE RESEARCH AND CLINICAL IMPLICATIONS

Summary

Obesity is a worldwide epidemic that has increased dramatically over the past decade. About 325,000 people die annually due to obesity related disorders, such as cardiovascular disease (CVD). Body weight is increasing due to many factors, particularly a lack of physical activity and unhealthy diets, which are producing increased incidence of Metabolic Syndrome, insulin resistance, diabetes and CVD. Abdominal obesity is a particular concern in the growing epidemic of obesity, and can cause increased cytokine release by the fat tissue leading to the previously mentioned disorders. Obesity, sleep loss and restriction have been associated with changes in energy balance and can result in overall weight gain. Sleep deprivation may cause changes in appetite regulation due to disturbances in glucose metabolism, as well as reducing ones motivation to exercise and increase dietary intake due to elevated leptin levels. Obesity is one of the strongest risk factors for OSAS including an increase in body weight parameters such as BMI, waist/hip ratio, neck circumference, percent body fat, and skin fold thickness have been confirmed in its association to OSAS. Adipose tissue is an endocrine organ that produces many adipokines, particularly leptin which is released during inflammation in response to dysregulation of many systems. Leptin is a peptide hormone produced by fat tissue that helps regulate body weight, metabolism, food intake, and reproductive function. The relationship and mechanisms associated with obesity and OSAS is understood slightly by leptin. Leptin reduces appetite and is one part of the dual control system of energy intake and expenditure regulation. Leptin is high in obese individuals and even higher in those with OSAS. It is not clear why high leptin levels do not lead to a starvation state therefore leading to weight reduction. In obese individuals with OSAS, it is believed that a leptin resistant state occurs and high leptin levels are not adjusted by appetite-promoting peptides. There have been few studies that have explored the relationship of OSAS severity and dietary intake. In sleep-deprived adults, there is a report of higher energy intake particularly of high-fat foods that can lead to obesity. However, there have been inconclusive results to show the relationship between increased energy intake and short sleep duration or sleep fragmentation.

Overweight, sedentary men with and without OSAS between the ages of 18-45 years who had increased energy intake and short sleep duration or sleep fragmentation were involved with this study. Subjects completed initial screening, informed consent, and completed the Epworth Sleepiness Scale before being tested for OSAS. Subjects were tested for OSAS according to the Embletta PDS, an overnight at home polysomnography. Body composition was assessed using the DXA, with total body fat, central adiposity and fat free mass evaluated. A leptin RIA was used to assess serum blood leptin levels following overnight fasting blood sampling. Nutritional analysis was done using 4 day diet records that were analyzed using Nutritionist Pro, with the healthy eating index (HEI) being calculated for each subject. Subject groups consisted of twelve, sedentary, overweight young men with OSAS (BMI: Mean ± SD = 30.5 ± 4.6 kg/m^2, age = 21.4 ± 3.6 yr) vs. twelve sedentary, overweight young men without OSAS (BMI: 30.3 ± 4.0 kg/m^2, age = 22.4 ± 2.3 yr). There were no significant differences among body indices such as total fat mass, lean body mass, intrabdominal fat, BMI, waist or waist circumference. There were no significant differences between the OSAS and non-OSAS groups among the macro and micronutrients measured, or for the HEI score. Young, normal weight men presented an average HEI of 63.5 which is an average of 12 points higher than the average HEI in the overweight groups [14]. The normal weight group consumed 13% more carbohydrates, and 10% more total fat than the overweight group. However, polyunsaturated fat was 50% higher in the normal weight group. Vitamin C and Vitamin E were over 50% higher in the normal weight group in comparison to the overweight groups. Finally, the normal weight group consumed less than half the amount of sodium than the overweight groups. Serum leptin levels were not significantly different between the OSAS and non-OSAS groups. Serum leptin levels in the OSAS group were 9.78 ng/mL vs. the non-OSAS group with leptin levels being 8.09 ng/mL. Normal weight, young men represent an average of 6.2 ng/mL for serum leptin, representing lower levels in comparison to the overweight with and without OSAS groups [15]. OSAS severity was predicted using two independent variables, HEI and BMI with RDI as the dependent variable as seen in **Figure 1 and Figure 2**. Prediction was not established between BMI and RDI, or HEI and RDI.

Future Research

To date, this is the first study to examine the association of obstructive sleep apnea syndrome on dietary patterns, leptin levels and body composition in young men. More research

needs to be conducted in this area. It is known that higher RDI levels are associated with many complications that include increased obesity and leptin levels. RDI severity should be researched further based on the predictive factors of the HEI and BMI. Elevated obesity and leptin levels are related to one another, but it is still unclear as to the exact mechanism that causes these elevated levels in OSAS. It is believed that leptin is elevated in OSAS due to sympathetic nervous activation, which can increase one appetite leading to obesity. OSAS individuals tend to be less physically active due to their excessive daytime sleepiness and fatigue, leading to obesity as well. Many studies presented in the current literature do no look at specific macro and micronutrient intake, but speculate that those with sleep deprivation tend to eat more caloric dense foods and increase caffeine intake. Future studies should focus strictly on overweight individuals, those with and without OSAS such as those presented in our study. Subjects should be meticulously directed to complete their 4-day diet records, focusing on instructing subjects to accurately measure portion sizes, and to eat as they normally would. Following nutritional analysis, nutritional intervention should be incorporated in the study. Subjects should review their nutritional analysis results with a registered dietician. This intervention should include physical activity and nutrition and follow up should be done. This follow up will be used to determine if severity of OSAS is improved due to a combination of nutritional and physical activity. A control group could be included to determine if a nutrition only intervention is still effective in improving OSAS severity. Leptin levels should be monitored throughout to determine if improvements in OSAS due to nutrition could help improve leptin levels and appetite regulation. Body composition markers including total fat mass, fat free mass, and particularly central adiposity should be monitored as well with DXA readings taken before and with follow up. These readings may help to support that improvements in dietary patterns may help to improve body composition indices, as well as OSAS severity and improve appetite regulation by decreased leptin circulation. The quality of life of those with OSAS needs to be a main focus of future research, along with methods of improving their mortality rate with treatment. It is important not to just treat OSAS with CPAP therapy, but to treat the underlying problems such as obesity by changing their lifestyle.

Clinical Implications

Starting with improving the increasing rates of obesity, physicians need to address this problem to help save many lives. Obesity is an underlying problem for many disorders including OSAS and cardiovascular complications. Assessing ones quality of life and improving their lifestyle choices may help to improve their co morbidities associated with OSAS. Behavioral and nutritional interventions along with physical activity regimens should be considered when providing CPAP treatment. Dietary management in those who are overweight, especially in OSAS is very important to improve their overall quality of life. By evaluating the overall trends in nutrition related to OSAS, it is evident that these individuals tend to consume high fat foods particularly high in saturated fat. OSAS individuals consume high carbohydrate food choices, and combined with their increased saturated fat intake, are not making smart food choices to help mange their disorder. Nutritional intervention is key to controlling overweight and obesity, as well as helping to control the increasing numbers of those with OSAS. Evaluating all gamuts of treatment beyond CPAP therapy, can help reduce strain on the economy due to obesity related disorders.

If results can be produced from long term research in the area of dietary effects on OSAS, it will be vital in implementing new treatment options to improve quality of life associated with OSAS. In a clinical setting, those at risk for OSAS and who may be diagnosed with a sleep disorder should undergo nutritional therapy along with the start of an exercise regimen.

APPENDIX A

Leptin RIA Detailed Procedures

1. Pipet 300µl of Assay Buffer to the Non-Specific Binding tubes, 200µl to the reference tubes, and 100µl to the remainder of the tubes.

2. Pipet 100µl of Standards and Quality Controls in duplicate.

3. Pipet 100µl of each sample in duplicate.

4. Pipet 100µl of ^{125}I-Human Leptin to all tubes.

5. Pipet 100µl of Human Leptin antibody to all tubes except Total Count tubes and Non-Specific Binding tubes.

6. Vortex, cover, and incubate overnight (20-24 hours) at 4°C.

7. Add 1.0 ml of cold (4°C) Precipitating Reagent to all tubes (except Total Count tubes).

8. Vortex and incubate 20 minutes at 4°C.

9. Centrifuge, 4°C, all tubes [except Total Count tubes (1-2)] for 20 minutes at 2,000-3,000 xg.

10. Immediately decant the supernate of all tubes except Total Count tubes (1-2), drain tubes for at least 15-60 seconds.

11. Count all tubes in a gamma counter for 1 minute. Calculate the ng/ml of Human Leptin in unknown samples using automated data reduction procedures.

APPENDIX B

RAW DATA

Table 1. Baseline Anthropometric Data in OSAS (Group 1) vs. Non-OSAS (Group 2)

Ss ID	Group	Age (Years)	Weight (kg)	Height (cm)	BMI (kg/m^2)	RDI (events/hr)
014	1	24.00	76.20	171.50	25.50	4.50
016	1	21.00	120.00	172.30	40.10	1.80
023	1	26.00	100.45	184.00	30.10	1.20
025	1	19.00	85.28	177.00	24.10	1.60
030	1	15.00	121.00	179.00	36.10	4.20
032	1	23.00	102.70	173.00	34.30	1.10
033	1	18.00	86.00	170.00	29.70	4.70
034	1	26.00	98.60	178.00	30.90	2.90
035	1	21.00	102.30	177.00	32.65	2.70
036	1	20.00	85.00	177.00	27.10	.60
038	1	18.00	88.00	174.00	29.10	.30
039	1	26.00	84.00	175.00	27.43	4.50
Mean		**21**	**95.7**	**175.7**	**30.6**	**2.2**
SD		**4**	**14.3**	**3.8**	**4.6**	**1.6**
003	2	19.00	96.20	182.90	28.80	8.50
010	2	22.00	77.27	173.00	25.83	5.00
011	2	25.00	103.64	182.00	31.30	21.50
015	2	20.00	79.54	176.00	25.70	6.80
018	2	25.00	101.60	182.40	30.30	5.50
021	2	23.00	84.10	172.00	28.41	13.30
027	2	20.00	121.81	178.00	38.40	54.00
028	2	26.00	80.90	168.00	28.66	11.50
029	2	21.00	91.36	185.00	26.70	6.00
031	2	23.00	106.36	172.50	35.60	19.90
040	2	24.00	89.14	175.00	29.09	16.90
041	2	21.00	122.70	187.00	35.10	8.50
Mean		**22**	**96.2**	**177.8**	**30.3**	**14.7**
SD		**2**	**15.5**	**5.9**	**4.1**	**13.6**

Table 1 (Con't)

	Group	Neck circumference (cm)	Waist circumference (cm)	Hip circumference (cm)
014	1	37.00	77.00	99.00
016	1	47.50	115.00	120.00
023	1	41.00	95.50	100.00
025	1	38.50	85.50	103.00
030	1	43.00	118.00	124.00
032	1	41.50	91.00	119.00
033	1	40.00	92.00	103.00
034	1	42.00	96.00	117.00
035	1	42.00	103.00	113.00
036	1	37.00	86.00	105.50
038	1	41.00	86.00	104.00
039	1	37.00	93.00	104.00
Mean		**40.6**	**94.8**	**109.3**
SD		**3.0**	**12.1**	**8.7**
003	2	41.60	114.50	119.00
010	2	38.10	86.40	98.00
011	2	43.00	104.50	115.00
015	2	36.50	85.00	103.50
018	2	42.00	92.00	114.50
021	2	39.70	96.30	112.00
027	2	41.50	100.00	221.00
028	2	37.00	90.00	107.00
029	2	38.00	84.00	109.00
031	2	42.00	105.00	224.50
040	2	39.00	96.00	109.00
041	2	42.00	100.00	129.00
Mean		**40.0**	**96.1**	**130.1**
SD		**2.3**	**9.2**	**43.9**

Table 1 (Con't)

	Group	ESS Score	Fat Mass (kg)	Lean Body Mass (kg)	Intrabdominal Fat (kg)	% Body Fat
014	1	11.00	13.643	51.949	33.89	20.00
016	1	8.00	39.190	80.776	106.27	31.80
023	1	12.00	24.818	74.414	82.14	24.30
025	1	11.00	14.931	64.143	49.78	18.30
030	1	1.00	41.052	76.478	89.73	34.20
032	1	8.00	26.366	73.805	63.57	25.30
033	1	11.00	21.225	61.966	52.42	24.60
034	1	8.00	26.918	69.587	73.60	27.00
035	1	8.00	23.143	77.750	61.19	22.20
036	1	10.00	17.169	66.295	50.92	19.80
038	1	12.00	20.146	67.119	56.15	22.30
039	1	5.00	23.709	57.827	70.38	23.70
Mean		**8.7**	**24.4**	**68.5**	**65.8**	**24.8**
SD		**3.2**	**8.4**	**8.6**	**1.9**	**4.8**
003	2	8.00	28.076	51.949	81.77	26.70
010	2	2.00	17.166	80.776	48.60	21.70
011	2	6.00	27.851	74.414	91.46	26.60
015	2	10.00	21.079	64.143	51.05	26.70
018	2	19.00	22.043	76.478	61.19	21.80
021	2	12.00	23.297	73.805	62.03	27.20
027	2	6.00	35.631	61.966	98.13	29.20
028	2	4.00	22.863	69.586	63.79	27.10
029	2	5.00	20.117	77.750	50.13	21.90
031	2	6.00	39.069	66.295	115.11	35.90
040	2	6.00	25.734	67.119	75.49	28.40
Mean		**7.9**	**26.9**	**68.5**	**77.4**	**27.1**
SD		**4.5**	**7.5**	**9.9**	**2.6**	**4.2**

Table 2. Serum leptin in OSAS (Group 1) vs. Non-OSAS (Group 2)

	Group	**Leptin (ng/ml)**
014	1	4.00
016	1	11.76
023	1	6.50
025	1	3.50
030	1	4.00
032	1	6.50
033	1	7.90
034	1	9.00
035	1	5.00
036	1	3.70
038	1	7.10
039	1	7.10
Mean		**8.09**
SD		**7.2**
003	2	11.50
010	2	3.49
011	2	6.10
015	2	10.30
018	2	5.38
021	2	11.98
027	2	11.30
028	2	9.50
029	2	7.50
031	2	10.80
040	2	6.80
003	2	13.98
Mean		**9.78**
SD		**4.2**

Table 3. Macronutrient Intake in OSAS (Group 1) vs. Non-OSAS (Group 2)

	Group	CHO (% of total kcal)	Fat (% of total kcal)	Protein (% of total kcal)	Saturated Fat (grams)	Monounsaturated Fat (grams)	Polyunsaturated Fat (grams)
014	1	53.8	19.0	27.1	34.46	24.26	11.03
016	1	60.1	20.5	19.4	19.62	16.99	8.21
023	1	45.2	38.2	14.8	26.78	18.08	13.15
025	1	49.7	36.7	13.6	26.79	15.62	7.68
030	1	57.4	34.4	8.2	48.71	8.72	3.99
032	1	37.3	36.6	18.6	41.35	32.05	12.65
033	1	61.2	21.5	17.3	18.20	11.26	6.39
034	1	48.5	34.7	14.8	45.12	32.47	15.29
035	1	51.5	33.5	15.1	33.90	17.65	7.39
036	1	43.7	36.1	20.2	22.40	17.78	10.72
038	1	40.4	43.8	15.8	36.65	25.22	12.38
039	1	38.2	47.0	14.8	53.11	25.51	12.48
Mean		**48.9**	**33.5**	**16.6**	**33.9**	**20.4**	**10.1**
SD		**8.2**	**8.9**	**4.5**	**11.5**	**7.5**	**3.3**
003	2	61.9	19.9	18.2	28.17	10.48	3.44
010	2	54.7	24.8	13.3	18.45	11.24	10.62
011	2	56.5	21.0	22.5	58.67	29.06	15.19
015	2	57.3	9.79	18.7	18.58	18.40	7.09
018	2	46.6	21.4	29.5	28.90	29.87	11.61
021	2	68.8	20.0	11.2	46.53	60.08	30.12
027	2	42.5	39.3	16.4	17.48	10.77	5.16
028	2	64.4	24.7	10.9	19.97	6.07	3.11
029	2	50.8	33.0	16.2	32.72	31.02	14.35
031	2	53.0	32.4	14.6	21.82	15.44	13.20
040	2	37.7	44.1	18.8	33.44	17.78	10.94
003	2	35.1	45.7	18.6	45.44	41.05	20.52
Mean		**52.4**	**28.0**	**17.3**	**30.8**	**23.4**	**12.1**
SD		**10.4**	**10.9**	**5.1**	**15.6**	**15.6**	**7.6**

Table 4 Micronutrient Intake in OSAS (Group 1) vs. Non-OSAS (Group 2)

	Group	Healthy Eating Index	Cholesterol (mg)	Sodium (mg)	Sugar (grams)	Alcohol (grams)	Iron (mg)	Chormium (µg)
014	1	46.00	545.47	3577.75	73.83	0	21.44	54.00
016	1	53.00	147.21	2765.04	42.45	0	11.21	0
023	1	41.00	159.75	3504.59	47.50	1.9	14.50	.05
025	1	43.00	140.30	3922.44	86.50	0	11.28	0
030	1	51.00	115.48	4710.17	254.06	0	17.69	.00
032	1	79.00	900.99	4562.62	90.54	0	19.60	.07
033	1	54.00	192.91	5179.31	176.65	0	15.82	.03
034	1	52.00	330.20	6762.61	127.44	0	20.33	.01
035	1	43.00	261.94	4648.06	110.46	0	13.86	0
036	1	74.00	182.54	4045.16	53.40	0	15.39	.03
038	1	68.00	187.13	4574.56	56.71	0	25.90	.05
039	1	51.00	302.51	5779.06	97.31	0	15.24	.02
Mean		**54.6**	**288.8**	**4502.6**	**101.4**	**0.16**	**16.8**	**6.0**
SD		**12.5**	**226.2**	**1073.8**	**61.4**	**0.55**	**4.3**	**5.9**
003	2	25.00	202.39	3780.04	73.83	0	10.86	.0
010	2	65.00	121.85	3410.67	42.45	7.3	19.46	.01
011	2	51.00	423.05	6603.29	47.50	0	21.19	0
015	2	28.00	298.82	3108.83	86.50	14.200	17.42	0
018	2	44.00	460.39	3952.42	254.06	2.570	24.69	0
021	2	33.00	223.35	3591.32	90.54	0	18.18	0
027	2	46.00	175.62	3128.69	176.65	1.800	18.39	.02
028	2	39.00	117.87	2208.82	127.44	0	19.06	.00
029	2	66.00	284.04	3623.42	110.46	0	37.36	.06
031	2	59.00	114.67	6095.88	53.40	0	20.35	.05
040	2	54.00	204.27	4618.68	56.71	0	16.07	.02
003	2	63.00	341.31	5492.84	97.31	.700	24.77	.02
Mean		**47.8**	**247.3**	**4134.5**	**95.5**	**2.2**	**20.6**	**0.02**
SD		**14.3**	**116.3**	**1315.3**	**68.5**	**4.3**	**6.4**	**0.02**

Table 4 (Con't)

	Group	Copper (mg)	Selenium (µg)	Iodine (µg)	Zinc (mg)	Magnesium (mg)	Vitamin C (mg)	Vitamin E (mg)
014	1	1.81	90.76	53.87	14.69	383.86	106.01	17.40
016	1	.75	62.90	38.42	7.95	195.66	54.85	5.59
023	1	.61	82.98	0	8.86	236.89	37.77	3.00
025	1	.39	58.74	0	1.98	153.37	11.82	.67
030	1	.66	47.86	0	3.04	131.55	302.67	.22
032	1	1.13	158.98	0	16.80	376.79	56.71	3.44
033	1	.75	76.03	0	8.50	204.80	39.24	1.62
034	1	.93	102.80	0	9.34	203.70	99.45	4.08
035	1	.81	53.16	0	9.39	129.26	39.26	1.09
036	1	.99	84.54	0	10.52	252.98	76.93	3.10
038	1	.88	104.79	0	14.79	250.17	55.56	.91
039	1	.47	63.66	0	10.90	106.49	15.43	1.98
Mean		**0.8**	**82.2**	**46.1**	**9.7**	**218.7**	**76.4**	**3.5**
SD		**0.3**	**30.5**	**10.9**	**4.3**	**76.4**	**77.4**	**4.6**
003	2	.53	49.03	29.46	5.44	98.88	42.93	3.11
010	2	1.42	85.88	0	9.53	326.30	56.61	6.35
011	2	.97	89.69	85.75	13.45	235.84	142.49	6.40
015	2	1.07	98.40	5.62	11.39	312.69	32.29	5.21
018	2	1.43	184.05	8.53	16.76	302.94	66.39	9.79
021	2	1.32	91.10	18.08	11.20	277.23	51.05	8.75
027	2	.48	41.90	0	12.56	153.56	36.21	1.23
028	2	.62	61.74	0	3.78	137.02	30.19	.10
029	2	.98	86.82	0	10.42	261.28	32.16	3.63
031	2	.83	82.88	0	6.33	194.02	94.08	3.40
040	2	.81	128.50	0	16.07	247.93	82.94	4.72
003	2	1.05	126.50	0	14.78	264.85	138.21	5.04
Mean		**0.9**	**93.8**	**29.4**	**10.9**	**234.4**	**67.1**	**4.8**
SD		**0.3**	**38.6**	**10.9**	**4.1**	**73.2**	**39.7**	**2.8**

APPENDIX C

Figure

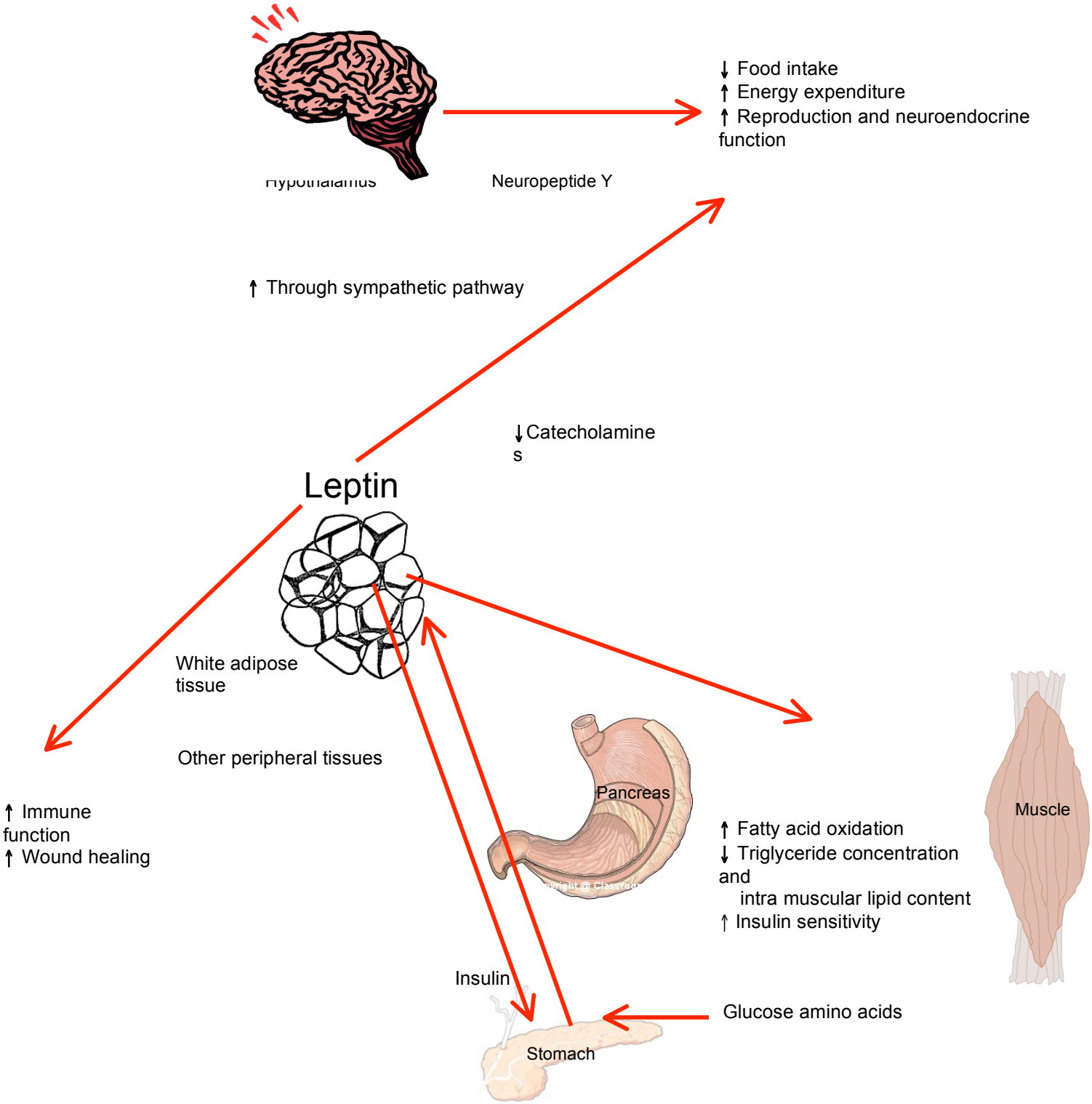

Figure 1. Schematic of leptin and its metabolic effects on the body systems based on Arora S Leptin and its metabolic interactions - an update. *Diabetes Obes Metab* 2008.

APPENDIX D

Institutional Review Board
Protection of Human Subjects Approval

Virginia Tech
VIRGINIA POLYTECHNIC INSTITUTE
AND STATE UNIVERSITY

Institutional Review Board

Dr. David M. Moore
IRB (Human Subjects) Chair
Assistant Vice President for Research Compliance
1880 Pratt Drive, Suite 2006, Blacksburg, VA 24061-0442
Office: 540/231-4991; FAX: 540/231-6033
email: moored@vt.edu

DATE: August 23, 2005

MEMORANDUM

TO: William G. Herbert Human Nutrition, Foods, & Exercise 0351

FROM: David Moore 

SUBJECT: **IRB Full Review Continuation**: "Risk Factors for cardiovascular and metabolic dysfunction in overweight adolescents vs. young adults at risk for sleep apnea syndrome(SAS)" IRB # 05-424 FR ref 04-370 FR

This memo is regarding the above referenced protocol which was previously granted expedited approval by the IRB on August 9, 2004. The proposed research, having been previously approved at a convened IRB meeting, required full IRB review prior to granting an extension of approval, according to the specifications authorized by 45 CFR 46.110 and 21 CFR 56.110. The above referenced protocol was submitted for full review continuation and approval by the IRB at the August 8, 2005 meeting. Pursuant to your request, I, as Chair of the Virginia Tech Institutional Review Board, have, at the direction of the IRB, granted approval for this study for a period of 12 months, effective August 9, 2005.

Approval of your research by the IRB provides the appropriate review as required by federal and state laws regarding human subject research. It is your responsibility to report to the IRB any adverse reactions that can be attributed to this study.

To continue the project past the 12 month approval period, a continuing review application must be submitted (30) days prior to the anniversary of the original approval date and a summary of the project to date must be provided. Our office will send you a reminder of this (60) days prior to the anniversary date.

Virginia Tech has an approved Federal Wide Assurance (FWA00000572, exp. 7/20/07) on file with OHRP, and its IRB Registration Number is IRB00000667.

cc: File

VirginiaTech

Office of Research Compliance
Institutional Review Board
2000 Kraft Drive, Suite 2000 (0497)
Blacksburg, Virginia 24061
540/231-4991 Fax 540/231-0959
e-mail moored@vt.edu
www.irb.vt.edu
FWA00000572(expires 1/20/2010)
IRB # is IRB0000667

DATE: September 10, 2008

MEMORANDUM

TO: William G. Herbert
Trent Hargens
Stephen Gulli

FROM: David M. Moore

SUBJECT: **IRB Exempt Approval:** "Cardiovascular and Metabolic Dysfunction in Adolescents and Young Adults at Risk for Sleep Apnea Syndrome", OSP #443968, IRB # 08-513

I have reviewed your request to the IRB for exemption for the above referenced project. The research falls within the exempt status. Approval is granted effective as of September 9, 2008.

As an investigator of human subjects, your responsibilities include the following:

1. Report promptly proposed changes in the research protocol. The proposed changes must not be initiated without IRB review and approval, except where necessary to eliminate apparent immediate hazards to the subjects.

2. Report promptly to the IRB any injuries or other unanticipated or adverse events involving risks or harms to human research subjects or others.

cc: File
OSP

Appendix E

Copyright Permission

Subject: FW: Permission to reproduce an diagram

Date: Monday, October 13, 2008 5:39 AM

From: Journals Rights <jrights@wiley.com>

To: "ejones4@vt.edu" <ejones4@vt.edu>

Conversation: Permission to reproduce an diagram

Dear Emily Paulus,

Thank you for your email request. Permission is granted for you to use the
material below for your thesis/dissertation subject to the usual acknowledgements and on the understanding that you will reapply for
permission if you wish to distribute or publish your thesis/dissertation
commercially.

Best wishes,

Lina Kopicaite
Permissions Assistant
Wiley-Blackwell
9600 Garsington Road
Oxford OX4 2DQ
UK
Tel: +44 (0) 1865 476158
Fax: +44 (0) 1865 471158
Email: lkopicai@wiley.com

-----Original Message-----
From: Emily Jones Paulus [mailto:ejones4@vt.edu]
Sent: 13 October 2008 00:45
To: Journals Rights
Subject: Permission to reproduce an diagram
I would like to make a diagram from an idea in another diagram. The title of the article
is "Leptin and its metabolic interaction-an update" by Arora S. It was published on Feb.
18, 2008 in the journal, Diabetes, obesity and metabolism. The article ran from pages
1-21 and the diagram I built my ideas from is on page 9. I have attached the diagram I
have done. I do need permission to use this diagram for my master's thesis that needs
to be submitted on October 31st. My contact information is:
Emily Paulus
304 Jamerson Court
Glen Allen, VA 23059 in the United States Work phone: 804-474-8884 Cell phone:

540-998-9004
Thank you,
Emily Paulus

APPENDIX F

Informed Consent

VIRGINIA POLYTECHNIC INSTITUTE AND STATE UNIVERSITY

Informed Consent for Participants in Investigative Project

Title of Study: Risk factors for cardiovascular and metabolic dysfunction in adolescents vs. young adults at risk for sleep apnea syndrome (SAS)

Location of Study: 231 War Memorial Hall and 225 Wallace Hall, Virginia Polytechnic Institute and State University, Blacksburg, Virginia

Investigators: William Herbert, Ph.D., Don Zedalis, MD, John Gregg, DDS, Ph.D., Sharon Nickols-Richardson, RD, Ph.D., Stephen Guill, MS, Trent Hargens, MS.

XI. Purpose of This Research

The purpose of you being in this study is to provide information on how young adults develop sleep apnea syndrome. Sleep apnea is a sleeping disorder that occurs when you stop breathing multiple times over the course of the night. The results of this study may help researchers identify risk factors for sleep apnea that can be identified and treated at a younger age. Before you begin the study, you will be asked some questions about your health history, complete forms on your quality of life and sleep habits, and have your weight and resting blood pressure measured in order to see if you meet the levels to be in this study. If you qualify, you will be asked to take an in-home, overnight sleep test to determine if, and to what extent, you may be affected by sleep apnea. Upon completing this test, you will then be asked to perform several exercise, blood vessel, body fat, and blood tests. All of these tests will take place on the Virginia Tech campus and will take a total of about 3 hours over the course of 3 days. If you decide to be in this study, your results may help researchers better understand how sleep apnea develops in your age group.

The scientific purposes for this study are: 1) to see how sleep apnea affects the heart and circulation, physical fitness, and risk factors for heart and metabolic disease; and 2) to identify risk factors for developing sleep apnea that may be present, yet unknown, in young adults.

To be in this study, you will be asked to make sure that you do not currently have, or have a history of, any of the following:

- Heart problems, including heart attack, chest pain that may be related to heart problems (this is called angina pectoris), surgery for your heart or its blood vessels, or heart failure;
- Chronic lung diseases (including asthma);
- Diabetes mellitus;
- Use of blood pressure medications or antihistamines (cold or allergy medicine);

- Bone or joint problems, muscular or bone conditions, or other conditions that would prevent you from doing vigorous exercise;
- Use of tobacco products use (only non-smokers can participate);
- Any problem affecting your breathing (cold, sinus infection, etc.) during the previous 6 weeks;
- Current pregnancy;
- Use of birth control pills;

If researchers are concerned by any part of your health history, we will ask you to contact your personal physician with a copy of this form in addition to the health history form. Your physician should review these and fax our office with his/her permission or refusal for you to participate in this study.

II. Procedures

You will be asked to complete the following procedures for this study:

Introduction, Informed Consent, and Advanced Screening (up to 3 meetings)
This session will last about 80 minutes. Before session 1, you will be provided a copy, either through mail, email, or access to the study website, of this informed consent form as well as a simple health history form. Please read these carefully and write down any questions you may have for the research team before you report to our lab for the first meeting.

You will then report to the Laboratory for Health and Exercise Science in 231 War Memorial Hall on the Virginia Tech campus. Once there, a researcher will read through this form with you and will answer any questions or concerns that you may have.

The researcher will also go over a more detailed health history form with you and may ask you more questions about your health. This allows researchers to identify if any pastor current health problems will place you in or keep you from being in this study. After these forms are completed and signed, you will be asked to sit quietly for 10 minutes and have your resting blood pressure taken. After this, a researcher will take your height,weight, neck, and waist measurements. If any of these numbers do not meet the study minimum, you will not be able to continue in this study. You will also complete more interviews and forms on your quality of life and sleep patterns.

Session 1 – Setup for At-Home Sleep Test

This session will last about 30 minutes and you will report to 231 War Memorial

Hall on the Virginia Tech campus. You will have another blood pressure measurement 135 taken. One of the researchers will then inform and instruct you about setting up and using a small pocket-sized recorder, the Embletta (At-Home sleep device, see attached picture). It is equipped with straps, wires, and small sensors. You will be asked to wear the Embletta for one entire night at home while you sleep. It measures your breathing activity, pulse, and blood oxygen levels. The Embletta is a harmless non-invasive monitor sometimes used by sleep doctors to screen people who may need more medical tests for possible nighttime breathing disorders. The researcher will make plans for you to take the Embletta home, assist you by phone if needed to properly set it up for one night, and make plans for you to return it the next day.

Session 2 – Blood Sample and Body Fat and Bone Health Tests

This session will last about 60 minutes. Within one or two weeks of your Embletta test, you will be asked to report to the 299 Wallace Hall on the Virginia Tech campus for more testing. On the first day, you will be asked to give a small amount of blood (~75ml, about 4 tablespoons), which will be taken from a blood vessel in your arm.

After having your blood drawn, you will undergo a dual energy x-ray absorptiometry (DXA) scan to measure the mineral content and density of your bones as well as body fat. This involves lying quietly for about 10 minutes on an exam table while the DXA scan slowly passes over your whole body. After this test, the researcher will set up a meeting date and time for the final day of testing.

Session 3 – Blood Vessel Health and Bicycle Exercise Tests

This session will last about 90 minutes. On this day, you will report to the Laboratory for Health and Exercise Science in 231 War Memorial Hall on the Virginia Tech campus. Once there, you will lie quietly for 10 minutes on a padded table and you will be given a simple, external measurement of blood vessel health. This involves having inflatable cuffs placed around your upper arm and wrist, in addition to an elastic band placed around your forearm.

Finally, you will perform an exercise test on a stationary bike. As you pedal longer on the bike, it will become harder to pedal. It is your goal to pedal as long as you can. Researchers will encourage and cheer you to do your best. After this test, you will rest quietly in the lab for 15 minutes to recover from the test and a researcher will provide you with several results from your tests. Both the blood vessel health and bicycle exercise tests are explained more in the next section.

More details about the **specific tests** are shown below:

a) Forms

In all, you will be asked to fill out several forms asking your opinion on several things. These include a detailed health history, a couple of forms about the quality of your sleep and daytime sleepiness, a form about your current quality of life, and forms about your daily physical activities. If any of these forms suggest a sleep problem other than sleep apnea, you will not be allowed to be in this study and we will suggest that you see a sleep physician for further testing and treatment.

b) Blood Pressure

You will have several blood pressures taken during this study. This involves you sitting quietly for 10 minutes. A cuff will be placed around your upper arm, between your shoulder and your elbow. The cuff will be pumped up to stop blood flow to your arm for a few seconds. The cuff pressure is slowly released and a researcher will read your blood pressure and remove the cuff from your arm. The cuff will get tight on your arm, but it only lasts a few seconds.

c) Other Physical Tests

Your height and weight will also be measured on a balance beam physician scale. A researcher will also use a tape measure to measure the size of your waist, neck, and hips.

d) At-Home Sleep Test

For this test, you will be given a recorder with straps, wires, and small sensors to take home. First, you will attach a flexible strap to your abdomen and chest to measure how they expand and contract when you sleep. You will also wear a nasal cannula, a device that attaches to your nostrils and measures if you are breathing. Finally, you will attach a small sensor to your finger that measures the amount of oxygen in your blood. You will wear this entire device for one whole night of your usual sleep.

e) Blood Sample

You will have blood samples drawn in order to look at blood glucose, lipids (fats), and several markers of blood vessel function. The total amount of blood that you will give will be small, i.e. less than 75 ml (about 5 tablespoons). A qualified technician will draw the blood

samples, and accepted medical procedures will be followed. A laboratory specialist will examine, process, and store your blood to be analyzed at the end of the study.

If a technician or other person who handles your blood sample is accidentally exposed to your blood, you will be required to have your blood tested for HIV/AIDS. This testing will be confidential and will be done at the Montgomery County Health Department. This test will cost $50 and funds provided by the research sponsor will cover this cost. It is required that you provide the Montgomery County Health Department with your social security number and your name; if you have a positive test for HIV/AIDS, and only then, this result must be reported to the State Health Department (this is a legal requirement). The names of persons with HIV/AIDS positive tests that are reported to the state remain confidential; however, this information will be placed in your permanent medical records. The test facility requires pretest and post-test counseling. They will contact you within 2 weeks to notify you that you must return there to receive your test results. No results will be given by phone.

f) Body Fat and Bone Health Test

Dual energy x-ray absorptiometry (DXA) will be used to measure your body fat. This test also tells us the mineral content and density of the bones in your arm and leg. Bone mineral content and density provides information on general bone health. The DXA is much like an X-ray machine. The dose of radiation that you will receive with this test is very small and no greater than you normally receive each day from your surroundings over the course of a year. The DXA will scan your entire body very slowly; so, you will need to lie on a table without moving for almost 10 minutes, while the DXA is passed over your entire body. You will feel no discomfort associated with this test.

Exposure to radiation will occur during DXA scans for measurement of your bone mineral density. Radiation exposure will occur from the DXA scans because the DXA machine uses x-ray technology. Radiation exposure is measured in □illiards (or mR). The total amount of exposure is 40 mR (whole body = 1 mR, lumbar spine = 7 mR, hip = 7 mR, forearm = 5 mR) or 20 mR at two testing times. This represents 4% of the estimated exposure to increase cancer risk in only 0.03% of the population. This dose is very small and poses minimal risk. The following table lists the radiation limits for an adult research participant according to the National Institutes of Health, Office for Protection from Research Risk (NIH-OPRR), compared to the exposure during this study.

NIH-OPRR Radiation Limits for an Adult Research Participant per Year	**Exposure During Participation in this Research Study**
Whole body (single dose) = 3,000 mR	Whole body (single dose) = 1 mR
Lumbar spine (single dose) = 5,000 mR	Lumbar spine (single dose) = 7 mR
Hip (single dose) = 5,000 mR	Hip (single dose) = 7 mR
Forearm (single dose) = 5,000 mR	Forearm (single dose) = 5 mR
CUMULATIVE EXPOSURE = 18,000 mR	**CUMULATIVE EXPOSURE = 20 mR**

Any individual may choose to not complete any one, combination, or all of these DXA scans. If in the event that any scan is unreadable or unusable, a replacement scan will not be conducted to avoid further exposure. I**f you are pregnant or think that you may be pregnant, you should not undergo DXA scans because radiation exposure from DXA scans may cause harm to your unborn fetus.** It is unknown how much or how little damage may occur to an unborn fetus during DXA scans. The risk of harm to an unborn fetus is unknown but is possible. It is best to not have DXA scans done if you think that you are or if you know that you are pregnant. In fact, before DXA scans are done, a pregnancy test kit will be completed for each adolescent or young woman who is post-menarcheal (have started menstrual cycles) or premenopausal (have not yet stopped having menstrual cycles) with a sample of her urine. If this pregnancy test is negative (or shows "not pregnant"), participation in the study may continue. If this pregnancy test is positive (or shows "pregnant"), participation in the study will not be allowed and you will be instructed to seek care from your Primary Care Physician or Obstetrician/Gynecologist. If the pregnancy test is positive, any and all costs related to this pregnancy will be borne by the individual and not by Virginia Tech. **If you are under the age of 18 and your pregnancy test is positive, your parent or guardian will be informed of this positive test result.** So that there is an equal opportunity to be in this study, any woman who is not pregnant and meets other study criteria will be given the chance to participate in this study if desired. DXA scans will be conducted in the BONE Laboratory, Room 299 Wallace Hall, on the Virginia Tech campus by an investigator who is a Licensed Radiologic Technologist – Limited in the Commonwealth of Virginia.

g) Blood Vessel Health Test

Plethysmography (PTG) is a simple test of the ability of your blood vessels to expand and contract. For this test, you will be asked to lie supine on a padded table for 10 minutes. Your forearm will be measured and a flexible band will be placed across the largest part of your forearm. A blood pressure cuff will be placed around your wrist and your upper arm. As the cuffs are pumped up, the flexible band placed around your forearm sends blood vessel measurements to the computer to which it is connected. These cuffs may be pumped up for up to 10 minutes and you may feel some slight discomfort and numbness in your fingers, which will go away quickly after the cuffs are removed.

h) Bicycle Exercise Test

Your exercise test will be on a stationary bike. We will measure the electrical output of your heart by placing 10 electrodes directly on your skin across your chest

and stomach. A female researcher will be available to place and remove electrodes for female subjects. During the test, researchers will measure your heart's electrical activity, heart rate, blood pressure, effort, and how much oxygen your body is using. To see how much oxygen you use, we will ask you to breathe into a rubber mouthpiece. During the bicycle test, you will breathe only through the mouthpiece and may experience some dryness in your mouth. The intensity of the cycling exercise will increase as you pedal, over about 14 minutes. At first it will be very easy and then become harder; during the last few minutes, the work will become very intense and should be a best effort on your part. It may be as hard as any exercise that you remember doing. The exercise test will last about 10 minutes.

The total time involved to complete all of the above procedures over the 3 or 4 days you are in the study is about 4 hours. If we find unusual results from any of these tests, we will suggest you see your personal doctor. We will provide you with specific information about these tests to give to him/her.

III. Risks

XI. *Blood Sample*

During the blood draws, you may have pain and/or bruising at the place on your arm where the blood is taken. In about 1 in 10 or 10% of the cases, a small amount of bleeding under the skin will cause bruises. The risk of a blood clot forming in the vein is about 1 in 200 (0.005%), while the risk of infection or significant blood loss is 1 in 1000 (0.001%). To reduce these discomforts, a trained phlebotomist (person skilled in collecting blood by needle) will draw your blood from a vein in your arm. The amount of blood taken is less than NIH guidelines for single blood draws.

b) Bicycle Exercise Test

There is a very small chance of abnormal changes during the bicycle exercise test. These changes may include abnormal blood pressure, fainting, heart rhythm disorders, stroke, heart attack, and death. The chances of serious heart problems during maximal exercise among adults who seem to be healthy is very small, e.g. risk of cardiac death is less than 1 per 10,000 in maximal treadmill exercise tests. The researcher present during your exercise test will have current certification from the American Heart Association in Basic Cardiopulmonary Life Support (BCLS) or the equivalent. A phone will be available to contact the local Emergency Medical System (EMS). The response time for our EMS, the Virginia Tech Rescue Squad, to reach the strength testing/training facility averages less than 5 minutes.

Every effort will be made to minimize abnormal responses to the exercise test by

a review of your health history in addition to close supervision of your response to the exercise test. If the health history form shows conditions that may make you more likely to have exercise-related complications, you cannot be in the study.

c) Body Fat and Bone Health Test

The amount of radiation that you will receive in the DEXA exam is less than the amount permitted by the Food and Drug Administration (FDA) per year. The amount you will receive is equal to 1/20 of a chest x-ray. The more radiation you receive over the course of your lifetime, the more likely your risk increases in developing cancer. The radiation in this study is not expected to greatly increase these risks, however the exact increase in such risk is not known. You should not be pregnant for this study because of risks from the DEXA scan radiation to the embryo or fetus.
All other tests in this study have very little risks. We believe the overall risks of you being in this study are small. It is not possible to identify all possible risks in a study, however the study staff will take all possible steps to lessen any risks to your well-being.

IV. Benefits of Your Participation in This Project

- You will be provided with the results from your exercise test, which can be used for evaluating the condition of your heart and lungs. The researchers suggest that you take a copy of the test to your personal physician to be placed in your permanent medical records.
- A physician will act as a research coordinator and stay in contact with you to monitor and manage your progress throughout the study.
- A trained nutritionist or dietitian will evaluate and make general recommendations to you about the type and amount of foods that you are eating. This information may be beneficial for your health and controlling risk factors for chronic diseases, such as coronary heart disease. Were you not in the study, this type of analysis normally costs $50 per evaluation.
- You will be provided with the results of your blood test, including blood glucose, total cholesterol, HDL (good) cholesterol, LDL (bad) cholesterol, and triglycerides.
- You will also be provided with the results of your DXA scan, including bone density measurements and body composition. These analyses normally cost $500.
- You will be given the results of your at home sleep test. We suggest that you take a copy of the home sleep report, the exercise test report, and the blood test report to your doctor. Should you have abnormally high scores on the at home sleep test, we will notify you and strongly encourage you and to see a sleep specialist. This is particularly important if you drive or operate heavy equipment, as excessive daytime sleepiness is associated with these higher scores. If your doctor notes a concern

after reviewing any of these tests, you and your doctor may decide that you should consult with a healthcare specialist. However, any and all costs related to such a referral and medical care will by paid by you and not by Virginia Tech, nor any of its agents, including the researchers.

XI. Extent of Anonymity and Confidentiality

The results of this study will be kept strictly confidential. At no time will the researchers release your individual results to anyone other than the researchers working on the project without your written consent. The information that you provide will have your name removed and only a subject number (excluding social security numbers) will identify you during analyses and written reports of this research. Your file will be kept in a locked file cabinet and your data will also be kept in a password secured electronic database in 213 War Memorial Hall.

XI. Compensation

You will receive the following for being in this study:

- For session 1 of this study (blood pressures, weight, interviews, forms, and the At-Home Sleep test), you will be paid $15.
- For session 2 of this study, you will be paid $15.
- For session 3 of this study, you will be paid $15.

XI. Freedom to Withdraw

Your participation in this study is completely voluntary. Your refusal to participate in this study will, in no way, affect your standing at Virginia Tech (if you are enrolled as a student). Once you agree to be in the study, you are free to stop at any time without penalty. To withdraw, please contact one of the listed investigators.

VIII. Injury during Participation in This Study

Neither the researchers nor the university have money set aside to pay for medical treatment that would be necessary if injured as a result of you being in this study. Any expenses that you have including emergencies and long-term expenses would be your own responsibility.

IX. Approval of Research

This research project has been approved, as required, by the Institutional Review Board for projects involving human subjects at Virginia Polytechnic and State University and the Department of Human Nutrition, Foods, and Exercise. IRB approval of this project is in effect from August 15, 2004-August 15, 2005.

X. Subject's Responsibilities

By being in this study, you accept that it is your responsibility to:

- Accurately and completely report your medical history;
- Refrain from participation in vigorous physical activity for the 24 hours prior to any measurement for this study;
- Consume no food, caffeine, or nicotine products during the 12-hour period before arriving at the testing lab;
- Remain in the testing and/or exercise area 15 minutes after each of the exercise testing periods;

Report any physical or medical problems that might occur outside the lab during the period of testing, even if you feel it is not related to the testing to: Carol Haskell (951-8814), Stephen Guill (231-6374/951-5665), Trent Hargens (231-6374/818-5884) or Dr. William Herbert (231-6565/951-0974).

XI. Subject's Permission

You have read and understand the informed consent and conditions of this research study. You agree to undergo all screening procedures described above prior to acceptance into this study. It is your right to withdraw from the study at anytime without penalty and that you can be dropped from the study by the investigators without you consent. You also understand the risks of your participation and the nature of any potential benefits. Any questions that you have asked have been answered to your complete satisfaction. If you have questions that arise at a later time, please contact one of the listed investigators. You hereby acknowledge the above and give your voluntary consent for participation in this study.

Questions/Response:

_____ _____
Signature Date

_____ _____
Witness (Research Coordinator) Date

Should I have any questions about this research or its conduct, I will contact:

Carol Haskell, MD 951-8814
Research Coordinator

William G. Herbert, Ph.D. 231-6565
Principal Investigator
Human Nutrition, Foods, & Exercise

Stephen Guill, M.S. 231-6374
Investigator

David M. Moore, Ph.D. 231-499
Chair, IRB, Research Division

Trent Hargens, M.S. 231-6374
Investigator

Kevin Davy, Ph.D. 231-3487
Departmental Reviewer

Nadine Guignel, B.S. 231-6375
Investigator