

**SKELETAL STATUS AND BONE TURNOVER IN OVERWEIGHT YOUNG MEN  
WITH AND WITHOUT SLEEP APNEA SYNDROME**

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# **SKELETAL STATUS AND BONE TURNOVER IN OVERWEIGHT YOUNG MEN WITH AND WITHOUT SLEEP APNEA SYNDROME**

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## **ABSTRACT**

Obesity is a worldwide epidemic increasing at an alarming rate among youth who are facing similar health problems as adults. Sleep Apnea Syndrome (SAS) is an underdiagnosed comorbidity of obesity, characterized by repetitive nocturnal interruptions in breathing. Obesity is associated with delayed skeletal maturation in overweight youth, but mechanisms contributing to this problem are unclear. Obesity and SAS both have been shown to disrupt regulatory hormones and cytokines that influence bone accretion during adolescence. **PURPOSE:** The purpose of this study was to assess the combined effects of excess body weight and SAS on bone mineral density (BMD) and content (BMC), bone turnover, and on the regulatory hormones leptin and IGF-1 known to potentially influence bone accretion during adolescence.

**METHODS:** Men aged 18-28 years were assigned to groups as follows: normal weight controls (CON: AHI <3, n=8); overweight without SAS (OWT: BMI < 26 kg/m<sup>2</sup> and AHI <3, n=9); and overweight with SAS (SAS: BMI >26 kg/m<sup>2</sup> and AHI >5, n=8). The apnea/hypopnea index (AHI) expresses the score for disrupted nighttime breathing events/hr and was obtained in this study with results from a home sleep screening test. Health history and Epworth Sleepiness Scale (ESS) questionnaires also were administered. Bone mineral parameters and body composition variables were measured with dual-energy X-ray absorptiometry. Serum osteocalcin, leptin, IGF-1, and NTx-1 were measured, respectively, by radioimmunoassay and enzyme-linked immunoabsorbent assay. **RESULTS:** Fat-free mass, intra-abdominal fat, and fat mass were higher in the SAS and OWT groups (p<0.03). ESS scores revealed that SAS individuals were sleepier than CON and OWT groups (p<0.009). Total body and site-specific BMD and BMC values (lumbar spine, hip, and forearm) were similar between groups and did not relate to the estimated AHI score. Serum OC and NTx-1 did not differ between groups. Leptin levels were 30% higher in OWT and SAS than in the CON group (p<0.02), but did not correlate with the AHI score. Across all subjects (n=25), only lumbar spine BMC (p<0.005) was correlated to AHI (r=-.52; p<0.01). The preponderance of this relationship between AHI and lumbar spine BMC was attributable to the close inverse association of these two variables within the SAS group (r = -.81; p<0.001). **CONCLUSION:** The effects of SAS were not influenced by the amount of whole-body, intra-abdominal adiposity or lean body mass. Neither leptin nor IGF-1 predicted bone status across all groups. Daytime fatigue and sleepiness, a cardinal symptom of SAS, combined with overweight may contribute to lower lumbar BMC by chronically reducing weight-bearing physical activity and thereby reduce exposure time for mechanical loading of the spine in affected individuals. Further research is needed to explore the biochemical, physiological, and apparently the physical activity implications of SAS on skeletal status and turnover.

## **DEDICATION**

I dedicate this thesis first and foremost to mom, dad, and my grandmother Ida for their financial support and love throughout those four years spent in Blacksburg. I also dedicate this thesis to my fiancé Michel for the laughs, encouragement, admiration, and all the love and strength you always give me.

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## CHAPTER I INTRODUCTION

### *Background*

Obesity is a metabolic pathology commonly manifested by accumulation of excess energy stores in the form of body fat. Most commonly, obesity is defined by a body mass index (BMI) greater than 30 kg/m<sup>2</sup>. This insidious disease has become one of the most critical health problems of the past 30 years (Dekkers et al., 2004). Low levels of physical activity and overconsumption of foods with high energy and fat content are leading to an increased incidence of metabolic and cardiovascular disease risk factors in youth and portend higher morbidity and mortality from these diseases in the years ahead. The third National Health and Nutrition Examination Survey (NHANES III; 1999-2000) states that about 64% of the US adult population is overweight or obese. The problem is not isolated to the U.S. To illustrate, the National French Office for Demographic Statistics (INSEE) reports that about 30-35 % of the adult population in Martinique, the home country of this investigator, is also overweight or obese. Unfortunately, the leading cause of death among adults living in Martinique is also attributed to cardiovascular diseases because of the cultural (i.e. increase in fast-food consumption) and societal (i.e. consumption of pre-cooked meals in spite of good availability of local products) changes in the island. Responsible for a significant increase in mortality, morbidity and decrease in the “Quality of Life”, obesity also carries severe multiple cardiovascular, metabolic, respiratory, mechanical, and psycho-social complications.

### Excess Adiposity & Health Risks

Health researchers and medical practitioners have searched for simple and definitive ways that may clearly define which overweight individuals are at greatest risk of developing premature metabolic and cardiovascular diseases. Use of BMI alone does not adequately allow assessment of fat distribution especially excess fat deposits around the vital organs. For instance, measurements of intra-abdominal adiposity (American Heart Association: > 102 cm for men, >88 cm for women) has become a useful predictor for cardiovascular disease risks. Many studies have also examined the relation of fat distribution to cardiovascular risk factors in the younger segment of our population, finding it more closely related to risk than percent body fat in children (Daniels et al., 1999 ; Moreno et al., 2002). Those cardiovascular risk factors are clustered during childhood and adolescence suggesting the high predisposition for cardiovascular diseases in later life (Chu et al., 1998 ; Moreno et al., 2002). Despite the expansive preventive measures targeting physical activity and better nutritional habits, overweight children often become overweight adults. Immediate consequences of being overweight during childhood are psychosocial and also include metabolic and cardiovascular risk factors, as seen in adults.

### Obesity and Bone Health

Both inherited risk factors and unhealthy environmental behaviors influence bone mineral acquisition and density during skeletal accretion in youth. The increased mechanical loading from increased body weight and increased lean muscle mechanical forces are hypothesized contributors to the higher bone mass observed in obese children. Children with excess body weight and higher BMI are expected therefore to have higher BMD. However, most studies have reported the opposite situation in obese children after adjustment for height and body weight. It

is still difficult to draw definite conclusions on the effect of obesity on young adult bone metabolism and strength during skeletal growth. Indeed, overweight children and young adults have higher vertebral BMD, whole-body bone dimensions and mass for chronological age (Goulding et al., 2000 ; Leonard et al., 2004). However, higher bone mineral density (BMD) and bone mass do not automatically correlate with increased bone strength. Overweight children and young adults were shown to have weaker bones relative to their body weight based on either predicted values (stature-for-age growth charts from the CDC; <http://www.cdc.gov/growthcharts/>) or a bone strength index (Goulding et al., 2000). These data are consistent with the hypothesis that obese children and young adults are skeletally advanced for their age. Nevertheless, none of the preceding cited studies have examined the relationships between fat mass, fat-free mass (LBM) and BMD as both variables are known to be strong predictors of bone mineral content (BMC) and site-specific BMD (Crabtree et al., 2004). Intermittent hypoxia also known to impact bone turnover was not controlled in any of the above studies. It is clear that prior research showing this low BMD in overweight children could potentially reflect the effect of sleep apnea syndrome (SAS) not previously recognized as a comorbidity of obesity.

#### Sleep Apnea Syndrome and Bone Health

Sleep apnea syndrome is a disorder characterized by occurrence of brief repetitive hypoxic states due to partial or complete obstruction of the upper airways causing interruption of the breathing pattern. It leads to diminution of partial blood oxygen saturation and frequent interruption of sleep. The long-term consequences of chronic intermittent hypoxia may have detrimental effects, including increased blood pressure and myocardial and cerebral infarction,

developmental and neurocognitive deficits (Neubauer, 2001). There is currently no published *in vivo* evidence of the effect of intermittent hypoxia on bone.

Sleep apnea syndrome frequently occurs as a comorbid condition of obesity and the incidence in children may be as high as in the adult population (Amin et al., 2004). Most importantly, current evidence suggests that obese young adults with SAS have an impaired bone accretion which affects their skeletal growth and development usually reported by a lower body stature for their age (Yilmaz et al., 2002). In many of these studies, the abnormal body growth usually refers to a smaller body stature for age. Those studies have only inferred a shorter height-for-age in children with SAS based on the assessment of the somatotrophic hormones GH and IGF-1. None have actually compared the children's body heights and weights to any recommended standardized growth charts (to date, the only standardized growth charts known come from the Center of Disease Control).

Hypoxia, even as it occurs intermittently in SAS, may have the potential to disturb bone metabolism. Proposed mechanisms, based on evidence from *in vitro* studies (Arnett and Spowage, 1996 ; Kaysinger and Ramp, 1998 ; Fujimoto et al., 1999) suggest that the increase in oxidative stress and extracellular acidification as seen under hypoxic conditions may modify regulation of bone modeling and remodeling. Those finding are consistent with the delayed body growth reported in children with SAS. Currently, there is no direct evidence to indicate if overweight and SAS may together detrimentally affect bone accretion during childhood and adolescence. However, obesity and SAS have both been shown to disrupt regulatory hormones and cytokines that influence bone accretion during adolescence (Gianotti et al., 2002). Thus, the

pathophysiology of these two disorders may operate independently to suppress bone modeling and remodeling during growth.

#### Adipocytes & Bone: Regulations Possibly Affected by Obesity-Related Dyslipidemia

Various adipocytokines known to be markers of obesity are continuously studied to understand better the neuroendocrine control and involvement of fat in the disorder. Leptin is among the most important and interesting cytokine studied so far. Leptin is a 16 kD protein secreted by the white adipose tissue, and its blood levels are directly related to body fat, especially central fat mass (trunk). Circulating leptin concentrations reflect the status of body energy reserves and subsequent energy balance: depletion of energy fat reserves inhibits leptin production, for example during prolonged fasting, while their repletion stimulates release of leptin (Ogawa et al., 2004). It is also well-known that leptin inhibits appetite via dual actions on specific neurons in the medial hypothalamus. Both types of neurons send either appetite-suppressing (anorectic) or appetite-enhancing (orexigenic) signals to neurons located in the lateral hypothalamus. Leptin also stimulates energy expenditure in normal weight individuals. In overweight and obese individuals, circulating leptin is markedly increased, yet its normal appetite-suppressing effects seem to fail. Individuals with excess body weight and especially visceral adiposity have a resistance to leptin thus preventing its role in the regulation of body weight by inhibiting food intake and stimulating energy expenditure (Ogawa et al., 2004).

Concentrations of circulating leptin in overweight SAS patients are significantly higher than age and BMI-paired non-SAS subjects. Whether high leptin levels may act in some manner to affect bone turnover and bone accretion is unknown. Current evidence suggests that leptin is

able to control bone metabolism through the hypothalamic relay. Experimentally obese mice deficient in leptin and leptin receptors had an increased bone mass, independently of obesity, while intracerebroventricular infusion of leptin in both mutant forms induced bone loss (Ducy et al., 2000). Authors of the above cited study did not find any leptin receptors on the osteoblasts thus stipulating that the inhibitory action of leptin on osteoblastic bone formation is mediated via a neuroendocrine pathway. Other studies have demonstrated that leptin is expressed in cultures of human osteoblasts and promotes bone mineralization (Reseland et al., 2001) and may also inhibit osteoclast generation (Holloway et al., 2002), but no studies have actually proved these findings in humans. The actual mechanism of action explaining the mediation of local leptin signal on the bone unit remains unclear. However, one viable hypothesis is that high leptin levels observed in obesity and SAS may increase adipocytes signaling to progenitor cells in such a manner to reduce formation of new osteoblasts and increase formation of other cell types. This ultimately could lower the capacity for bone accretion throughout adolescence in obese children. Leptin is not the only hormone disturbed in obesity since there is evidence of somatotrophic hormonal imbalances (GH and IGF-1) which may also influence bone accretion during adolescence.

#### Disturbances in GH & IGF-1 in Obesity and Implications for Bone

Abnormal nocturnal GH secretion resulting from intermittent nocturnal hypoxemia is the most frequently postulated cause explaining abnormalities in body weight and shorter body stature. Current research has been vague on specific and accurate methods used to assess this 'growth retardation' in children since no current studies make use of standardized growth charts of stature- and body weight-for-age. Most of the studies reporting these abnormal variables in

overweight children with SAS could potentially reflect the reduced effect of IGF-1 on skeletal muscle mass hypertrophy, well known for its osteogenic effects in bone. In fact, overweight children with SAS have lower blood levels for GH and IGF-1 than do overweight children without SAS, especially during sleep hours (Yilmaz et al., 2002). Those results are consistent with the observed effects of IGF-1 *in vitro* studies, in which this peptide functions as an important local acting regulator of osteoblast proliferation and differentiation from progenitor cells.

Through direct actions on LBM, IGF-1 is a possible indirect mediator between muscle mass and bone density. Growth hormone acts to promote growth effects in skeletal muscle through IGF-1, the latter being directly responsible for increasing myocyte differentiation and skeletal muscle hypertrophy (Semsarian et al., 1999). These somatotrophic effects on skeletal muscle are achieved by increased protein biosynthesis, tissue growth and amino acids incorporation into proteins; this may well result in direct positive effects on the internal tension and mechanical loads placed on the bones to which they attach, ultimately responsible for higher bone mass and bone strength. In addition, more and more evidence indicates the possible relationship between muscle mass and bone density especially since LBM is a strong independent predictor of site-specific bone mineral density and related fractures (Crabtree et al., 2004). In obese individuals, IGF-1 levels are decreased and this possibly might result in diminished accumulation of LBM. The reduction in muscle mass may lessen tension loads on bones to which the muscles attach and ultimately result in lower bone accretion during adolescence and young years. Rosen (2004) also suggests that IGF-1 is responsible for paracrine

and neural control of bone remodeling because of the presence of cell surface receptors on osteoblasts stimulating DNA synthesis and matrix production locally. Whether the different IGFs participate directly or indirectly in bone metabolism in humans remains an important issue since all of the reported effects of IGF have been demonstrated using *in vitro* or mouse models. It is unknown how intermittent hypoxia can impact LBM, but lower levels of IGF-1 anticipated in overweight individuals with SAS might explain the accelerated observed bone resorption. Measurements of bone biomarkers will help to evaluate possible acute effects of SAS on bone turnover, allowing inferences about effects of this disorder on bone remodeling. In addition, BMD and BMC will provide information about possible long-term effects of SAS on skeletal mass and density in areas of interest for assessment of osteoporosis risk in later life.

#### *Statement of problem*

Published evidence indicates that there is an independent and direct association between sleep apnea and visceral adiposity, and that the combination of these two pathophysiological conditions leads to increased risk for atherosclerotic cardiovascular disease (Vgontzas et al., 2000). It is also well-known that SAS simultaneously worsens the biochemical and physiological regulatory hormones that are present in overweight adolescents. To date, no studies have assessed bone turnover in young adults who have been chronically exposed to the intermittent hypoxic of SAS. Much more is known about the interactive effects of SAS and excess visceral adiposity on increasing risk of CVD. However, little, if anything is known about the potential interactive effects of obesity and SAS, with its effects of intermittent nocturnal



hypoxia and excess sympathetic activation, on bone health at the point in life where bone mass is rapidly accruing.

### *Significance of Study*

This is an exploratory study to determine if obesity, or obesity in combination with obstructive sleep apnea, may contribute to unfavorable effects on bone turnover and skeletal mass and density in young adults approaching the age of skeletal maturation. Targeting this age group is particularly important because of the rising medical costs associated with therapeutic preventions and treatments related to both obesity and osteoporotic fractures. Anthropometric assessments of site-specific BMD for the radius, hip, lumbar spine, total body and visceral adiposity will provide useful information of bone health under simultaneous influences of SAS and obesity, as these sites are important in later life for assessing risk of osteoporotic fracture. The hormonal data from leptin and IGF-1 may suggest whether factors directly or indirectly influencing bone regulation are modified by being overweight or the combination of overweight and SAS in young adults.

### *Research Aims*

This study is part of a larger study exploring the physiological, biochemical, and clinical consequences associated with overweight in adolescents and young adults, with or without SAS. A first study aim is to assess the combined effects of excess intra-abdominal fat (or excess total body weight) and SAS on bone turnover and BMD in overweight young adults. The second aim

of this study is to understand better the effects of LBM on these bone variables in these individuals.

### *Specific Research Hypotheses*

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

- (1) greater intra-abdominal fat mass and lower fat-free mass than similarly overweight young adults without SAS and than normal weight young adults without SAS;
- (2) lower BMD and BMC values for hip, wrist, spine, and total body than overweight and normal weight young adults without SAS;
- (3) suppressed bone turnover (reflected by osteocalcin and N-telopeptide of type I collagen cross-links biomarkers) in comparison to overweight and normal weight young adults without SAS;
- (4) elevated serum leptin and reduced serum IGF-1 levels than overweight and normal weight young adults without SAS;

### *Delimitations*

The primary delimitations of this study were: (1) All subjects were volunteer aged 18-26 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 bone metabolism; (3) Subjects had not been exercising regularly during the 12 months preceding the study; (4) All dual X-Ray absorptiometry measurements are analyzed by

the same technician to minimize inter-tester variation; (5) blood collection after overnight fasting was performed by the same technician for the same reason as DXA.

### *Limitations*

The primary limitations of this study were: (1) Subjects were volunteers who might not be representative of the entire student population; (2) Medications and items related to physical history were self-reported; (3) There were no behavioral interventions throughout the study.

### *Basic Assumptions*

- (1) Subjects with different SAS scores were grouped due to a limited number of overweight subjects who will be selected with SAS;
- (2) None of the control subjects had SAS and the same exclusion criteria applied to this group (i.e., no prescribed medications and no medical problems known to affect bone metabolism).

### *Definitions of terms*

1. Bone mass: total amount of trabecular and cortical bone tissue at sites of observations; expressed as bone mineral content (BMC) (g).
2. Bone Mineral Density (BMD): in grams of mineral per area or volume ( $\text{g}/\text{cm}^2$ ) and in any given individual is determined by peak bone mass and amount of bone loss.
3. Dual-energy X-ray absorptiometry (DXA): A two-dimensional x-ray that measures BMD, BMC, body composition, and bone width. It is the current gold standard measure of BMD.

4. Insulin-like growth factor-1 (IGF-1): growth hormone that is structurally similar to the insulin molecule, which circulates in blood as a form bound to various proteins, with the physiological effect of increasing muscle mass and bone acquisition.
5. Leptin: polypeptide hormone of 16 KDa released by white-adipose cells; important component in the long-term regulation of body weight and more recently found to possibly control bone metabolism.
6. Osteoblast: connective tissue cell that builds bone.
7. Osteoclast: large multinucleated cell of hematopoietic origin that degrades and resorbs bone. This is important to the development, growth, maintenance, and repair of bone.
8. Osteocalcin: biomarker utilized to monitor bone formation. Osteocalcin is a 49-residue polypeptide and is a specific product of the mature osteoblast.
9. N-telopeptide of Type I Collagen Cross-links (NTx): N-terminal telopeptides released during bone resorption; provides a specific indicator of bone resorption.
10. Resorption: The loss of bone by osteoclastic degradation.

### *Summary*

In addition to the increased risk for developing hypertension, diabetes or coronary artery disease, overweight children and adolescents (especially those with visceral obesity) who also have SAS may be at higher risk for suppressed skeletal development during the years when bone accrual occurs but should be increasing most rapidly. With increasing rates of osteopenia and osteoporosis (NOF, 2000) in adulthood, it is important to understand factors that may result in

less than optimal attainment of bone mass upon achieving adulthood. So far, most research has focused on understanding the interactive effects of SAS and excess visceral adiposity on increasing risk of cardiovascular disease (CVD) in children, but little is known about the potential interactive effects of obesity and intermittent nocturnal hypoxia on bone health at the point in life where bone mass peaks.

## CHAPTER II REVIEW OF LITERATURE

### *Introduction*

Obesity is a metabolic and pathophysiological disorder characterized by an excessively high amount of body fat deposits in relation to the lean body mass (LBM). Individuals with body mass index (BMI) between 25 and 29 kg/m<sup>2</sup> or greater than 30 kg/m<sup>2</sup> are considered overweight and obese, respectively. This pathology is associated with an array of disorders such as hyperlipidemia, hypertension, type 2 diabetes, coronary heart disease and stroke, osteoarthritis, and sleep disorders, etc. In the past, the status of overweight or obesity was mainly considered a pathology of adults, not a paediatric condition. Across the globe, the prevalence of overweight and obesity in children and young adults has increased exponentially over the last 30 years; especially the last 10 years. Using standard BMI-for-age growth charts, the Centers for Disease Control and Prevention (CDC) ([www.cdc.org](http://www.cdc.org)) estimated that 30% of children and adolescents were overweight, half of them being obese. As seen in adults, the immediate consequences of being overweight during childhood are psychosocial and also include cardiovascular, skeletal, and respiratory disease risk factors. Past puberty, obese children and adolescents are also more likely to remain obese as adults. Genetic and behavioral factors, as well as environmental (societal and familial) influences contribute to the increased incidence of metabolic and cardiovascular disease risk factors and subsequently higher morbidity and mortality in the years ahead. Children and adolescents are also at increasing risk for poor skeletal accretion and development if their environment and habits (Calcium intake and physical activity) are not

properly monitored. Achievement of peak bone mass, critical during the pubertal years of development for later life, may be compromised in obese children and adolescents. This review of the current literature will give better insight of the problems associated with bone health in young adults.

### *Status of overweight and obesity*

#### Defining the Disease

According to the Center for Disease Control (CDC), the U.S. spends approximately 92 billion dollars on direct and indirect obesity-related disorders. Direct medical costs may include preventive, diagnostic, and treatment services related to obesity. Indirect costs relate to morbidity and include the value of income lost from decreased productivity, restricted activity, absenteeism, and bed days. Indirect costs associated with mortality include such factors as the value of future income lost by premature death. Data from the third National Health and Nutrition Examination Survey (NHANES III, 1999-2002) from the CDC indicated that 60 million individuals over age 20 have a BMI of  $> 30 \text{ kg/m}^2$ , which represents an increase in prevalence of 50% since 1994. The CDC uses standard BMI-for-age charts to classify children and adolescents at risk for being overweight (BMI-for-age between the 85<sup>th</sup> and 95<sup>th</sup> percentile) and overweight (BMI-for-age greater than 95<sup>th</sup> percentile).

#### Epidemiology

Based on those standardized cut-offs points, the prevalence of obesity among children and adolescents aged 6-19 years old has tripled since 1980, reaching an outrageous 18 million (about 30%) being overweight or obese. As seen in adults, non-Hispanic African-Americans and

Mexican-American adolescents (21 and 23% respectively) between 6 and 19 have a higher incidence and prevalence of overweight and obese than non-Hispanic white adolescents (14%). Mexican-American children (22%) ages 6-11 are also more likely to be overweight than non-Hispanic African-American children. Between 2002 and 2004, the Center for Physical Activity and Health Evaluation in Fort-de-France, Martinique, followed about 800 6-years old school-based children (46 % girls and 54 % boys) to evaluate and try to improve their physical and psychological capacities as well as bring awareness to adults on the importance of regular exercise at younger ages. After a two-year follow-up, approximately 12% of children were overweight, with half being obese. Applied to the whole island, it would estimate that about 15 % of children ages 2-15 yr old would be overweight and obese in Martinique. It is very alarming considering that the proportion of overweight and obese children in the United States is 184 times the proportion of children in Martinique but yet, half of Martinique's children already overweight and obese! With chronic conditions such as diabetes and hypertension so prominent in adults, more needs to be done to protect the young population already at risk for further complications in later life.

### Clinical Manifestations

Unfortunately, past puberty, overweight adolescents are more likely to stay obese (Gunnell et al., 1998). A 57-yr follow-up British study examined 1165 males and 1234 females ages 2-15 at time of first examination to observe the relationship between childhood overweight and adult all-cause and cardiovascular diseases. Body mass index was calculated on the basis of the British standard values of BMI-for-age. For instance, overweight subjects had a BMI-for-age



greater than 75<sup>th</sup> percentile while obese subjects had a BMI-for-age greater than 90<sup>th</sup> percentile. All-cause (hypertension, stroke, type 2 diabetes, dyslipidemia, ischemic heart disease) cardiovascular mortality was associated with higher childhood BMI for both genders. Children (males and females) with BMI-for-age greater than 75<sup>th</sup> percentile had a hazard ratio (95% CI) for all cause-mortality between 1.5 and 2.0 for cardiovascular diseases and ischemic heart diseases compared to children with normal BMI-for-age (25-49<sup>th</sup> percentile). Yet, male subjects were twice more likely to die from cardiovascular diseases and up to five times from ischemic heart diseases. Those findings agree generally with reported higher cardiovascular mortality rates of adult males. It also suggests that overweight children and adolescents are already highly predisposed to many cardiovascular risk factors (Chu et al., 1998 ; Csabi et al., 2000 ; Moreno et al., 2002).

In the Taipei heart study by Chu and colleagues (681 boys and 685 girls, 12-16 yr), overweight and obese boys in contrast to their age-paired lean counterparts had significantly higher blood glucose concentrations, systolic blood pressure ( $121.1 \pm 11.8$  vs.  $112.8 \pm 12.5$ ,  $P < 0.001$ ), and diastolic blood pressure ( $66.7 \pm 8.8$  vs.  $72.8 \pm 10.8$ ,  $P < 0.001$ ). Total cholesterol, HDL cholesterol, LDL cholesterol, apolipoprotein A-I and B concentrations were also significantly more elevated for the obese boys ( $P < 0.01$ ). Obese boys had also a higher prevalence (70%) of clustered risk factors for elevated blood pressure, dyslipidemia, and glucose concentration. Because standard cut-offs points for hypertension, hyperlipidemia, or hyperglycemia are not yet clearly established in children, abnormal status was defined as clinical measures greater than or equal to the age- and gender- specific 90<sup>th</sup> percentile. With similar

findings, Csabi and colleagues (2000) also reported the presence of the metabolic CVD risk factors clustered in childhood and strong predictors of adulthood obesity. Even though BMI is a simple tool commonly used to define obesity, other studies have put a greater emphasis on the body fat distribution and regional body fat, especially the abdominal adiposity given by waist circumference or quantification of fat in this region by data from whole body scans (Daniels et al., 1999 ; Moreno et al., 2002). Both of these studies indicated that fat distribution (android and gynoid model) correlates strongly and independently to CVD risk factors compared to BMI or percent body fat.

For instance, the American Heart Association ([www.aha.org](http://www.aha.org)) uses the waist circumference associated with abdominal adiposity as one major criterion to identify the metabolic syndrome or clustering of cardiovascular and metabolic risk factors. Among five criteria, men and women with a waist circumference greater than 102 and 88 cm, respectively, are at increased risk for CVD. For instance, the waist is measured at the narrowest part of the torso above the umbilicus and at mid-point between the lowest rib and the iliac crest. In clinical settings, waist measurement highly predicts one's predisposition to obesity and surrounding comorbidities, especially type 2 diabetes. Koh-Banerjee et al. (2004) investigated the possible role of change in body composition and type 2 diabetes. They reported that men who increased their waist circumference by 14.6 cm (weight gain >19 kg) doubled their risk for type 2 diabetes (RR = 2.1; 95% CI; 1.5-3.7). In the study cohort, a waist gain greater than 2.5 cm could explain about 20% of all new cases of diabetes. A large waist circumference often associated with the accumulation of adipose cells around the viscera also results in excessive production of

inflammatory cytokines exacerbating such disorders as type 2 diabetes/hyperinsulinemia or atherosclerosis (Vgontzas et al., 2000).

#### Endocrine Dysfunctions: Leptin and IGF-I

Various adipocytokines e.g. adiponectin, ghrelin, cholestocytokinin, satietin, bombesin, leptin are continuously studied to understand better the endocrine regulation of energy turnover, fat storage and biological factors influencing eating behavior. Yet, leptin is the cytokine most extensively explored so far. Leptin is a 16 kD peptide hormone secreted by the white adipose tissue with reproductive, metabolic, and hematopoietic functions (Casanueva , 1999 ; Casanueva and Dieguez, 1999 ; Sahu, 2003). Leptin correlates positively with total body fat stores; higher leptin is produced by larger adipocytes or larger concentration of adipocytes at the site of measurement. After release in the bloodstream, leptin is transported to the central nervous system especially to the hypothalamus where it binds to surface receptors on specialized orexigenic (appetite-enhancing) and anorectic (appetite-suppressing) neurons involved in the control of appetite (Bates and Myers, 2003 ; Sahu, 2003). Overall, leptin reduces food intake and body weight in lean individuals by inhibiting the orexigenic neurons and activating the anorectic neurons. It would be wise, therefore, to assume that individuals with higher abdominal adiposity, and increased leptin levels, will have enhanced appetite-suppressing signals to the hypothalamus. However, overweight and obese individuals have such elevated leptin levels that leptin no longer suppresses appetite, resulting in a leptin resistance (Ogawa et al., 2004). *In vitro*, genetically modified mice without leptin or leptin receptors became obese, secondary to increased feeding and decreased energy utilization (Ducy et al., 2000) as illustrated on **Figure 1** (see Appendix C).

In a study by De Marinis (2004), 15 obese females, ages 23-54 yr, with a BMI greater than  $42 \text{ kg/m}^2$ , who underwent biliopancreatic diversion, exhibited higher plasma leptin levels at baseline compared with control subjects ( $63.77 \pm 4.62$  vs.  $27.8 \pm 1.21 \text{ } \mu\text{g/l}$ ,  $P < 0.0001$ ). This biliopancreatic diversion (BD) consisted of the partial removal of part of the stomach. This surgical operation reduced partially both the gastric volumes to 200-400 ml and the length of the digestive tract to 200 cm. As a consequence, both pancreatic and biliary secretions were reduced and did not facilitate absorption of nutrients from the ingesta. A year after the surgery, post-BD subjects had significantly lower plasma leptin levels compared to before the surgery ( $63.77 \pm 4.62$  vs.  $9.7 \pm 0.59 \text{ } \mu\text{g/l}$ ,  $P < 0.0001$ ) and controls ( $P < 0.0001$ ). Sixteen to 24 months post-surgery, body weight significantly decreased in all patients (mean BMI:  $28.29 \pm 0.89$  vs.  $44.02 \pm 1.45 \text{ kg/m}^2$ ,  $P < 0.0001$ ). Fat mass was also significantly lower after the surgery ( $50.34 \pm 2.03$  vs.  $80.05 \pm 3.57 \text{ kg}$ ,  $P < 0.0001$ ). Leptin was positively correlated with fat mass, but only before surgery ( $r^2 = 0.75$ ,  $P < 0.0001$ ). Findings from this study support the hypothesis that leptin levels help the body regulate energy balance and body weight.

Besides its properties in appetite regulation, leptin signaling may contribute to the development of atherosclerosis and hypertension (Nishina et al., 2003 ; Reilly et al., 2004). Reilly and colleagues (2004) determined the association between plasma leptin levels and coronary artery calcification (CAC), a measure of coronary atherosclerosis in type 2 diabetes subjects. A strong positive association was found after controlling for BMI, waist circumference, plasma C-reactive protein and measures of subclinical vascular diseases ( $P < 0.01$ ). One major weakness of this study was the failure to control for hypertension, one major risk factor for

atherosclerotic disease. To clarify the mechanism of blood pressure elevation in obesity, Nishina and colleagues (2003) observed 109 obese children aged 6-15 yr old with a family history of hypertension and 83 simply obese children in the same age range. Systolic blood pressure (SBP) was measured three times in a seated position on the right arm using an automated blood pressure recorder. Only the third measurement was used in this study. Hyperinsulinemia was the most important predictor of elevated SBP ( $P < 0.01$ ) for both groups of subjects followed by hyperleptinemia ( $P < 0.05$ ). Risks factors contributing to hypertension in obese adults are very similar in children. A family history of hypertension, overactivity of the sympathetic nervous system, and abnormalities in the vascular system may contribute to this debilitating health condition.

Many more adipocytokines are disturbed in human obesity but several important hormones such as growth hormone (GH) and insulin-like growth factor-1 (IGF-1) are also affected. Growth hormone is a 190 amino acids peptide hormone synthesized and produced by somatotroph cells in the anterior pituitary. It plays a major role in metabolic and growth functions. Growth hormone directly controls lipolysis by stimulating the break down of triglycerides and suppressing their ability to accumulate in adipose cells (Boguszewski et al., 2005). Growth hormone directly suppresses the abilities of insulin to stimulate the uptake of glucose in peripheral tissues and enhances glucose synthesis in the liver. Growth hormone also indirectly stimulates the proliferation of the cartilage cells at the epiphyseal plates of long bones via IGF-1, a hormone secreted by the liver. IGF-1 also controls lean muscle mass growth by stimulating the differentiation and proliferation of myoblasts and uptake of amino acids resulting

in protein synthesis (Galvin et al., 2003). In human obesity, the GH/IGF-1 axis is disrupted at different levels: (1) GH daily production rate and half-life are reduced and its clearance is accelerated.(2) Plasma IGF-1 is decreased but the literature is conflicting on IGF-1 since normal, increased or decreased IGF-1 levels have been reported in obese subjects.

Frystyk et al. (1995) conducted a study with 33 moderately obese subjects ( $25 < \text{BMI} < 30$ ), 28 morbidly obese subjects ( $\text{BMI} > 30$ ) and 31 controls ( $\text{BMI} < 25$ ) to investigate the effect of obesity on circulating levels of free and total IGF-1 and IGF-2. They isolated the serum free or bioactive IGF-1 with radioimmunoassay after previous acid-ethanol extraction to avoid interferences with the binding proteins. Blood collection was performed after overnight fasting. In contrast to the controls, serum free IGF-1 increased by 47 % for the moderately obese subjects (mean  $\pm$  SEM:  $690 \pm 90$  vs.  $470 \pm 50$  ng/L,  $P < 0.05$ ) and 72% for the morbidly obese subjects ( $810 \pm 90$  vs.  $470 \pm 50$  ng/L,  $P < 0.05$ ). They found an inverse correlation between IGF-1 and IGFBP-1 ( $r = -0.47$ ,  $P < 0.001$ ). The suppression of the IGFBP-1 was mainly due to the properties of insulin in reducing the hepatic production of the IGFBPs. Therefore, hyperinsulinemia and an increased peripheral insulin resistance may be key controllers of free IGF-1 levels. Alternatively, in the study by DeMarinis et al (2004) previously mentioned, IGF-1 was lower for the obese subjects compared to the controls ( $17.48 \pm 0.61$  vs.  $26.26 \pm 0.88$ ,  $P < 0.0001$ ). They suggested that insulin directly impacted GH release and IGF-1 in a similar fashion. Maccario et al. (1999) also found a reduction in IGF-1 levels in their obese patients compared to the controls. They concluded that IGF-1 levels undergo an age-related reduction since present findings indicated a clear negative independent correlation between IGF-1 and age, in obese and normal subjects.

Nevertheless, more research needs to identify correctly the independent contributors to the secretion of IGF-1.

### Skeletal Disturbances: Physical and Endocrine Characteristics

Achievement of PBM during skeletal accretion in youth is a critical step to predict bone mineral density (BMD) and bone mineral content (BMC) in adulthood. Known inherited risks factors (small frame or being too thin, family history of osteoporosis, being a female) and environmental behaviors (low calcium intake under the recommended dietary intake, eating disorders leading to abnormal menstrual cycle and hormonal imbalances, physical inactivity, current cigarette smoking and excessive alcohol consumption) can detrimentally and irreversibly disturb bone acquisition at a time when optimal BMD and BMC must be achieved. Obesity is also a major contributor to skeletal accretion in adults as well as youth. There is a general premise that obesity protects against bone fragility and fractures due to the increased mechanical loading from increased body weight and increased lean muscle mechanical forces. Leonard et al. (2004) determined the effect of obesity on BMC and BMD relative to age, height, and maturation given by the Tanner stage in 103 obese children aged 4-20 yr. Using sex-specific multivariate regressions, obesity (BMI > 95<sup>th</sup> percentile) was associated with an increase vertebral bone density and whole-body bone area after adjustment for age, height, and Tanner stage (all  $P < 0.05$ ). No adjustments for body weight were made, so it is difficult to believe that they were able to relate obesity status to BMD and BMC simply based on age, height, and Tanner stage.

Most of the current literature disagrees with this hypothesis and many studies have

reported the opposite situation after adjustment for height and body weight (Goulding et al., 2000 ; Eliakim et al., 2001 ; Nagasaki et al., 2004). Goulding et al. (2000) recruited 200 girls and 136 boys aged 3-19 yr to determine if overweight (BMI > 85<sup>th</sup> percentile) or obese (BMI > 95<sup>th</sup> percentile) fracture-free children and adolescents had lower BMD and BMC than children of normal adiposity. All children were Caucasian and free of medication affecting both body and bone growth. BMC and BMD were measured by dual-energy X-ray absorptiometry (DXA). Relative to their chronological age (based on pubertal development), BMC was higher in overweight children in comparison to the lean counterparts (1291 vs.1080 g, P < 0.005). Only obese boys had higher BMC in contrast to the controls (1579 vs.1400, P < 0.003). However, those observed values relative to body weight and after age adjustment were lower than the predicted values when compared to children of normal adiposity (2.5-10 % less, P < 0.05). They concluded that overweight and obese children were skeletally advanced based on an increased BMC for their age with significant lower bone mass for body weight. This mismatch between elevated body weight and bone development put them at increased risk for fractures and knee pains. Their predicted values were only based on regressions from the total samples of each gender. This is a major drawback for this study because a standard tool to accurately predict of bone mass and bone area was not used. Those predicted values apply only to the sample under study with little reproducibility in different studies. Nevertheless, this study further supports that high body weight in children is a risk factor for low bone mass and fractures.

Russell et al. (2001) found the same relation between skeletal maturation and adiposity in overweight African American children. The difference between bone age and chronological age



was significantly correlated with DXA fat mass ( $r > 0.46$ ,  $P < 0.001$ ). The excess body fat accounted for the advancement in skeletal maturation of prepubertal African American children. Similarly, Nagazaki et al. (2004) also found that obese Japanese children, boys and girls, had lower BMD relative to their bone and chronological age than prepubertal matched-peers ( $P < 0.05$ ). Obese children are skeletally more mature than their lean peers for chronological age. However, obese children also have lower BMD and BMC relative to their body weight, translating in a mismatch between their high body weight and actual bone development during growth. Obese children fail to adapt sufficient and adequate bone development to cope with this excess weight. It follows that considerable strains are placed on bones and joints, increasing the likelihood of sustaining fractures after a fall.

More research needs to be done with a more standard and common approach to establish the effect of obesity on bone development in children. The use of gender- and ethnic-specific standards is recommended when interpreting pediatric bone densitometry data. For the purpose of this study, we will be assuming that the obese subjects have lower BMD and BMC for their body weight. In addition, most of the participants will be in the process of achieving peak bone mass or are already at this point of their skeletal accretion. Measurements of BMC and BMD may therefore allow us to draw possible conclusions on the mineral accrual and bone gain during childhood.

Current *in-vitro* evidence suggests that leptin and IGF-1 may control bone metabolism through different hypothalamic and mechanistic relays. For instance, Ducy et al. (2000) created leptin-deficient and leptin receptor-deficient obese mice to determine if leptin, independent of

obesity, could potentially control bone metabolism. Both mutant forms of mice had increased vertebral and femoral bone mass due to the enhanced osteoblastic bone formation without changes in osteoclastic activities. Furthermore, the intracerebroventricular administration of leptin in the leptin-deficient and receptor-deficient mice caused bone loss. They did not find any evidence of increase osteoblast number or leptin receptors on osteoblasts in both types of mutants, reinforcing that the inhibitory actions of leptin are mediated through a neuroendocrine pathway. In contrast to these findings, Reseland et al. (2001) found that leptin was released in cultures of normal human osteoblasts, indicating its function in osteoblastic cell growth, cell hyperplasia, and bone mineralization. Using similar methods, Holloway et al. (2002) reported that leptin could inhibit osteoclast generation via increased osteoprotegerin, a potent inhibitor of osteoclastogenesis. Taken together, these *in-vitro* results agree on a direct or indirect regulatory control of leptin in bone metabolism, but further research needs to be done to clarify if leptin controls bone locally or via a neuroendocrine loop in obesity.

Insulin-like growth factor-1 is another key “player” involved in bone remodeling. Found in high concentrations in the serum, IGF-1 is produced by bone cells through *de novo* synthesis and released from the matrix, indicating that IGF-1 acts primarily in an autocrine way to regulate bone microenvironment (Rosen, 2004). To examine the local effects of IGF-1 on bone, Zhao et al. (2000) used a specific osteocalcin (OC) promoter to overexpress IGF-1 in mice osteoblasts (OC\_IGF-1). They measured femoral BMD in groups of 6-week-old female control and OC\_IGF-1 transgenic mice. Trabecular bone volume significantly increased by 30 % in the 6-week transgenic mice compared to the controls ( $9.11 \pm 0.55$  vs.  $7.23 \pm 0.56$  mm<sup>3</sup>, P = 0.039) but

not for the 3- and 24- weeks old mice. Simultaneously, the rate of bone formation increased while the mineralization lag time (time it takes for the newly formed osteoids to mineralize) was reduced. Interestingly, the positive changes observed in trabecular bone volume for the OC-IGF-1 mice at 6 weeks reverted to control levels by 24 weeks, indicating a reduced sensitivity to the effects of IGF-1 or a down-regulation of the IGF-1 receptor. Rosen (2004) developed a molecular model involving two IGF binding proteins (IGFBP-5 & IGFBP-4) and a transforming growth-factor (TGF- $\beta$ ), initiated by osteoclastic matrix resorption. Their hypothesized model suggests that bone resorption starts the IGF-dependant mechanism, triggering a site-specific bone replacement during remodeling.

Another possible mechanism may lie directly in IGF-1 functions. In normal conditions, IGF-I directly stimulates skeletal muscle mass by increasing protein biosynthesis, skeletal muscle hypertrophy, and myocyte differentiation (Boguszewski et al., 2005). For instance, LBM is a well-known predictor of BMC and site-specific BMD (Lim et al., 2004; Rauch et al., 2004). To determine the most important variables influencing BMC in pediatric health, Crabtree et al. (2004) scanned 646 school children and 43 children aged 5-18 yr with chronic diseases including osteogenesis imperfecta, spinal muscular atrophy and fragility fractures treated with biphosphonates. Lumbar spine and whole body BMC, LBM, and fat mass were measured using a Lunar DXA beam scanner. Stepwise linear regression analysis found that LBM was the strongest single predictor for whole body and lumbar spine BMC (total body BMC:  $r^2 = 0.95$ ; lumbar spine BMC:  $r^2 = 0.89$ ) for the healthy children. LBM was best predicted by height ( $r^2 = 0.95$ ). For the children with chronic diseases, they calculated Z scores based upon the control data for BMC

relative to LBM ( $Z_{BMC/LBM}$ ) and LBM relative to height ( $Z_{LBM/HT}$ ) to investigate the relationship between LBM and BMC. Both the spinal muscular atrophy (SMA) and fragility fractures (FF) groups exhibited reduced  $Z_{LBM/HT}$  ( $Z_{LBM/HT} = -1.8$  vs.  $1.4$  for the controls) translating in early signs of sarcopenia or inadequate muscle development. The osteogenesis imperfecta (OI) and FF group showed markedly reduced whole and lumbar spine BMC for LBM ( $Z_{BMC/LBM} = -2.5$  vs.  $1.6$  for the OI group;  $Z_{BMC/LBM} = -2.1$  vs.  $1.3$  for the FF group). Whether IGF-1 may have been the mediator between muscle mass and bone density is unknown but it is a likely possibility based on experimental models. Taken together, IGF-1 may mediate its anabolic activity in the skeleton in both a direct/autocrine (via local promoter) and an indirect/endocrine (by way of LBM) way but mechanisms so far have been contradictory and poorly understood. More research needs to be done to understand the mechanisms of action of IGF-1 on bone *in vitro* and *in vivo*. To date, there is no *in-vivo* evidence of direct control of IGF-1 on bone density in lean or obese young adults at time close to peak skeletal accretion. Intermittent hypoxia/hypopnea also known to influence bone development in experimental studies was not controlled any of the previously reported studies. It is clear that prior research showing low BMD in overweight young adults could potentially reflect the effect of SAS.

### *Sleep Apnea Syndrome (SAS)*

#### Defining the disease

Sleep apnea syndrome is a serious condition characterized by occurrence of brief repetitive interruptions of breathing during sleep. It occurs in two different types: central and

obstructive. Far more common, obstructive sleep apnea is caused by a blockage of the upper airways resulting in poor air flow in and out of the nose or mouth and reduction in the blood oxygen saturation of  $\geq 4\%$  ([www.sleepfoundation.org](http://www.sleepfoundation.org)). Several physical and mechanical problems may be responsible for these interruptions in breathing during sleep. For the great majority of individuals with SAS, the muscles located in the throat, neck, and tongue “excessively” relax and collapse, partially blocking the opening of the airway. Furthermore, the muscles of the soft palate at the base of the tongue relax and sag, reinforcing the difficulties in breathing. People with SAS are often not aware of the consequences associated with this condition such as heavy snoring, periods of complete absence of breathing, and frequent arousals. To compensate for the reduced air flow, the brain briefly arouses people with SAS, consequently resulting in fragmented sleep of poor quality.

### Etiology

According to the National Institutes of Health, SAS affects more than 12 million Americans. Currently, approximately 24% American men and 9% American women suffer from sleep apnea. In children, the prevalence of the disease is similar to adults ranging from 2 to 4% but experts suggest that as many as 11% of American children may suffer from sleep-disordered breathing problems. The disorder is also much more common in African Americans, Hispanics, and Pacific Islanders. People who have sleep apnea may be more prone to car accidents. According to the Medical Center of the University of Maryland, as many as 200,000 automobile accidents and about 1500 deaths are caused by sleepiness. People with SAS have two to three times as many car accidents. The most-at-risk population group was the commercial truck

drivers. Howard and colleagues (2004) measured the prevalence of sleep-disordered breathing in 2342 Australian commercial drivers. About 60% had a sleep disordered breathing and close to 16 % had sleep apnea. Sleepiness associated with sleep apnea should be therefore strictly monitored to reduce the number of accidents and related deaths while driving.

Being overweight, having high blood pressure, a family history of sleep apnea, some anatomical and geometrical abnormalities especially small sizes of the airways in the nose, mouth, throat, and the jaws are among the most frequent physical risks factors reported. Of these factors, obesity is the most important predictor because it is present in roughly 70% of the patients with the disorder (Malhotra and White, 2002). Lately, greater emphasis has been put on the relation between visceral or intra-abdominal adiposity and sleep apnea (Chin et al., 1999 ; Vgontzas et al., 2000 ; Teixeira et al., 2001 ; Vgontzas et al., 2003). Schafer and colleagues (2002) assessed if the most traditional risks factors for CVD were associated with the degree of severity of sleep apnea given by the apnea-hypopnea index (AHI), the average number of apneas and hypopneas per hour of sleep for each subject. Among those risks factors, they focused on the regional body fat distribution especially the intra-abdominal adiposity and looked at correlations between this previous variable and AHI scores. They evaluated 81 obese class I male participants between 27 and 75 yr old (mean age: 55 yr; BMI >30 kg/m<sup>2</sup>) with suspected SAS. Sleep analysis consisted of an overnight polysomnography within the sleep laboratory. Body fat distribution was determined by a bioelectrical impedance analysis. Sleep apnea was considered to be mild with an AHI between 10 and 20, moderate with AHI between 20 and 40 and severe with an AHI >40. The total amount of intra-abdominal fat was significantly correlated with AHI ( $r = 0.30$ ,  $P <$

0.025). Linear regressions analysis on subjects according to their AHI scores would have had power and significance to the reported results. Furthermore, they did not have any control normal body weight subjects as well as obese without sleep apnea to determine if AHI scores were independent from obesity. Similarly, Vgontzas et al. (2000) reported significantly greater amount of visceral fat in the sleep apneics compared to obese controls ( $P < 0.05$ ). Visceral fat was also strongly correlated to the indexes of sleep apnea ( $r^2 = 0.70$ ,  $P < 0.01$ ).

### Clinical Manifestations

Sleep apnea is associated with conditions responsible for the leading causes of morbidity and mortality in adults including hypertension, cardiovascular and cerebrovascular diseases (Chin et al., 1999; Vgontzas et al., 2000). During sleep, intermittent bouts of apnea and hypopnea cause acute blood pressure perturbations ultimately resulting in sustained elevated mean arterial pressure, chronic elevated sympathetic nervous system tone, and failure of the baroreceptor function to maintain blood pressure at steady-state. The prevalence of SAS patients with cardiovascular and cerebrovascular disease is frightening: about 50% patients with hypertension and up to 60% patients who had a stroke are more likely to have sleep apnea (Lattimore et al., 2003). A study by Moller et al. (2003) determined whether patients with SAS ( $n = 24$ ; age range: 25-70 yr olds;  $BMI = 30 \text{ kg/m}^2$ ) had abnormal blood pressure and abnormal activity in the vasoactive hormones part of the renin-angiotensin system. Compared to controls, SAS patients had significantly higher systolic and diastolic BP during daytime (SBP:  $130 \pm 2.4$  vs.  $150 \pm 2.6$ ,  $P < 0.001$ ; DBP:  $78 \pm 1.7$  vs.  $90 \pm 2.5$ ,  $P < 0.001$ ) and nighttime ( $P < 0.001$  for all).

Plasma angiotensin was twice as high in the sleep apneics as in the control subjects ( $7.8 \pm 1.0$  vs.  $13.3 \pm 1.6$ ,  $P < 0.01$ ). This study reinforces that systemic hypertension is much more common in overweight patients with SAS, and is probably due to SAS (Caples et al., 2005). Unfortunately, SAS is also affecting younger generations in a similar way. To demonstrate that children with SAS already exhibit abnormally elevated BP and BP variability, Amin and colleagues (2004) measured 24-hour ambulatory blood pressure in 60 overweight children with either primary snoring or SAS (mean age:  $10.8 \pm 3.5$  years old). All the children with SAS ( $n = 39$ ) had a greater BP variability during wakefulness and sleep ( $P < 0.0001$ ), a smaller nocturnal dipping of BP ( $P < 0.01$ ), and a higher SBP night/day ratio testifying of the attenuated reduction of BP at night ( $P < 0.02$ ). As seen in adults, the frequency of sleep apnea, oxygen desaturation, and repetitive arousals contributed to the development of abnormal BP.

Besides hypertension, epidemiological studies continuously provide evidence that sleep apnea worsens the development of atherosclerosis in the coronary and cerebral arteries, suggesting that SAS is an independent predictor of coronary heart disease and stroke (Lattimore et al., 2003 ; Wolk et al., 2003). To determine if there is a link between the severity of SAS and the atherosclerotic progression, Kaynak et al. (2003) followed 114 male patients, aged 40-65 yr, referred for evaluation of snoring habits and sleep quality during a twelve-month period. Based on their polysomnographic respiratory distress index (RDI), subjects were divided into three groups: the first group consisted of habitual snoring subjects ( $n = 37$ ,  $RDI \leq 5$ ), the second group of mild-moderate apneics ( $n = 41$ ,  $5 < RDI < 30$ ), and the last group of severe apneics ( $n = 36$ ,  $RDI \geq 30$ ). They measured the intima-media thickness (IMT) as the maximum wall thickness at



the thickest point, not including plaques, but also the presence of plaques as a localized thickening of  $> 1.2$  mm. Severe SAS patients had significantly higher IMT values compared to the habitual snorers ( $1.91 \pm 0.39$  vs.  $1.37 \pm 0.46$  mm,  $P < 0.001$ ) but not to the mild-moderate patients. Similar patterns were found with the presence of plaques with 28% in the snoring only group, 59% in the mild-moderate SAS group, and as high as 89% in the severe SAS group ( $P < 0.001$ ). Multiple linear regression best predicted the plaque formation with age and RDI ( $P = 0.01$ ) while IMT was significantly associated with age and BMI only ( $P < 0.02$ ). This study strongly suggests that SAS is a predisposing factor of atherosclerosis and worsens plaque formation when associated with higher RDI. Circumstantial diseases such as congestive heart failure, pulmonary hypertension, and cardiac arrhythmias are thought to be associated to SAS but a strong cause-effect relationship remains to be proven. The current literature generally suggests also that many cytokines (leptin and IGF-1) already disturbed in obesity are exacerbated in SAS delineating possible confounding effect of obesity but the independent role of SAS and obesity on peptide hormones should not be excluded.

#### Endocrine Dysfunctions: Leptin and IGF-1

It is generally reported that elevated leptin levels are associated with SAS and/or obesity (Vgontzas et al., 2000; Harsch et al., 2003; Barcelo et al., 2005). Mary et al. (2000) investigated the role of leptin resistance in 30 SAS patients BMI-matched with 30 non-SAS patients. Compared to the controls, SAS patients had significantly higher leptin levels ( $9.18 \pm 4.24$  ng/mL vs.  $6.54 \pm 3.81$  ng/mL,  $P < 0.001$ ). After a 6-month treatment with nasal continuous positive airway pressure (CPAP), leptin concentration was decreased ( $P = 0.01$ ), independently of BMI.

Harsch et al. (2003) found similar results as CPAP-treated male patients exhibited reduced leptin levels without any change in BMI. Because BMI did not change after treatment, it strongly suggests that the leptin is not the only mediator in explaining sleep apnea in overweight and obesity, but can predict SAS independently of obesity. To further confirm this point, Manzella et al. (2002) investigated the possible associations between SAS and hyperleptinemia in 20 overweight and obese subjects (age:  $50.8 \pm 6.7$  yr, BMI =  $30.9 \pm 4.2$  Kg/m<sup>2</sup>, SaO<sub>2</sub> =  $95.4 \pm 2.1\%$ ). Among many variables, BMI correlated positively with fasting plasma triglycerides ( $r = 0.67$ ;  $P < 0.001$ ) and leptin ( $r = 0.64$ ;  $P < 0.002$ ). Multivariate analysis predicted 95% of the AHI variability with plasma soluble leptin receptor ( $r = 0.76$ ;  $P < 0.001$ ) but not BMI. They concluded that the reduced amount of leptin receptor was associated with sleep apnea, independently of obesity status. However, as stated earlier, SAS is strongly associated with visceral fat accumulation rather than whole body fat given by BMI, yet the great majority of studies have looked at the association between SAS and BMI.

To date, only one study has reported evidence of SAS in lean subjects to determine the contribution of SAS and obesity on plasma leptin levels. Barceló and colleagues (2005) compared leptin levels between 24 obese (BMI > 30 kg/m<sup>2</sup>) and 24 nonobese (BMI < 25 kg/m<sup>2</sup>) subjects with SAS, and 19 obese and 18 nonobese control subjects without SAS. In the SAS-free disorder group, obese subjects had significantly higher leptin levels compared to the controls ( $24.7 \pm 3.5$  vs.  $5.5 \pm 0.5$  ng/ml,  $P < 0.001$ ). To evaluate the effects of SAS, they compared the leptin levels between the nonobese control subjects without SAS and the nonobese patients with SAS. Leptin was also increased in the latter ( $11.5 \pm 1.6$  vs.  $5.5 \pm 0.5$  ng/ml,  $P < 0.01$ ) but to a

smaller extent than for the obese and nonobese control group. This study provides strong evidence that elevated leptin is mostly associated with obesity and to a lesser degree to sleep apnea. It is also well-known that leptin activates the sympathetic nervous system to increase energy expenditure, thermogenesis, and reduce food intake. Elevated leptin levels are known to cause an increase activity of the sympathetic nervous system in obesity (Yildiz et al., 2004). Because hypoxia and hypercapnia seen in SAS are also responsible for sympathetic nervous system overactivation, it is hypothesized that obesity and SAS share simultaneous factors altering both ventilatory control and producing this sympathetic tone. It is also known that the amount of leptin produced in our body is proportional to the amount of white-adipose tissue and can be reversed in fasting conditions, suggesting a strong dependence to body weight status. Taken together, leptin may be a key factor simultaneously altering ventilatory control and producing sympathetic overactivity in both obesity and sleep apnea, making it difficult to completely isolate the elevated level of the cytokine to either SAS or obesity alone. More needs to be done to clearly identify what is responsible for elevated leptin levels in obesity combined or not with SAS.

While extensive research has been conducted on leptin, not much has been done on the effects of GH/IGF-1 in SAS. Contradictory results reaffirm that the regulatory mechanisms of GH mediated by IGF-1 in SAS are still not completely understood. Yilmaz et al. (2002) examined the influence of tonsil and adenoid hypertrophy (TAH) in 32 children (31% girls and 69% boys) aged 4-8 yr with SAS. After an overnight fasting, the children were given a breakfast of 450 to 500 kcal one hour before blood was collected for IGF-1 and IGFBP-3. The goal of this

step was to avoid fasting status to interfere with the serum levels of IGF-1 and IGFBP-3. All children underwent dissection tonsillectomy and curettage adenoidectomy. Blood samples were obtained before the operation and repeated at 3 to 6 months after the operation. Radioimmunoassay was used to analyze IGF-1 and IGFBP-3. Levels of IGF-1 increased significantly by 35% from  $85.1 \pm 54.6$  ng/mL to  $115.6 \pm 66.2$  ng/mL ( $P < 0.001$ ), suggesting blunted baseline levels. One major weakness from this study comes from the lack of descriptive characteristics (height, weight, percent body fat, BMI) that could help understand the wide range of disparate values obtained for IGF-1 and IGFBP-3. Similarly, Gianotti and colleagues (2002) reported lower basal IGF-1 levels in SAS obese patients compared to weight-matched patients with simple obesity ( $17.5 \pm 1.9$  nmol/L vs.  $21.3 \pm 1.6$  nmol/L,  $P < 0.05$ ) and lean controls ( $28.0 \pm 2.1$  nmol/L,  $P < 0.001$ ). Simple obesity was associated with reduced IGF-1 levels between obese and lean individuals ( $P < 0.03$ ).

It is again difficult to dissociate the effects of obesity or obesity related disorders and SAS to IGF-1 disturbances. Many studies suggest that the change in insulin level starts out the cascade of impaired GH and IGF-1 production in SAS. As a matter of fact, insulin is able to inhibit GH synthesis and release, suggesting an IGF-1-like effect (De Marinis et al., 2004). Patients with SAS have reportedly higher prevalence of insulin resistance and diabetes mellitus than normal body weight individuals, explaining the negative metabolic control of GH/IGF-1 axis in the disorder (Caples et al., 2005). In addition, recent evidence in experimental rat models showed that hypoxia was responsible for an acute but reversible suppression of GH release, and reduced GH mRNA expression in the anterior pituitary (Zhang and Du, 2000 ; Gutierrez et al.,

2003). As stated earlier, GH/IGF-1 directly controls lipolysis by enhancing triglyceride breakdown and suppressing the ability to accumulate in adipose cells. It is therefore important to further explore the consequence of impaired GH and IGF-1 in increasing the risk of cardiovascular events as well as mortality. While the emphasis has been put mainly on the increasing role of sleep apnea in cardiovascular disease, more and more *in-vitro* evidence suggests that SAS may exert some potential detrimental effects on bone accretion and metabolism.

#### Physiological and Endocrine effects of SAS/hypoxia on bone

Even though no current *in vivo* evidence suggests that SAS may directly or indirectly control bone formation and resorption, many experimental studies support that bone metabolism is disturbed in hypoxic conditions. Indeed, hypoxia is well-known to be associated with the extracellular acidification responsible for bone resorption more precisely, stimulation of the resorptive activity of the osteoclasts (Kaysinger and Ramp, 1998 ; Fujimoto et al., 1999). A study by Arnett et al. (1996) was conducted to determine the effects of small extracellular pH changes, independent of hypoxia on rat osteoclasts *in vitro*. Disaggregated osteoclasts obtained from neonatal rat long bones were cultured for about 24 h on bovine cortical bone disks in a  $\text{HCO}_3^-/\text{CO}_2^-$  buffered medium with added protons and hydroxyl ions. They used the pit counting technique to measure the degree of bone resorption. Above a pH of 7.30, little or no resorption occurred in the culture medium. Between 7.0 and 6.9, osteoclasts exhibited progressive to maximum resorptive action but the most sensitive change occurred at a pH of about 7.1 ( $P < 0.02$ ). A minimal change in the pH of less than 0.05 units was responsible for a doubling rate of

the pit formation ( $P < 0.02$ ), emphasizing on the possible effect of pH changes on bone cellular activities.

A few years later, Arnett et al. (2003) investigated the effects of different levels of oxygen tension (atmospheric to very low level: 20, 12, 5, 2, 1, 0.2 % O<sub>2</sub>) on the formation and action of osteoclasts from 7-day mouse marrow cultures. At 20 % O<sub>2</sub> level, no significant changes in osteoclasts size and number were reported. Conversely, cultures maintained in 12, 5 or 2 % O<sub>2</sub> showed significant osteoclast hypertrophy and hyperplasia. Indeed, resorption was increased 4, 9, and 21-fold, respectively, compared to the control ambient O<sub>2</sub> culture ( $0.001 < P < 0.05$ ). In 13-day cultures, osteoclast formation increased 3.5-fold and resorption by 9.5-fold. Hypoxia did not seem to alter the resorptive activity of mature osteoclasts to a great extent, confirming the negative role of hypoxia on osteoclast formation. In conclusion, both hypoxia and hypoxia-related acidosis may be important regulators of bone resorption, only emphasizing on the importance of adequate vasculature in bone. The reduction in oxygen saturation of  $\geq 4$  % observed in SAS may be responsible for lower site-specific BMD and BMC.

As reported previously, bone metabolism in obesity is under the control of multiple endocrine markers such as leptin and IGF-1. Both hormonal concentrations also disturbed in SAS may therefore play a role in bone metabolism. To date, there is no direct evidence of sleep apnea/hypopnea on BMD and BMC in humans, making the current study quite novel. Furthermore, no current study interested in the role of sleep apnea in bone impairment has looked at the bone biomarkers (osteocalcin and N-telopeptide of type I collagen cross links) to monitor rate of bone formation and resorption.

## *Instrumentation and key parameters*

### Body composition

With the increasing rate of obesity and its consequences, especially visceral obesity, numerous techniques are used for screening body tissues to make subsequent health risks predictions. Despite allowing direct measurements of soft tissues, computed tomography (CT) or magnetic resonance imaging (MRI) are quite expensive, time-consuming, and expose the subject to high doses of radiations. Simple anthropometric measurements such as waist-to-hip ratio, waist and hip circumference are easy and cheap tools but do not allow differentiation between visceral and subcutaneous fat (abdominal fat). While the DXA machine has the same weakness as the previously cited techniques, it is accurate, simple and the radiation dose is minimal. Snijer et al. (2002) investigated the potential use of DXA combined with anthropometric measurements for visceral adiposity prediction compared to computed tomography. Scanning of 150 patients, aged 70-79 showed that total abdominal fat was strongly correlated with same readings by CT ( $r = 0.93$ ). The combination of DXA measurements with anthropometry improved the prediction of visceral fat by only 4 %. Those results suggest that DXA is as effective as CT in predicting visceral fat. Similarly, Glickman et al. (2004) found that DXA lumbar L<sub>1</sub>-L<sub>4</sub> region of interest proved to be as reliable and accurate as CT to determine abdominal adiposity.

Despite the higher prevalence and incidence of women with osteoporosis, about 20% men in the United States are affected by osteoporosis ([www.nih.gov](http://www.nih.gov)). Like women, non-Hispanic white and Asian men have the higher prevalence of osteoporosis compared to African-American and Hispanics (7, 4, and 3% respectively). Both genders combined, osteoporosis

causes more than 1.5 million hip, wrist, and vertebral fractures each year, because of the fragile and porous bone structure in these areas. The DXA machine allows with great accuracy the evaluation of whole-body and regions of interest BMD and BMC and may serve in indirect estimation of an individual fracture risk. Because of its simplicity, accuracy, and low cost, DXA is currently the standard method used in clinical evaluation of BMC and BMD (Madsen et al., 1997). Measurements of BMD give information about the total amount of bone for the surface area measured while BMC accounts for whole-body or site-specific bone mass. However, as it measures in two-dimensional planes, DXA does not allow determining bone mechanical strength and fracture risk directly, suggesting inability to assess volumetric bone density ( $\text{g}/\text{cm}^3$ ). Nevertheless, the World Health Organization (WHO) uses measurements from the DXA to define patients at significantly increased fracture risk. Furthermore, no previous study involving sleep apneics patients has evaluated BMD and BMC, suggesting that measurements of BMC and BMD using DXA in the context of the disorder is completely novel and a starting point to better understand the potential effects of SAS on bone.

### Biomarkers

#### *EIAs for bone turnover: Osteocalcin and NTx*

Bone turnover refers to the rate at which bone formation and bone resorption occurs. Biochemical tests of bone turnover provide different information from BMD and BMC measurements since it measures, at the bone cellular level, how fast the skeleton is remodeling. These markers precisely detect the acute changes taking place in the bone environment. Monitoring *in-vivo* the enzymes and other biochemical markers released from the osteoblasts and



osteoclasts gives precise information about the rate of bone turnover and occurrence of fractures. In clinical settings, it should complement bone mass tests in predicting the risk of future and on-going bone loss (Eyre, 1997). Yet, use of such bone biomarkers for individual diagnosis of disease remains debatable due to differences between ethnic groups, gender, secular changes, physical activity level, seasons, number of subjects, length of the study, techniques used, study design, and time of the sample collection (Heshmati et al., 1998 ; Garnero 2004). Previous authors reported intra-subject serum variability between 5 and 10%, and up to 20% for the urinary samples. Bone turnover also follows a circadian rhythm with bone resorption peaking at night and to a smaller extent, bone formation. Studies involving bone biomarkers measurements performed on men are limited. However, we now recognize the potentially damaging effects of sleep apnea combined with obesity on bone metabolism, suggesting the need for more extensive use of the bone biomarkers in this group.

Osteoblasts synthesize and release different proteins measured as markers of bone formation including alkaline phosphatase, procollagen I extension peptides, sialoprotein, and osteocalcin. Osteocalcin is the most abundant noncollagenous 5800 kDa protein in bone (Craciun et al., 2000). Activation of its glutamic residues are vitamin K-dependant. Between 70 and 90% (adults and the young, respectively) of the osteocalcin produced is incorporated into the bone matrix where it binds to the calcium phosphate crystals of the hydroxyapatite (Swaminathan, 2001). There, it forms approximately 1% of the organic matrix of bone. Consequently, only a small amount of newly produced osteocalcin is released into the circulation but it is sufficient to reflect the spillover of osteoblast activity. In clinical research studies,

osteocalcin is the ideal bone biomarker to measure because of its exclusive specificity to the process of bone formation compared to the procollagen I extension peptides also found in the skin and soft tissues (Eyre, 1997). For adult males, the normal reference range for osteocalcin is between 8.0-52.0 ng/mL.

During bone resorption, osteoclasts are responsible for the dissolution of the mineral phase (crystals of hydroxyapatite) by acidification of the inorganic phase of bone, the secretion of proteases for the degradation of the organic matrix, and the digestion of the fragments of collagen. The formation and activation of osteoclasts are directed by the osteoblasts, but only calcitonin directly inhibits osteoclasts after binding to specific surface receptor (Selander et al., 1996). Osteoclasts produce many markers of bone resorption including acid phosphatase, hydroxyproline & hydroxylysine (propeptides derived from the N or C terminal ends of the type I procollagen molecule), collagen cross-link molecules (pyridinoline and deoxypyridinoline), cross-linked telopeptides of collagen I. Type I collagen is the most abundant organic component of the bone matrix and also the most useful marker of bone resorption used in research clinical settings. Following the protease degradation, the amino-terminal and carboxyterminal cross-link forming sites of the type I collagen are released as peptide-attached cross-links called telopeptides, and then identified with specific antibodies (Swaminathan, 2001). The two resulting molecules N-terminal telopeptides (NTx) and C-terminal telopeptides (CTx) are identified in urine and serum by enzyme-linked immunoassays (ELISA). There are debating issues about the specificity of NTx assays to bone because they measure all collagen type I degradation products, but NTX remains the best marker of bone resorption (Swaminathan, 2001).

No current study has evaluated the bone turnover status in subjects with SAS, making the current study completely novel. Uses of both OC and NTX assays may help us to understand better the health consequences of such a debilitating disease.

#### *RIAs for endocrine functions: leptin and IGF-1*

Leptin is stable for at least five freeze/thaw cycles whether in serum, plasma, and cerebrospinal fluid. Following its discovery, leptin was isolated through an immunoprecipitation/Western blot technique using antibodies raised against the first 20-amino terminal amino acids of leptin. However, this technique was only semi-quantitative and the need for more accurate and precise ways to isolate leptin became quickly apparent. A more precise, quantitative radioimmunoassay kit for leptin was first developed by Linco Research Inc. More than 80% of the current literature involving circulating leptin levels in humans has used this assay (Wallace, 2000). This assay is also highly sensitive to human leptin since the antibody used is raised against highly purified Human Leptin. Radioimmunoassay isolation of leptin is therefore highly appropriate and adequate to use for this study. Concentrations of IGF-1 are also determined by radioimmunoassay. In males aged 16-24 yr, the normal reference range is between 23.7-101.4 nmol/L. The hormonal data from leptin and IGF-1 may suggest whether factors directly or indirectly influencing bone regulation are modified by being overweight or the combination of overweight and SAS in young adults.

#### Risk for SAS

##### *Embletta at-home night time screening device to estimate AHI*

The Embletta PDS (Portable Diagnostic System) is a pocket-sized digital recording

device that makes ambulatory sleep studies convenient and reliable. This device records up to twelve hours of comprehensive respiratory data that can be easily reviewed and analyzed using the Somnologica Embletta software. It consists of a nasal cannula for air flow, chest and abdomen straps to detect movements, a finger pulse oximeter to measure blood oxygen level, and a microphone placed in the mid-region of the neck to assess snoring pattern. For best results, SAS is usually diagnosed by in-clinic overnight sleep recording or polysomnography. However, sleep clinics offer only limited bed capacities resulting in waiting list of undiagnosed patients. This procedure is also expensive to both patients and healthcare systems (Dingli et al., 2003). At-home sleep devices such as the Embletta offer screening possibilities for reduced costs. In addition, it gives sufficient information for interpretation by a trained sleep technician.

### *Summary*

Achieving maximum bone accretion during childhood and adolescence is critical to preserve bone mass and integrity and minimize osteoporotic fracture risk later in life. Environmental, hormonal, and genetic factors are known to influence bone mineralization. For instance, simple obesity is associated with increased BMD. Unfortunately, obese children with high BMD paradoxically fail to reach adequate bone mass to carry this excessive body weight, raising questions asking why there should be a mismatch between skeletal growth and weight. Obesity is associated with disturbed levels of cytokines and hormones involved in the control of bone metabolism. Through a cascade of signaling neuroendocrine messengers and pathways not currently well understood, abnormally elevated leptin levels may exert some anti-osteogenic

effects on regulation of bone metabolism and bone development. Reduced IGF-1 may as well contribute to the shorter stature and delayed skeletal growth attained in obese children. Sleep apnea syndrome, a frequent comorbid disorder of obesity in adults, may have the same prevalence among overweight children and exert independent effects on suppressing bone development as well. Sleep apnea syndrome is a condition characterized by occurrence of brief repetitive interruptions of breathing during sleep, resulting in poor air flow in and out of the nose or mouth and often associated with reduction in blood oxygen saturation of  $\geq 4\%$  or hypoxemia. Based on findings from experimental studies, intermittent hypoxia is a detrimental environment to bone accretion. Prior research reporting low BMD in overweight young adults could potentially reflect the effect of sleep apnea syndrome. Sleep apnea also augments increases in leptin and reductions of IGF-1, beyond the changes in these cytokines associated with obesity alone. With growing concern for our young population already at risk for cardiovascular diseases it is important to better understand, if disturbed metabolic regulation associated with obesity-related SAS may compromise bone regulation in young adults and lead to reduced bone accretion at skeletal maturation.

CHAPTER 3  
JOURNAL MANUSCRIPT

*Skeletal Status and Bone Turnover in Overweight Young Adults with and without Sleep Apnea  
Syndrome*

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## Abstract

Obesity is a worldwide epidemic increasing at alarming rates among youth who are facing similar health problems as adults. Sleep Apnea Syndrome (SAS) is a pernicious underdiagnosed comorbidity of obesity, characterized by repetitive nocturnal cessations of breathing.

Obesity is associated with delayed skeletal maturation in youth. Therefore, the combined effects of excess body weight and SAS on bone turnover, bone mineral density (BMD) and content (BMC) for the whole body, lumbar spine, hip, and forearm were investigated. The potential contributions of regulatory hormones leptin and IGF-1, known to potentially influence bone accretion during adolescence were further examined.

Men, aged 18-28 years, were assigned into normal weight controls (CON: AHI <3, n=8), overweight without SAS (OWT: AHI <3, n=9), and overweight with SAS (SAS: AHI >5, n=8) based on estimates of the apnea/hypopnea index (AHI) obtained with a home sleep screening device and BMI. Health history and Epworth Sleepiness Scale (ESS) questionnaires were also administered. Bone mineral parameters and body composition variables were measured with dual-energy X-ray absorptiometry. Serum osteocalcin, leptin, IGF-1, and NTx-1 were measured by radioimmunoassay and enzyme-linked immunoabsorbent assay, respectively.

Fat-free mass, intra-abdominal fat, and fat mass were higher in the SAS and OWT groups ( $p < 0.03$ ). ESS scores revealed that SAS individuals were sleepier than CON and OWT groups ( $p < 0.009$ ). Total and site-specific BMD and BMC values were similar between groups and did not relate to the estimated AHI score. Serum OC and NTx-1 did not differ between groups. Leptin levels were about 30 % higher in OWT and SAS than in the CON group ( $p < 0.02$ ), but did not correlate with the estimated AHI score. Correlational analyses including all subjects indicated that only lumbar BMC ( $p < 0.005$ ) was negatively correlated ( $r = -.52$ ;  $p < 0.01$ ) to AHI. This relationship was mainly found in the SAS group ( $r = -.81$ ;  $p < 0.001$ ).

Sleep apnea syndrome in combination with obesity is negatively associated with lumbar spine BMC. The involvement of leptin and IGF in this relationship remains unclear. More research is needed to understand better the relation of SAS and bone health.

**Key words:** Sleep apnea syndrome, Bone mineral content, leptin, Insulin-like growth factor-1, Osteocalcin, N-telopeptides cross-link-1

## **Introduction**

Maximizing achievement of peak bone mass during childhood and adolescence is important to prevent osteoporotic-related fractures and excessive bone loss later in life. According to the mechanostat theory, changes observed in bone strength during growth are secondary to the increased loads generated by large muscles to which they attach. In other words, an increase in mechanical loading from muscle strength should influence bone mineral acquisition and increase bone mineral density (BMD) at sites of attachment. In older adults, lean body mass (LBM) might not be the best predictor of bone strength and the fat-bone relationship seems to prevail more in this age group as higher amount of fat tissue increases the release of cytokines known to negatively affect bone strength [1]. Obesity is known to increase skeletal mass due to skeletal loading. However, it has been reported that obese children have lower BMD relative to the excess body weight for chronological age [2]. Mechanisms explaining this relationship in obesity remain unclear, but evidence suggests a possible hormonal mediation between fat mass, LBM and bone [1, 2].

Leptin, a product of the *ob* gene, is secreted by the white adipose tissue and correlates positively with total body fat stores. More leptin is produced by larger adipocytes or larger concentration of adipocytes at the site of measurement [3 , 4]. Evidence suggests that leptin is able to control bone metabolism but its mechanisms of actions are conflicting. Ducy et al. [5] reported that obese mice deficient in leptin and leptin receptors had an increased bone mass, independent of obesity, while intracerebroventricular infusion of leptin in both mutant forms induced bone loss. Based on previous findings, high leptin levels observed in obesity could



increase adipocyte signaling to progenitor cells in such a manner to reduce formation of new osteoblasts. Other lines of evidence suggest that the bone marrow stromal cells are able to differentiate into osteoblasts as well as adipocytes, chondroblasts, and myoblasts. Cells of osteoblastic lineage do express leptin surface receptors, enabling osteoblastic differentiation and promotion of bone mineralization. Taken together, leptin possibly exerts dual actions depending on skeletal maturity and signaling pathways [6 , 7].

Through direct actions on LBM, IGF-1 is another possible indirect mediator between muscle mass and bone density since LBM is a strong independent predictor of BMD and related fractures [8]. Growth hormone promotes its growth effects in skeletal muscle through IGF-1, the latter being directly responsible for increasing myocyte differentiation and skeletal muscle hypertrophy [9]. In obese individuals, IGF-1 levels are decreased, and this could potentially result in diminished accumulation of lean muscle mass. The reduction in muscle mass may lessen tension loads on bones to which the muscles attach and ultimately result in lower bone accretion in youth.

Intermittent hypoxia, also known to impact bone turnover, may clearly reflect the effect of sleep apnea syndrome (SAS) not previously recognized as a comorbidity of obesity, especially intra-abdominal adiposity [10-15]. Sleep apnea syndrome is a serious condition characterized by occurrence of brief repetitive interruptions of breathing often with dips in oxygen saturation of blood (>4%) during sleep. The collapse of soft tissues in the upper airways results in poor air flow, leading to intermittent episodes of hypoxia. Indeed, hypoxia is well-known to cause extracellular acidification responsible for bone resorption, particularly stimulation of the

resorptive activity of the osteoclasts [16 -18]. To date, there is no direct evidence of sleep apnea on bone mineral status in humans. Yet, it is important to examine if SAS combined with obesity may exacerbate the changes in bone environment seen in overweight. As mentioned previously, bone metabolism in obesity is under the control of multiple endocrine factors, such as leptin and IGF-1. Both of these hormones, if also disturbed as a function of SAS, may therefore play an important role in chronically modifying bone turnover, particularly important during growth, resulting in less than optimal bone accretion at skeletal maturity. Measurements of the bone biomarker levels will help to evaluate possible acute effects of SAS on bone turnover, allowing inferences about effects of this disorder on bone remodeling.

Thus, we investigated the influences of excess body fat, with and without the presence of SAS on BMD and BMC measures of interest, on serum biomarkers of bone turnover, and on two serum cytokines, leptin and IGF-1, which may have potential regulatory effects on bone.

## **Materials and Methods**

### *Study Sample*

Twenty-seven young men, 18-28 years old, from the Virginia Polytechnic Institute and State University (VPI & SU) campus and surrounding areas were recruited by electronic mailings, personal contact, newspaper announcements, and flyers for participation. Subjects were financially compensated for their participation and received comprehensive individualized results upon completion. The subjects were equally categorized into three different groups according to BMI classification and results from the at-home sleep test: (1) controls (CON: n = 8, BMI <26 kg/m<sup>2</sup>, no SAS), (2) overweight and obese without SAS (OWT: n = 9, BMI >26

kg/m<sup>2</sup>), and (3) overweight and obese with SAS (SAS: n = 8, BMI >26 kg/m<sup>2</sup>). We chose to define our groups using a BMI cut-point of 26 Kg/m<sup>2</sup> based on recent evidence suggesting that being slightly overweight was not associated with excess mortality [19]. Participants with BMI <18 kg/m<sup>2</sup> were excluded from the study. Other exclusion criteria included any acute respiratory infections during the previous 6 weeks, diagnosed or medically-treated cardiovascular, pulmonary, or metabolic disorders, receiving any prescribed vasoactive medications, cigarette smoking, any sleep problems potentially related to emotional health (assessed by responses to sleep survey items that affect sleep status), and participation in moderate to vigorous physical activity > 2 times a week for at least 20 minutes. Self-reported height and weight obtained from the medical history questionnaire were used to calculate BMI as one of the screening tools. All potential subjects provided the informed consent. Researchers covered any concerns and questions from the informed consent form and additional questionnaires prior to participation in this study. This research project was approved, as required, by the Institutional Review Board for projects involving human subjects at VPI & SU and the Department of Human Nutrition, Foods, and Exercise.

### *Anthropometrics*

Weight and height were measured to the nearest 0.1 kg and 0.1 cm, respectively, using the stadiometer (Detecto, Webb City, MO). For accuracy purposes, BMI was then recalculated and used in data analyses rather than the subjects' self-reported values. Neck, hip, and waist circumference measures were obtained also during the first visit in the laboratory using a retractable ruler tape.

### *Embletta PDS*

The Embletta PDS [Portable Diagnostic System, ResMed Corp, Poway, CA] is a pocket-sized digital recording device that makes ambulatory sleep studies convenient and reliable. This device records up to twelve hours of comprehensive respiratory data that could be easily reviewed and analyzed using the Somnologica Embletta software. It consists of a nasal cannula for air flow, chest and abdomen straps to detect movements, a finger pulse oximeter to measure blood oxygen level, and a microphone placed in the mid-region of the neck to assess snoring pattern. The device was worn during a weeknight of normal sleep and values were used to estimate AHI scores. The Embletta PDS assesses quite accurately apnea/hypopnea events [20] but a trained sleep technician over-analyzed all scores obtained from the device for optimal results.

#### *Questionnaires*

Subjects were administered a detailed health history form, through self-report and interview by researchers. The health history form provided information on basic demographic and health variables. Subjects completed one standardized sleep questionnaire, the Epworth Sleepiness Scale (ESS), and the Veteran's Specific Activity Questionnaire (VSAQ). The ESS is a simple tool to assess patterns of daytime sleepiness, a strong predictor of SAS. The VSAQ provides a quick and simple assessment of the participant's perception of their own physical fitness level. Each participant was instructed on the procedure for maintaining a 4-Day Diet Record at home and for returning this record to the investigator at the specified intervals.

#### *Body composition*

Dual-energy X-ray absorptiometry (DXA; QDR-4500A, Hologic, Inc., Bedford, MA)

was used to measure LBM, fat mass, percent body fat, intra-abdominal fat, BMD ( $\text{g}/\text{cm}^2$ ) and BMC (g) for the whole body, total forearm (radius + ulna), non-dominant total proximal femur (hip), and standard lumbar spine (L1–4). Intra-abdominal fat was defined as fat within the cavity formed by the back and abdominal wall musculature. All DXA scans were conducted by one licensed investigator to eliminate inter-tester variation. Quality control scans of an anthropometric phantom lumbar spine were conducted prior to each testing session. Quality control of soft tissue mass was ensured by scans of an external wedge composed of aluminum and Lucite calibrated against stearic acid and water (Hologic, Bedford, MA). In our laboratory, the coefficients of variation (CV) for the whole body, total proximal femur, femoral neck, Ward's triangle, and lumbar spine BMD were 0.58%, 0.89%, 3.0%, 3.11%, and 0.63%, respectively [22].

#### *Markers of bone turnover and hormone measurements*

Subjects were informed to avoid any physical activity, caffeine, and alcohol consumption at least 24-hr prior to their scheduled blood sample collection. Blood samples were scheduled between 0745 and 0930 h to avoid inter-subject diurnal variation and most preferably within 2 hours of awaking. Samples were collected in serum vacutainer tubes by a trained phlebotomist and allowed to stand 30 min at room temperature. The serum was separated by centrifuge for 15 min at  $1,048 \times g$  and  $4^\circ\text{C}$ . Pippetted serum into microcuvettes was stored at  $-80^\circ\text{C}$  until assays were performed. For the current study, serum samples were analyzed OC, NTx, leptin, and IGF-1. The OC assay is an enzyme immunoassay utilizing two antibodies with an intra-assay CV of 2% (Mid-Tact Human Osteocalcin RIA Kit; Biomedical Technologies Inc). The concentration of

OC in the sample is proportional to the absorbance and values obtained by comparison to a standard curve prepared on the same assay plate. All samples were analyzed in duplicate. Serum NTx levels were measured by a competitive enzyme-linked immunoabsorbent assay (Osteomark NTx Serum; Wampole Laboratories). NTx measurements were reported as bone collagen equivalents (BCE). The NTx intra-assay CV was 8.5 % respectively.

The human leptin RIA kit (Linco Research Inc., St-Charles, MO) was used to assess serum leptin levels through competitive binding between <sup>125</sup>I-human leptin tracer and our unknown samples. The leptin intra-assay CV was 11%. Serum IGF-1 was quantified by radioimmunoassay according to the methods of Weber et al [23]. The IGF-1 intra-assay CV was 8 %.

#### *Statistical analysis*

All statistical analyses were performed using SPSS version 13.0 statistical software (SPSS Inc., Chicago, IL). Group comparisons (CON, OWT, and SAS) were performed using one-way ANOVA. When significance was obtained, post-hoc statistical tests were performed to identify the groups that differ from one another. Pearson product correlations were calculated to examine the relationships between entered variables of interest. A two-tailed level of significance was set at  $p < 0.05$  for all tests.

## **Results**

#### *Subjects characteristics*

Baseline physical characteristics and body composition variables including age, height, weight, BMI, neck, waist, and hip circumference, fat mass, intra-abdominal fat mass, lean body

mass, AHI and ESS scores are shown in **Table 1**. Body weight, BMI, neck, waist, and hip circumference were all significantly lower in the CON group compared to both OWT and SAS groups ( $p < 0.005$ ). Fat-free mass, intra-abdominal fat, and fat mass were higher in the SAS and OWT groups ( $p < 0.03$ ). The estimated AHI scores were higher by 22 % in the SAS group ( $p < 0.005$ ) in comparison to the CON and OWT groups, while the latter two groups were not different on this measure. Based on ESS scores and as anticipated, subjects in the SAS group were sleepier than both CON and OWT groups ( $p < 0.009$ ). The AHI scores were unrelated to any of the physical characteristics in the overall sample of subjects ( $n = 25$ ) (i.e. height, age, body weight, BMI, neck, waist, and hip circumference, fat mass, intra-abdominal fat, and fat-free mass).

#### *Bone densitometry*

As shown in **Table 2**, there were no differences between groups in total BMD or BMC and in site-specific lumbar (L1-L4), hip, and forearm (radius + ulna) BMD and BMC between groups. Estimated AHI was negatively correlated to lumbar BMC ( $r = -0.52$ ;  $p < 0.01$ ;  $n=25$ ), while no other bone mineral measure of interest showed any such association. Overall correlation was much stronger in the SAS group ( $r = -.82$ ;  $p < 0.001$ ). Both patterns are illustrated in **Figure 1**. There was a trend between total BMC and the AHI scores for the study population ( $r = -.40$ ;  $p < 0.053$ ;  $n=25$ ) but relationship is diminished after statistically controlling the association for body height.

#### *Differences in bone biomarkers*

Serum OC and serum NTx did not differ between groups as reported in **Table 2**. Normal

ranges for OC and NTx are 2.5-14 ng/mL [24] and 5.4-24.2 nm BCE [25], respectively. No differences were noted for the marker of bone formation, osteocalcin in the SAS group.

#### *Differences in hormonal measures*

**Figure 2** depicts values by group for IGF-1 and leptin levels. Normal fasting ranges for IGF-1 and leptin are 109-294 ng/mL [23] and 2.0-5.6 µg/L [26], respectively. Serum IGF-1 levels were not different across groups, while serum leptin was higher than CON in both the OWT ( $p < 0.001$ ) and SAS ( $p < 0.02$ ) groups. Intra-abdominal fat positively predicted leptin levels ( $r = 0.84$ ;  $p < 0.001$ ) across all subjects ( $n = 25$ ), but intra-abdominal fat was a stronger predictor of the cytokine than BMI score ( $r = 0.84$  vs.  $0.77$ ) as illustrated in **Figure 3**. Neither of these cytokines, however, were correlated with AHI scores.

#### **Discussion**

Achievement of peak bone mass during skeletal accretion in youth is a critical step to predict BMD and BMC in adulthood. Even though no current *in-vivo* evidence suggests that SAS may directly or indirectly control bone formation and resorption, many experimental studies support that bone metabolism is disturbed in hypoxic conditions [27 -31].

The primary aim of this study was to assess to the effects of SAS on bone status and bone turnover. We believe this may be the first human study to examine these factors in the setting of overweight individuals with SAS. We did not demonstrate any changes of bone parameters, when analyses were performed across the groups. However, estimated AHI scores were negatively correlated to lumbar spine BMC ( $p < 0.005$ ). We also found a trend in the



association for total BMC and AHI scores which disappeared after height adjustment. Even if body size was similar between groups, within-group differences might explain why total BMC and estimated AHI scores were related only before height adjustment. Overweight individuals with SAS may experience greater bone resorption than overweight alone and lean controls. Sleep apnea syndrome is marked by repetitive intermittent bouts of apnea/hypopnea events during which air flow is obstructed therefore reducing blood oxygen saturation levels. This hypoxic environment is known to disturb bone cellular activities locally.

Previous animal and laboratories studies support the findings in the current analysis. Arnett et al. [16] investigated the effects of different levels of oxygen tension (atmospheric to very low level: 20, 12, 5, 2, 1, 0.2 % O<sub>2</sub>) on the formation and action of osteoclasts from 7-day mouse marrow cultures. At 20% O<sub>2</sub> level, no significant changes in osteoclasts size and number were reported. However, cultures maintained with O<sub>2</sub> levels at only 2-12% showed significant osteoclast hypertrophy and hyperplasia. Indeed, under those lower levels of chronic hypoxia resorption was increased 4, 9, and 21-fold, respectively, compared to the control ambient O<sub>2</sub> culture (0.001 < P < 0.05). To replicate the effects of repetitive intermittent hypoxia levels in humans is practically impossible for ethical reasons and does not provide information about oxygen tensions in tissues. In the current study, lowest oxygen (O<sub>2</sub>) saturation levels were significantly lower in the OWT groups compared to both the CON and SAS groups (p < 0.02). No correlation was found between lowest O<sub>2</sub> saturation levels and any bone parameters measured. Only 4 subjects displayed significant lower O<sub>2</sub> saturation levels than 90% for more than 5 minutes. It may be important therefore to have low O<sub>2</sub> saturation levels sustained for a

minimal amount of time to noticeably detect changes in bone status.

One possible explanation for the detrimental effects associated with hypoxia reported in previous studies may be that hypoxia causes extracellular acidification, directly responsible for bone resorption and more precisely, stimulation of the resorptive activity of the osteoclasts [17 , 18]. In the current study, it was not possible to assess pH levels that would characterize the changes in bone microenvironment and therefore relevant to osteoclast function. Furthermore, pH changes related to hypoxia would be acute and may reflect general acid-base balance fluctuations. However, we quantitatively assessed osteoblastic and osteoclastic activity by measuring osteocalcin and NTx-1 in circulating serum. Both bone biomarkers are widely accepted as highly specific and representative of osteoblast and osteoclast activity. Little evidence to date demonstrates that osteoblasts function is influenced by hypoxia [18] . The current analysis did not find differences across groups for either of these bone biomarkers. One possible explanation is that subjects have not been exposed enough to repetitive situations of apnea/hypopnea to detect accumulative damages in the bone microenvironment that might be associated with long-term intermittent nocturnal hypoxemia. Severity and duration of the disease may therefore be important to consider before changes of the bone cellular activities might be detected. Another possibility explaining the lack of change is that blood samples might not have been collected close enough to the time of the last hypoxic event. However, CPAP treatment performed right after time of awakening did not affect hormonal measurements of interest [31]. Therefore, time of measurement was not an issue in the current study.

Besides the influence of the repetitive hypoxia specifically reported at the lumbar spine, it is possible that the low lumbar spine BMC reported in individuals with SAS is related to low fitness levels. It may be possible that overweight subjects with SAS don't have the drive to stay physically active. Removing the gravitational load from weight-bearing activities suppresses mechanical loading on bone and results in bone loss, particularly at the lumbar spine. This consensus is believed to be especially true in cyclists [32 , 33]. Correlations were run therefore between VSAQ scores, ESS scores, and lumbar spine BMC. However, low lumbar BMC were not fitness related. Scores from the VSAQ are very subjective and may be biased by the fact that subject may overestimate its physical activity level. Physical activities questionnaires would have provided more accurate measurement of overall fitness levels. Values of peak relative oxygen consumption relative to the whole body ( $pVO_2$ : mL/kg/min) and LBM ( $pVO_{2LBM}$ : mL/kg<sub>LBM</sub>/min) were obtained from a bicycle exercise test. Such values may reflect fitness levels more accurately than scores from the VSAQ. Levels of peak  $VO_2$  were significantly higher in the controls compared to OWT and SAS groups ( $P < 0.008$ ). However, they did not correlate with any bone parameters. Peak  $pVO_{2LBM}$  did not help to predict any of the bone measurements. Further research should explore the relationship between low lumbar spine BMC and fitness levels in obesity.

Bone metabolism in obesity is influenced by multiple endocrine factors such as leptin and IGF-1[34 -38] with both hormonal levels also being disturbed in SAS. In the current study, IGF-1 levels were not different across groups. Correlational analyses also showed no relationship between IGF-1 levels and the estimated AHI scores. Previously, we hypothesized that

overweight individuals with SAS may have lower circulating IGF-1, resulting in lower muscle mass and subsequent bone density due to the low skeletal loading. In the present analysis, however, we found that fat-free mass was higher in both the OWT and SAS groups ( $p < 0.03$ ) but did not correlate with levels of IGF-1. This finding suggests that hypoxia and sympathetic overdrive in SAS might not directly affect IGF-1. It is possible that the increased fat-free mass observed among overweight subjects is, after all, a function of increased mechanical and tension loading from carrying excess body weight. Previous investigators have reported that, although growth hormone (GH) levels are clearly blunted in obesity, the response of IGF-1 in obesity is unclear, i.e. findings reported that it is low, unchanged, or increased [34, 39, 40]. Another possible reason for the lack of differences in IGF-1 levels is that IGF-1 travels in two forms in the body, free and bound to IGF-1 binding proteins. Consequently, evaluation of IGF-1 in blood serum may not accurately indicate total IGF-1 at time of measurement. Roles of other cytokines should be explored to aid in understanding the relationship between disorders on bone health. Many studies suggest that the change in insulin level starts the cascade of impaired growth hormone and IGF-1 production in SAS. Patients with sleep apnea syndrome have reportedly higher prevalence of insulin resistance and diabetes mellitus than normal body weight individuals [41]. Adiponectin, a product of white-adipose tissue, is another cytokine-hormone that needs more attention in SAS. Adiponectin possesses insulin-sensitizing properties and it is commonly reported that low levels of the hormone are associated with insulin resistance and diabetes [28 , 31]. In SAS, adiponectin levels seem to be lower than normal lean controls and may be responsible for the impaired GH and IGF-1 production. No one has explored this venue

yet but it is a plausible mechanism to explain the effects of IGF-1 on bone.

It is generally reported that elevated leptin levels are associated with SAS and obesity [27, 31, 42]. In the current study, leptin levels were 3 to 4 times higher in the overweight with and without SAS compared to the controls but similar between simple overweight and overweight with SAS groups. Elevated leptin levels may therefore be independent of SAS. Results found in the current study are in agreement with some previous research showing that SAS does not help to predict higher leptin levels in overweight individuals [29, 42]. It could be possible that the sympathetic neural activation in SAS suppresses leptin production, thus not noticeably detectable in SAS. Previous research has shown that leptin levels are also increased [43, 44] during nocturnal night of sleep deprivation, daytime sleep, and narcolepsy in healthy lean adults without SAS. Those findings suggest that leptin levels may increase independently of SAS and obesity. More research needs to be done on leptin because its mode of actions in sleep fragmentation and sleep-disordered breathing are still very uncertain.

Overweight individuals with SAS in the present study had similar amount of intra-abdominal fat mass compared to their BMI-matched counterparts. It has been documented that leptin levels are proportional to the amount of white-adipose tissue that release the hormone but also that white-adipose tissue is found in higher amount in the abdominal region. Vgontzas et al. [11] examined whether patients with greater whole body or visceral adiposity were predisposed to sleep apnea. The study sample consisted of 14 obese sleep apneics [age:  $46.6 \pm 3$  yr (mean  $\pm$  sem); BMI:  $38.4 \pm 1.6$  Kg/m<sup>2</sup>; AHI:  $48.7 \pm 5.6$  events/h of sleep], 11 obese and 12 controls. The authors found that the sleep apnea patients had a significantly higher amount of intra-abdominal

fat compared to obese and controls. Furthermore, only intra-abdominal fat positively correlated to indexes of sleep disordered breathing. Total body fat and BMI did not significantly correlate with AHI scores. In the present analysis, leptin was positively correlated with intra-abdominal fat ( $r = 0.84$ ;  $p < 0.001$ ) in subjects with SAS but they did not have higher intra-abdominal fat than the overweight individuals without SAS. It can therefore possibly explain why we did not find significantly higher leptin levels in the SAS group. More research needs to be conducted to elucidate the contribution of SAS and obesity in the control of the hormone.

One major limitation of the current study was the small sample size. We are able to include only 25 subjects categorized in 3 different groups. Subjects in this sample with SAS might not have been sufficiently exposed to SAS long enough to detect changes or differences in body composition variables. For the purpose of this study, we used a portable device to diagnose sleep apnea syndrome. However, the gold standard for SAS diagnosis is polysomnography which allows technicians to record accurately a number of physiological variables during sleep, i.e. brain electrical activity, eye and jaw muscle movement, leg muscle movement, electrocardiogram, airflow, respiratory effort, and oxygen saturation. The portable device used in the current study only allowed assessment of the respiratory effort, oxygen saturation, and airflow, thus limiting the quality and accuracy of the data collected. However, Dingli et al. [20] conducted a study to examine the diagnostic accuracy of the portable device, the Embletta used in the present study compared to a polysomnography test in 61 patients. Authors concluded that the Embletta classified correctly the great majority of the patients ( $n = 32$ ) with SAS previously diagnosed with laboratories test. Overall, the Embletta might allow well-enough to properly

categorize subjects but all tests were re-interpreted by a trained sleep technician.

Only three subjects with SAS had estimated AHI scores above 10, meaning that more than 60% were classified with only moderate SAS. Previously, we mentioned that degree of severity of the disease may be an important factor to detect significant changes associated with SAS. A larger group of severe SAS patients would have likely affected the outcomes of analysis since those subjects with the highest scores had the lowest values for all the bone mineral content and bone mineral bone density variables. None of the subjects had had a previous sleep-disordered breathing test and most subjects were still very young, thus precluding the potential for long-term or cumulative effects that might have further clarified pathophysiologic consequences of SAS [45]. Finally, inclusion of a treatment condition for subjects with SAS might have been advantageous, as this could possibly have demonstrated amelioration of SAS effects and thus provided further understanding of disease consequences of interest in this study. Nevertheless, the current study is very unique and novel, as no other studies to date have explored the issue of bone health in SAS combined with obesity in young male adults.

In conclusion, this is the first study to identify negative associations between sleep apnea syndrome and lumbar BMC. More research is needed to understand better the mechanical and biochemical factors involved in the relationship between fat mass, fat-free mass and bone in obesity and SAS.

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**Table 1.** Mean ( $\pm$  SD) Physical and Physiological Characteristics for Controls (CON), Overweight with (OWT) and without sleep apnea syndrome (SAS)

Variables	CON (n=8)	OWT (n = 9)	SAS (n = 8)	<i>p</i> -value
Age (years)	22.6 $\pm$ 3.1	22.0 $\pm$ 2.9	22.1 $\pm$ 3.1	0.909
Height (cm)	176.5 $\pm$ 4.5	178.0 $\pm$ 6.2	177.0 $\pm$ 7.8	0.370
Weight (kg)	74.0 $\pm$ 5.6	107.4 $\pm$ 17.5	92.7 $\pm$ 7.8	0.001 <sup>a</sup>
BMI (Kg/m <sup>2</sup> )	23.6 $\pm$ 2.1	33.9 $\pm$ 5.7	29.6 $\pm$ 2.3	0.001 <sup>a</sup>
Neck circumference (cm)	36.9 $\pm$ 1.4	42.0 $\pm$ 4.1	40.1 $\pm$ 1.9	0.004 <sup>b</sup>
Hip circumference (cm)	97.1 $\pm$ 4.7	113.6 $\pm$ 10.9	110.9 $\pm$ 5.2	0.001 <sup>b</sup>
Waist circumference (cm)	81.9 $\pm$ 5.7	102.6 $\pm$ 13.1	97.6 $\pm$ 8.9	0.001 <sup>b</sup>
AHI scores (events/hr)	3.0 $\pm$ 1.7	2.8 $\pm$ 1.3	13.4 $\pm$ 11.2	0.004 <sup>c</sup>
ESS scores	6 $\pm$ 3.6	8.6 $\pm$ 4.2	13.1 $\pm$ 4.9	0.009 <sup>c</sup>
Fat mass (Kg)	14.0 $\pm$ 4.3	27.6 $\pm$ 13.3	24.1 $\pm$ 5.2	0.010 <sup>b</sup>
Fat-free mass (Kg)	56.7 $\pm$ 3.3	70.9 $\pm$ 8.7	66.2 $\pm$ 8.5	0.002 <sup>b</sup>
Intra-abdominal fat (Kg)	3.7 $\pm$ 1.3	8.6 $\pm$ 2.9	7.0 $\pm$ 1.6	0.001 <sup>b</sup>

Values are expressed as mean  $\pm$  SD; *p*-values for Post hoc tests between groups.

BMI: Body Mass Index; ESS scores: Epworth Sleepiness Scale scores.

<sup>a</sup> Significantly different between all groups

<sup>b</sup> OWT and SAS significantly different from CON groups

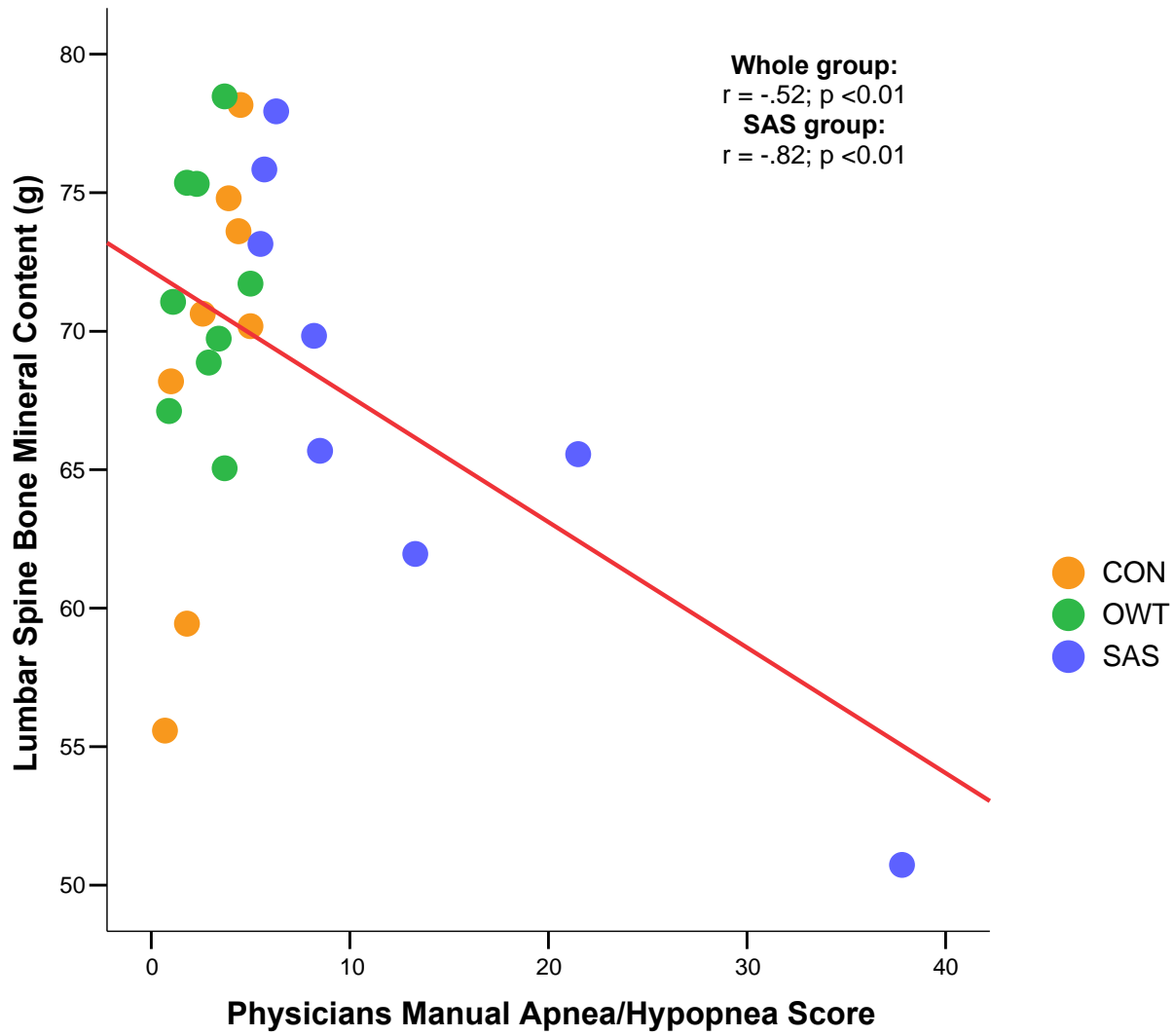
<sup>c</sup> OWT significantly different from CON and OWT groups

**Table 2.** Mean ( $\pm$ SD) of Bone Densitometry and Serum Bone Biomarkers for Controls, Overweight with and without SAS

Variables	CON (n=8)	OWT (n = 9)	SAS (n = 8)	<i>p</i> -value
<b>BMC (g)</b>				
Total body	2,629 $\pm$ 173	2,930 $\pm$ 85	2,863 $\pm$ 155	0.141
Lumbar spine (L1-L4)	68.8 $\pm$ 7.7	71.4 $\pm$ 1.4	67.6 $\pm$ 3.1	0.530
Total proximal femur	15.7 $\pm$ 2.2	16.9 $\pm$ 3.3	17.6 $\pm$ 2.9	0.264
Femoral neck	5.2 $\pm$ 0.6	5.7 $\pm$ 0.8	5.6 $\pm$ 0.8	0.378
Trochanter	9.6 $\pm$ 1.4	10.2 $\pm$ 2.3	10.9 $\pm$ 2.0	0.392
Ward's Triangle	0.9 $\pm$ 0.2	1.03 $\pm$ 0.2	1.1 $\pm$ 0.1	0.088
Total forearm	16.4 $\pm$ 2.3	18.4 $\pm$ 2.5	17.0 $\pm$ 4.9	0.630
Ultradistal	3.0 $\pm$ 0.5	3.28 $\pm$ 0.6	3.0 $\pm$ 0.6	0.587
Mid	9.3 $\pm$ 1.0	10.6 $\pm$ 1.2	9.8 $\pm$ 2.9	0.383
Proximal	4.1 $\pm$ 0.8	4.5 $\pm$ 0.7	4.2 $\pm$ 1.4	0.654
<b>BMD (g/cm<sup>2</sup>)</b>				
Total body	1.19 $\pm$ 0.02	1.23 $\pm$ 0.03	1.23 $\pm$ 0.44	0.597
Lumbar spine (L1-L4)	1.02 $\pm$ 0.04	1.08 $\pm$ 0.03	1.04 $\pm$ 0.03	0.481
Total proximal femur	2.40 $\pm$ 0.34	2.71 $\pm$ 0.46	2.73 $\pm$ 0.30	0.302
Femoral neck	0.89 $\pm$ 0.11	1.00 $\pm$ 0.16	0.98 $\pm$ 0.09	0.218
Trochanter	0.76 $\pm$ 0.10	0.84 $\pm$ 0.13	0.86 $\pm$ 0.14	0.252
Ward's Triangle	0.75 $\pm$ 0.13	0.87 $\pm$ 0.17	0.89 $\pm$ 0.07	0.094
Total forearm	1.90 $\pm$ 0.29	1.98 $\pm$ 0.28	1.98 $\pm$ 0.39	0.860
Ultradistal	0.51 $\pm$ 0.11	0.53 $\pm$ 0.11	0.54 $\pm$ 0.14	0.868
Mid	0.66 $\pm$ 0.05	0.69 $\pm$ 0.06	0.69 $\pm$ 0.08	0.568
Proximal	0.73 $\pm$ 0.13	0.76 $\pm$ 0.11	0.75 $\pm$ 0.17	0.920
<b>Markers of bone</b>				
Osteocalcin (ng/mL)	17.9 $\pm$ 2.9	20.2 $\pm$ 2.0	20.8 $\pm$ 5.2	0.641
NTx (nM BCE)	20.5 $\pm$ 2.6	17.9 $\pm$ 1.4	19.5 $\pm$ 1.2	0.330
OC/NTx	0.87 $\pm$ 1.1	1.13 $\pm$ 1.4	1.07 $\pm$ 4.3	

Values are expressed as mean  $\pm$  SD; *p*-values for Post hoc tests between groups.

NTx = serum N-telopeptide; BCE = bone collagen equivalents.



**Figure 1.** Relationship between Lumbar Spine BMC and estimated AHI scores

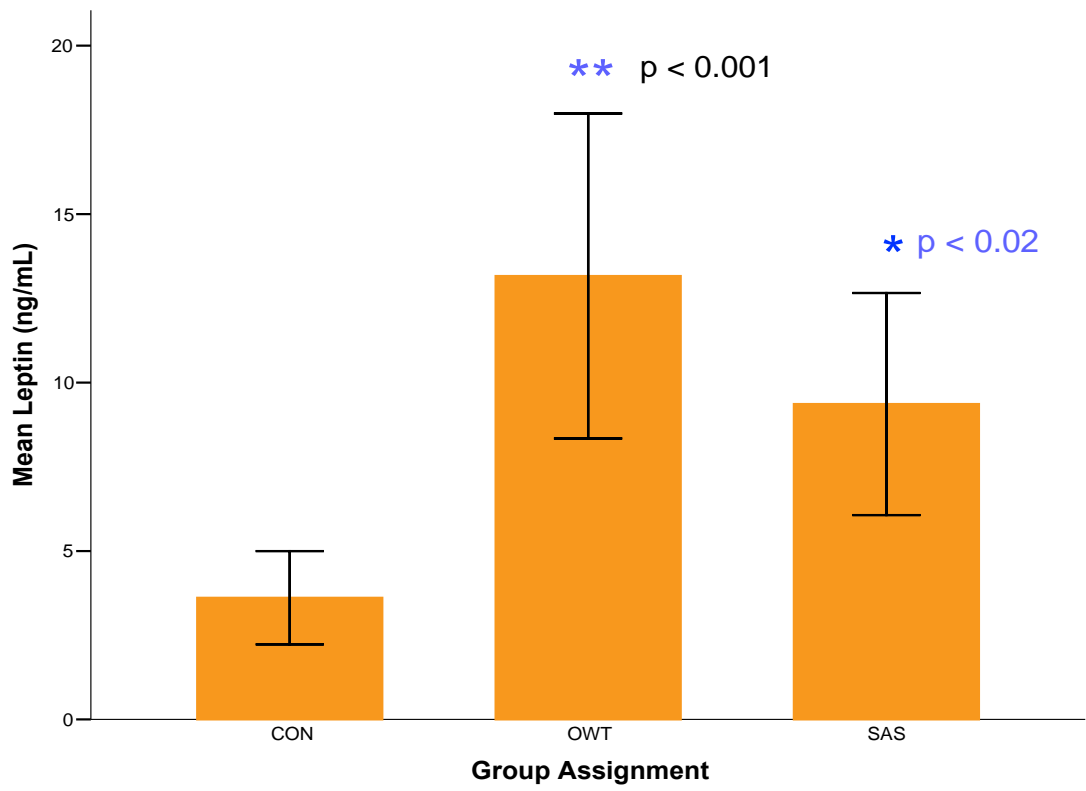
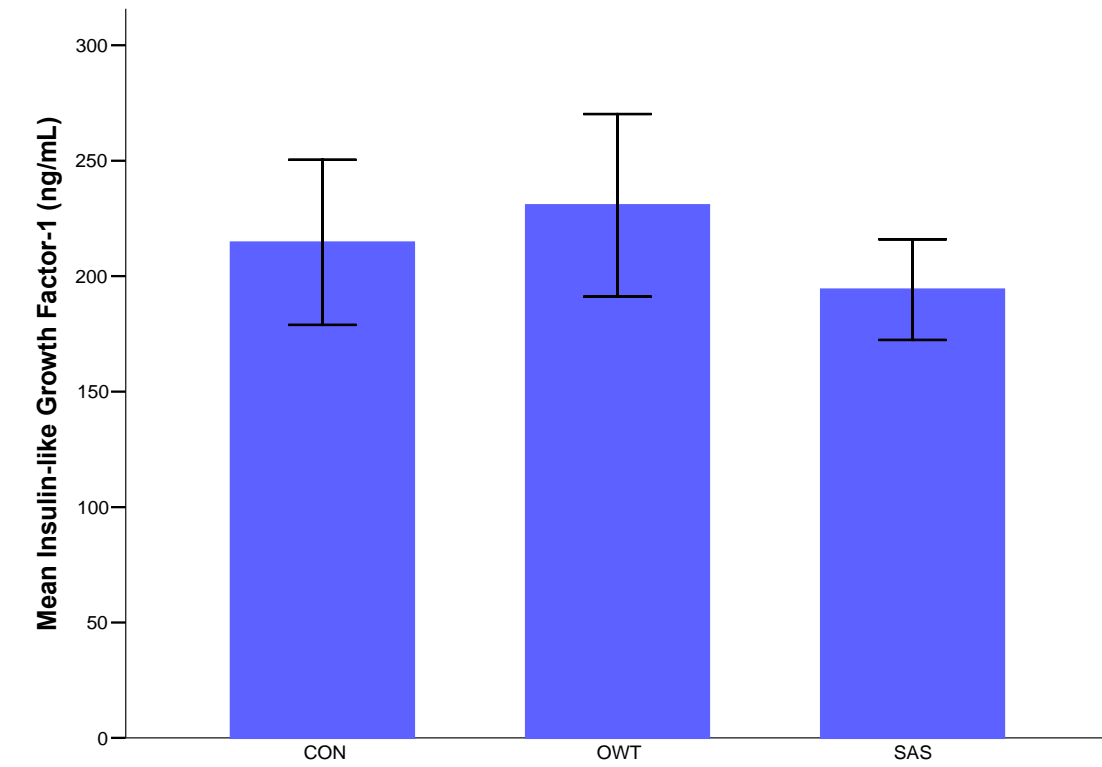
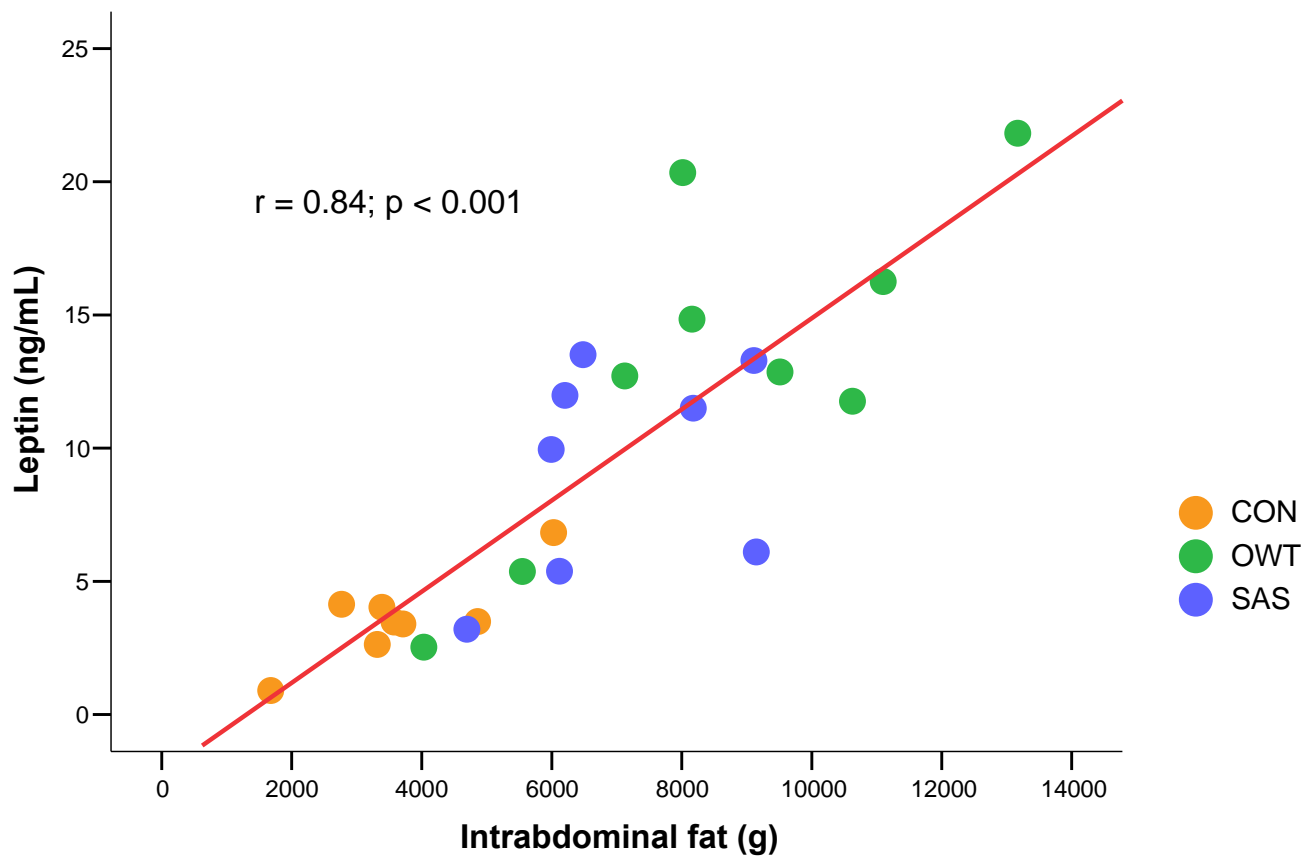


Figure 2. IGF-1 and Leptin differences between groups



**Figure 3.** Relationship between Leptin and Intra-abdominal fat across groups

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## CHAPTER 4 SUMMARY, CLINICAL IMPLICATIONS, AND FUTURE RESEARCH

### **Summary**

Obesity is a worldwide epidemic that continues to increase among youth who are facing similar consequences and health problems as adults. Sleep apnea syndrome (SAS), a frequent comorbid disorder of obesity in adults, may have the same prevalence among overweight children and exert independent effects on suppressing bone development as well. Sleep apnea syndrome is a condition characterized by occurrence of brief repetitive interruptions of breathing during sleep, resulting in poor air flow in and out of the nose or mouth and often associated with reduction in blood oxygen saturation of  $\geq 4\%$  or hypoxemia.

Besides the increased risk for developing hypertension, diabetes or coronary artery diseases, overweight children and adolescents (especially those with visceral obesity) who also have sleep apnea syndrome may be at higher risks for suppressed skeletal development during the years when bone accrual occurs and should be increasing most rapidly. With increasing rates of osteopenia and osteoporosis (NOF, 2000) in adulthood, it is important to understand factors that may result in less than optimal attainment bone mass upon achieving adulthood. So far, most research has focused on understanding the interactive effects of SAS and excess visceral adiposity on increasing risk of cardiovascular disease in children, but little is known about the potential interactive effects of obesity and intermittent nocturnal hypoxia on bone health at the point in life where bone mass peaks.

Achieving maximum bone accretion during childhood and adolescence is critical to

preserve bone mass and integrity and minimize osteoporotic fracture risk later in life.

Environmental, hormonal, and genetic factors are known to influence bone mineralization. For instance, simple obesity is associated with increased BMD. Unfortunately, obese children with high BMD paradoxically fail to reach adequate bone mass to carry this excessive body weight, raising questions about a mismatch between skeletal growth and weight. Obesity is associated with disturbed levels of cytokines and hormones involved in the control of bone metabolism. Through a cascade of signaling neuroendocrine messengers and pathways not currently well understood, abnormally elevated leptin levels may exert some anti-osteogenic effects on regulation of bone metabolism and bone development. Reduced IGF-1 may as well contribute to the shorter stature and delayed skeletal growth attained in obese children.

Based on findings from experimental studies, intermittent hypoxia is a detrimental environment to bone accretion. Prior research reporting low BMD in overweight young adults could potentially reflect the effect of sleep apnea syndrome. Sleep apnea may also augment leptin and reduce IGF-1, beyond the changes in these cytokines associated with obesity alone. With growing concern for our young population already at risk for cardiovascular diseases, it is important to better understand if disturbed metabolic regulation associated with obesity-related SAS may compromise bone regulation in young adults and lead to reduced bone accretion at skeletal maturation.

We investigated the combined effects of excess body weight and SAS on bone turnover, BMD and BMC for the whole body, spine, hip, and forearm, since both obesity and SAS have been shown to impair body growth in youth. We further examined the potential contributions of

regulatory hormones leptin and IGF-1, known to potentially influence bone accretion during adolescence.

Men, aged 18-28 years, were assigned into normal weight controls (CON: AHI <3, n=8), overweight without SAS (OWT: AHI <3, n=9), and overweight with SAS (SAS: AHI >5, n=8) based on estimates of the apnea/hypopnea index (AHI) obtained with a home sleep screening device and BMI. Health history and Epworth Sleepiness Scale (ESS) questionnaires were also administered. Bone mineral parameters and body composition variables were measured with dual-energy X-ray absorptiometry. Serum osteocalcin, leptin, IGF-1, and NTx-1 were measured by radioimmunoassay and enzyme-linked immunosorbent assay.

Fat-free mass, intra-abdominal fat, and fat mass were higher in the SAS and OWT groups ( $p < 0.03$ ). ESS scores revealed that SAS individuals were sleepier than CON and OWT groups ( $p < 0.009$ ). Total and site-specific BMD and BMC values were similar between groups and did not relate to the estimated AHI score. Serum OC and NTx-1 did not differ between groups. Leptin levels were about 30 % higher in OWT and SAS than in the CON group ( $p < 0.02$ ), but did not correlate with the estimated AHI score. Correlational analyses including all subjects indicated that only lumbar BMC ( $p < 0.005$ ) was negatively correlated ( $r = -.52$ ;  $p < 0.01$ ) to AHI. Sleep apnea syndrome in combination with obesity may detrimentally affect bone by accelerating the rate of bone resorption but the involvement of regulatory cytokines in this relationship remains unclear.

### **Future Research**

To date, this is the first study to examine the potential effects of sleep apnea syndrome

combined with excess body weight on bone turnover, bone mineral density and bone mineral content in humans. More research needs to be conducted in this area. Elevated AHI scores seemed to cause greater bone resorption at the lumbar site implying that degree of severity of the disorder might be an important predictor of bone metabolism. Assessment of bone turnover in combination with other hormones and unexplored cytokines known to be disturbed in SAS may provide better understanding of the mechanisms controlling this relationship. The following research initiatives could help clarify the detrimental effects of hypoxia on bone cells and the mechanistic roles of cytokines and hormones known to disturb bone metabolism.

1. It is well-known that bone resorption is accelerated in hypoxic environment by increasing the activity, number, and size of bone-resorbing osteoclast cells. The majority of experimental studies conducted in laboratories have demonstrated that osteoclastic activity is enhanced in severe hypoxic environment (between 10-20 %) but also lessens when exposed to mild or moderate conditions (below 5 %). Therefore, it would be ideal to test the consensus that severity of sleep apnea given by estimated AHI scores might impact bone turnover in a similar fashion. An ideal longitudinal study would be to compare five equal groups of 30 individuals, aged 18-45 categorized as normal weight controls (AHI <5), as overweight & obese without SAS, overweight & obese low-SAS (5 < AHI < 10), moderate-SAS (10 < AHI < 15), and severe-SAS (AHI > 15). Subjects would also be meticulously chosen and assigned to groups based on BMI levels, intra-abdominal fat, and fat mass to avoid possibilities of classifying subjects in the wrong groups or borderline subjects to fit in more than one group. The purpose of this study would be to examine the combined effects of SAS and obesity on bone turnover and bone cellular

activities across different age groups with different levels of disease severity for twelve months. Bone turnover would be assessed by osteocalcin and NTx. However, since it takes up to 8 months for the bone cycle to occur, monthly blood collection for one full year would be performed.

2. The great majority of individuals with SAS are also overweight or obese, sleep apnea being more and more recognized as a comorbidity of obesity. Following a healthy lifestyle by increasing intake of whole-grains, fruits, and vegetables, decreasing consumption of saturated fats in addition to regular physical activity are proven strategies in weight management. Currently, continuous positive airway pressure (CPAP) treatment is the most common and successful treatment currently known for sleep apnea syndrome. While weight management strategies ought indirectly to improve bone health by preventing or slowing down bone loss, no evidence can justify the effectiveness of CPAP on bone. It would be beneficial to assess the effects of weight loss alone or combined with CPAP in individuals with severe SAS and advancement in bone loss. The study would have five different groups of individuals, aged 18-45 with same categorization criteria mentioned above. Subjects would be randomly assigned to treatment groups including weight loss, CPAP, or weight loss + CPAP for at least twelve weeks and followed up to one-year after the treatment. The outcomes measured would be evaluation of changes in bone mineral density and content. Bone biomarkers would also be measured weekly during treatment and monthly during follow-up.

3. Experimental studies have already demonstrated that hypoxic state directly results in greater bone resorption via hyperplasia and hypertrophy of osteoclasts and accelerating their resorptive

activity. It is important to better understand the local mechanisms by which SAS/intermittent hypoxia may affect bone metabolism in human. We hypothesized that leptin and IGF-1 would be the key mediators between fat mass, lean body mass, and bone mineral density and content in SAS and obesity. We were unable to find such associations. It is therefore possible that there are some other hormones involved in this relationship. One peptide-hormone that deserves more attention is adiponectin. Low adiponectin levels are associated with insulin resistance and may impair the normal functioning of GH/IGF-1 axis involved in muscle growth. In addition, adiponectin seems to be more bone-specific than leptin, the hormone currently mostly studied. Furthermore, adiponectin possesses structural similarities to RANK-L and osteoprotegerin, two proteins involved in the regulation of osteoclastogenesis. Adiponectin controls a transcription factor critical for osteoclastogenesis which may explain how adiponectin affects bone and may better explain the fat- and lean body mass-bone relationship. The study would measure adiponectin, RANK-L and osteoprotegerin levels between five groups with similar criteria as above but also measure leptin levels and compare which hormone is a better predictor of fat tissue on bone.

### **Clinical Implications**

The current study provided evidence that SAS may have some detrimental effects on the skeleton. Findings may explain why obese children with high BMD paradoxically fail to reach adequate bone mass. Achieving maximum bone accretion during childhood and adolescence is critical to preserve bone mass and integrity and minimize osteoporotic fracture risk later in life. Physicians and pediatricians should be aware that obesity in youth does have implications on

bone health due to the mismatch between skeletal growth and body weight, but this may well be influenced and exacerbated by SAS.

If results can be reproduced with additional long-term research, it would have very important implications on implementing new treatment options to prevent or reverse physiological and neurological damages associated with SAS and simultaneously slow the process of bone resorption. In clinical settings, patients at risk and already diagnosed for sleep apnea syndrome should likely also be automatically screened for low bone mineral density and vice-versa.



CHAPTER V  
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APPENDIX A  
Biomarkers and Hormonal Assays



Subjects were informed to avoid any physical activity, caffeine, and alcohol consumption at least 24-hr prior to their scheduled blood sample collection. Blood samples were scheduled between 0745 and 0930 h to avoid inter-subject diurnal variation and most preferably within 2 hours of awaking. Samples were collected in serum vacutainer tubes by a trained phlebotomist and allowed to stand 30 min at room temperature. Then, the serum was separated by centrifuge for 15 min at 2500 rpm and 4°C. Pippetted serum into microcuvettes was stored at -80°C until assays were performed. For the current study, serum samples were analyzed for leptin, IGF-1, OC, and NTx.

Circulating osteocalcin is thought to reflect that portion of newly synthesized protein that does not bind to bone but is released directly into the circulation. Osteocalcin may circulate both as the intact molecule and as major N-terminal fragment. The OC radioimmunoassay used in this study measures both substrates inclusively (Mid-Tact Human Osteocalcin EIA Kit; Biomedical Technologies Inc., Stoughton, MA). An antibody is immobilized on the wells of the 96-well microtest plate. Samples and a biotinylated non-specific polyclonal antibody made against the 30-40 region are incubated in the test wells. After a wash, a second incubation is done with a streptavidin-horseradish peroxidase conjugate and the enzyme activity subsequently determined. The concentration of osteocalcin is proportional to the absorbance measured at 450 nm and values are obtained by comparison to a standard curve prepared on the same plate on an ELISA plate reader. The OC assay had an intra-assay CV of 11 %.

Generation of the cross-linked N-telopeptides of type I collagen (NTx) molecule is mediated by osteoclasts on bone found in serum as a stable product of degradation. Serum NTx

was measured by a competitive-inhibition enzyme-linked immunoabsorbent assay (Osteomark NTx Serum; Wampole Laboratories, Princeton, NJ). Diluted samples were added to the microplate wells, followed by a horseradish peroxidase labeled monoclonal antibody. Serum NTx in the individual competes with with the NTX epitope in the microplate well for antibody binding sites. Following a wash step, the amount of labeled antibody bound is measured by colorimetric generation of a peroxidase substrate. Absorbance was determined spectrophotometrically at 450 nm and NTx concentrations were calculated using a standard calibration curve. The NTx assay intra-assay CV was 8 %.

The IGF-1 was measured according to the procedures described by Weber et al. (1998). The hormone was first separated from 100 µl blood samples by using 900 µl of acid-ethanol solution. After 1 hr incubation time at room temperature, the samples were well-mixed by centrifugation in the cold room for 10 min. Addition of 200 µl of 0.855 M/l Tris Base neutralizing solution to the supernatant allowed the reaction to neutralize the samples. After resting for 1 hour and centrifugation, the IGF buffer was added on 3 consecutive d in different steps. Concentrations of calibrators (0.06, 0.17, 0.55, 0.64, 0.76, 1.47, 3.5, 4.67 ng/ml) were used to determine the standard curve. Unknown sample correlations were then calculated from the standard curve. The average coefficient of variation was 12 %.

The Human Leptin RIA Kit (Linco Research Inc., St. Charles, Missouri) was used to assess serum leptin levels through competitive binding between <sup>125</sup>I-Human Leptin tracer and unknown samples. Leptin antibody and <sup>125</sup>I-leptin tracer were added to the unknown samples (100 µl of each), vortexed, covered, and incubated at 4°C overnight allowing for the antigens to

compete for the binding sites on the antibody. On the second day, cold (4°C) precipitating reagent (1.0 ml) was added to all tubes containing unknown samples, vortexed, and incubated for 20 minutes at 4°C. Tubes were centrifuged for 20 minutes at 4°C at 2, 500 rpm and the supernatant was immediately decanted by one-time inversion to avoid the slipping of the pellets. The tubes were then counted in a gamma counter for 1 minute. A standard curve was generated using 100 µl standards (0.5, 1, 2, 5, 10, 20, 50, 100 ng/ml). The average coefficient of variation was 11.3%.

APPENDIX B  
Raw Data

Table 1. Baseline Anthropometric Characteristics

	Group	Age (Years)	Weight (kg)	Height (cm)	BMI (kg/m <sup>2</sup> )	AHlz
CON001	1	21	68	172.7	22.8	2.6
CON002	1	19	67.2	173.99	22	1.8
CON006	1	25	75.3	182.25	22.5	3.9
CON009	1	24	81.6	182	24.6	1
CON010	1	19	67.73	181.1	20.2	0.7
CON004	1	24	76.2	171.5	25.5	4.5
CON005	1	28	78.9	175.3	25.8	4.4
CON008	1	21	77.27	173	25.83	5
<b>Mean</b>		<b>22.6</b>	<b>74.0</b>	<b>176.5</b>	<b>23.7</b>	<b>3</b>
<b>SD</b>		<b>3.2</b>	<b>5.6</b>	<b>4.5</b>	<b>2.1</b>	<b>1.7</b>
2	2	23	73.5	166.4	27	3.7
4	2	27	134.4	175	43.9	0.9
5	2	21	112	177.8	35.5	1.1
6	2	18	108.9	184	32.2	2.3
9	2	18	112	178	35.5	3.7
12	2	23	108.2	185.42	31.6	3.4
14	2	22	88.18	183	26.3	2.9
16	2	21	120	172.3	40.1	1.8
20	2	25	109.1	182	32.94	5
<b>Mean</b>		<b>22.0</b>	<b>107.4</b>	<b>178.2</b>	<b>33.9</b>	<b>2.8</b>
<b>SD</b>		<b>3.0</b>	<b>17.5</b>	<b>6.2</b>	<b>5.7</b>	<b>1.3</b>
3	3	19	96.2	182.9	28.8	8.5
7	3	19	96.6	174	31.6	8.2
8	3	18	90	185.5	26	6.3
11	3	25	103.64	182	31.3	21.5
13	3	26	84.09	175	27.5	5.7
17	3	22	85.91	162	32.7	37.8
18	3	25	101.6	182.4	30.3	5.5
21	3	23	84.1	172	28.41	13.3
<b>Mean</b>		<b>22.1</b>	<b>92.8</b>	<b>177.0</b>	<b>29.6</b>	<b>13.4</b>
<b>SD</b>		<b>3.1</b>	<b>7.8</b>	<b>7.8</b>	<b>2.3</b>	<b>11.2</b>

Table 1 (Con't)

	neck circumference (cm)	waist circumference (cm)	hip Circumference (cm)
CON001	35.5	80	95
CON002	35.5	75	90
CON006	36.5	90	92
CON009	38	86	104
CON010	35.5	75.5	97
CON004	37	77	99
CON005	39	85.5	102
CON008	38.1	86.4	98
<b>Mean</b>	<b>36.9</b>	<b>81.9</b>	<b>97.1</b>
<b>SD</b>	<b>1.4</b>	<b>5.8</b>	<b>4.7</b>
2	37	82	95
4	48.8	123	130
5	42	98.5	109.5
6	41	98.5	123
9	41	115	118
12	38.5	92.8	102
14	38.5	92.8	108
16	47.5	115	120
20	44	105.5	117
<b>Mean</b>	<b>42.0</b>	<b>102.6</b>	<b>113.6</b>
<b>SD</b>	<b>4.1</b>	<b>13.1</b>	<b>10.9</b>
3	41.6	114.5	119
7	39.5	102	110
8	39.5	88.3	105
11	43	104.5	115
13	38	89.5	104.5
17	37.7	94	107
18	42	92	114.5
21	39.7	96.3	112
<b>Mean</b>	<b>40.1</b>	<b>97.6</b>	<b>110.9</b>
<b>SD</b>	<b>1.9</b>	<b>8.9</b>	<b>5.2</b>

Table 1 (Con't)

	VSAQ scores	Average O <sub>2</sub> saturation (%)	Lowest O <sub>2</sub> saturation (%)	Relative Peak VO <sub>2</sub> (mL/kg/min)	Relative Peak VO <sub>2</sub> (mL/kg <sub>LBM</sub> /min)	ESS scores
CON001	12	96.1	89.0	38.45	48.7	7
CON002	12	97.5	87.0	46.10	55.1	9
CON006		97.5	92.0	32.50	41.5	0.5
CON009	9	95.7	91.0	25.40	37.0	7
CON010	11	97.8	87.0	29.50	36.3	5
CON004	10	95.3	85.0	31.40	39.8	11
CON005		96.3	89.0	27.10	39.9	7
CON008	9	94.9	85.0			2
<b>Mean</b>	<b>10.5</b>	<b>96.4</b>	<b>88.1</b>	<b>32.9</b>	<b>42.6</b>	<b>6.0</b>
<b>SD</b>	<b>1.4</b>	<b>1.1</b>	<b>2.6</b>	<b>7.2</b>	<b>6.8</b>	<b>3.6</b>
2	8	94.9	85.0	28.9	37.8	12
4	9	94.4	78.0	23.9	37.7	7
5	10	94.6	87.0	25.9	39.9	15
6	9	90.8	63.0	25.8	38.9	1
9	9	94.9	84.0	22.7	36.5	8
12	9	95.7	89.0	26.3	44.3	9
14	9	96.3	91.0	24.5	34.6	5
16	9	95.2	70.0	19.6	29.2	5
20	6	95.4	86.0	24.5	36.4	8
<b>Mean</b>	<b>8.7</b>	<b>94.7</b>	<b>81.4</b>	<b>24.7</b>	<b>37.3</b>	<b>7.8</b>
<b>SD</b>	<b>1.1</b>	<b>1.6</b>	<b>9.4</b>	<b>2.6</b>	<b>4.1</b>	<b>4.1</b>
3	9	96.1	91.0	29.6	38.5	12
7	6	96.7	90.0	23.5	37.1	8
8	9	94.8	80.0	35.3	44.4	17
11	8	95.7	86.0	26.8	37.4	6
13		97.2	91.0	34.8	49.4	12
17	13	95.0	88.0	31.3	49.8	19
18	9	94.4	88.0	27.5	36.8	19
21	10	97.0	91.0	35.3	37.1	12
<b>Mean</b>	<b>9.1</b>	<b>95.9</b>	<b>88.1</b>	<b>30.5</b>	<b>41.3</b>	<b>13.1</b>
<b>SD</b>	<b>2.1</b>	<b>1.1</b>	<b>3.8</b>	<b>4.4</b>	<b>5.7</b>	<b>4.9</b>

Table 2. Hormones and Biomarkers of Bone Turnover

	IGF-1 (ng/ml)	leptin (ng/ml)	OC (ng/ml)	NTx (nM BCE)
CON001	242.6	3.47	27.94	23.29
CON002	214.6	0.9	11.8	19.19
CON006	228.6	3.4	8.39	24.64
CON009	158.6	6.83	10.14	18.66
CON010	238	4.14	31.17	21.72
CON004	280	4.03	20.58	20.31
CON005	154	2.63	16.31	16.75
CON008	200.6	3.49	16.68	19.16
<b>Mean</b>	<b>214.6</b>	<b>3.6</b>	<b>17.9</b>	<b>20.5</b>
<b>SD</b>	<b>42.8</b>	<b>1.7</b>	<b>8.2</b>	<b>2.6</b>
2	163.3	2.53	22.6	15.84
4	205.3	21.82	7.07	10.84
5	196	14.84	24.71	20.79
6	345.3	20.34	22.9	22.18
9	228.6	16.25	29.14	23.05
12	238	5.37	17.85	20.07
14	233.3	12.71	18.93	12.72
16	261.3	11.76	19.49	18.25
20	205.3	12.86	19.44	17.31
<b>Mean</b>	<b>230.7</b>	<b>13.2</b>	<b>20.2</b>	<b>17.9</b>
<b>SD</b>	<b>51.4</b>	<b>6.3</b>	<b>6.1</b>	<b>4.2</b>
3	177.3	11.5	13.93	17.1
7	224	13.29	24.26	16.25
8	209.97	3.2	26.3	19.98
11	205.3	6.1	22.93	23.01
13	219.3	9.95	13.37	16.29
17	158.6	13.51	22.52	24.63
18	158.6	5.38	17.39	22.66
21	200.6	11.98	26.05	16.21
<b>Mean</b>	<b>194.2</b>	<b>9.4</b>	<b>20.8</b>	<b>19.5</b>
<b>SD</b>	<b>26.1</b>	<b>3.9</b>	<b>5.2</b>	<b>3.5</b>



Table 3. Fat mass, Lean body mass, Intra-abdominal fat, % body fat

	fat mass (g)	LBM (g)	intra-abdominal fat (g)	% body fat
CON001	12128.3	53994.5	3576	17.7
CON002	7240.7	56303.2	1677.7	10.9
CON006	14294.8	59028.5	3710.9	18.8
CON009	21923.8	55953.9	6029.7	27.2
CON010	11421	55028.9	2766.3	16.6
CON004	13649.2	51949.4	3389.8	20
CON005	14054.5	62214.9	3316.4	17.8
CON008	17166.9	59255.1	4860.6	21.7
<b>Mean</b>	<b>13984.9</b>	<b>56716.1</b>	<b>3665.9</b>	<b>18.8</b>
<b>SD</b>	<b>4290.7</b>	<b>3289.7</b>	<b>1308.5</b>	<b>4.6</b>
2	13802	57464	4033.2	18.7
4	47624.2	85109.9	13168.3	35.2
5	30995.2	72412.9	8157.8	29.1
6	34254.7	72275.2	8017.1	31.2
9	3866.7	69904	11099.2	35.3
12	21887	64106.5	5548.7	24.7
14	24197	62358.3	7122.7	27
16	39190.5	80776.3	10627.1	31.8
20	32233.5	73452	9509.9	29.6
<b>Mean</b>	<b>27561.2</b>	<b>70873.2</b>	<b>8587.1</b>	<b>29.2</b>
<b>SD</b>	<b>13278.0</b>	<b>8737.7</b>	<b>2849.0</b>	<b>5.2</b>
3	28076.9	73922.6	8177.2	26.7
7	32708	61504.2	9110.3	33.7
8	15974.4	71563.4	4694.6	17.6
11	27851.2	74308.7	9146.6	26.6
13	22211.8	59128.8	5992.3	26.3
17	20927.7	54031.5	6481.8	27.2
18	22043.7	75568.5	6119.1	21.8
21	23297.2	59586.3	6203	27.2
<b>Mean</b>	<b>24136.4</b>	<b>66201.8</b>	<b>6990.6</b>	<b>25.9</b>
<b>SD</b>	<b>5189.3</b>	<b>8500.3</b>	<b>1623.8</b>	<b>4.6</b>

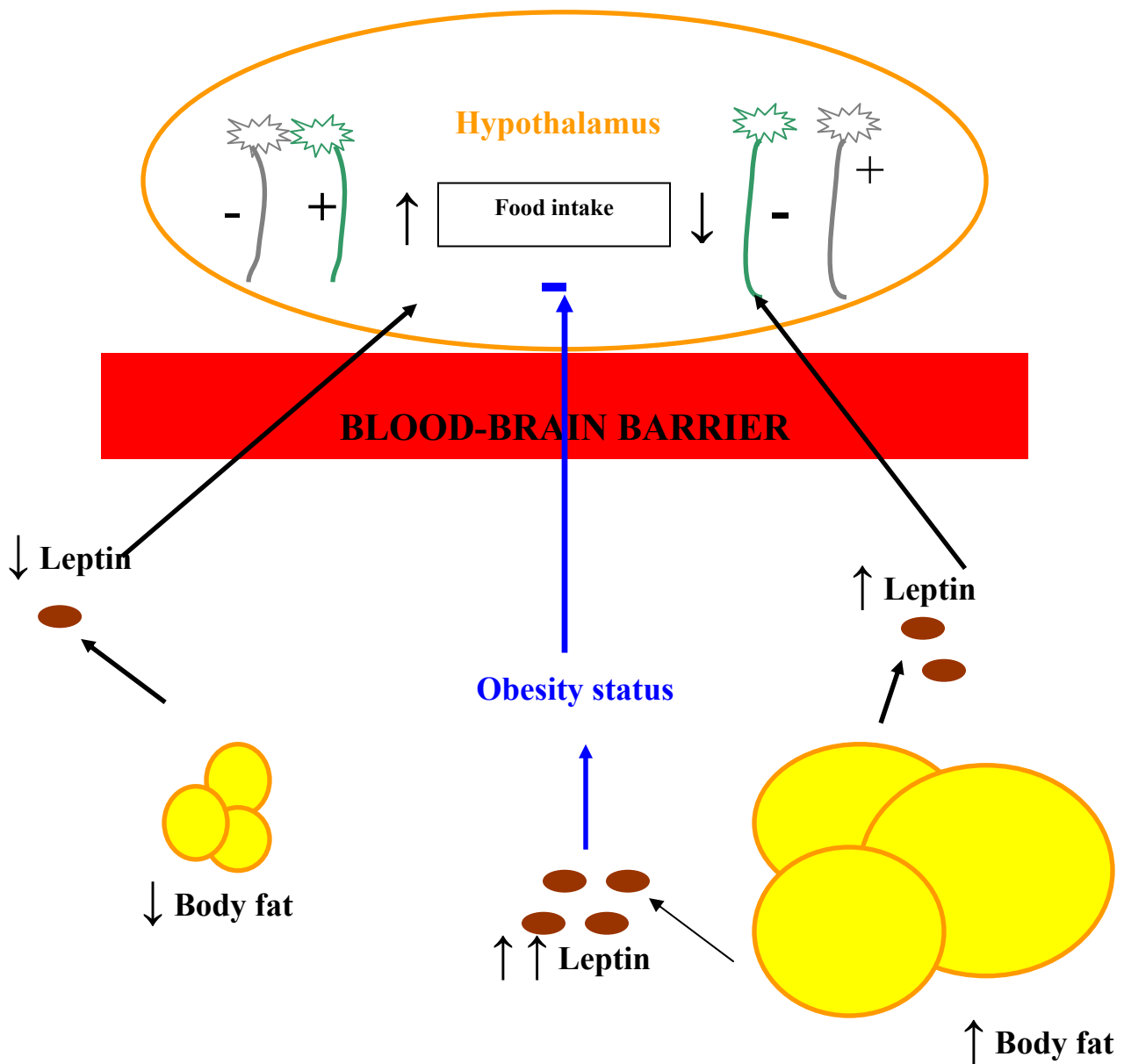
Table 4. Total and Site-specific Bone Mineral Density

	total BMD (g/cm <sup>2</sup> )	lumbar BMD (g/cm <sup>2</sup> )	Forearm BMD (g/cm <sup>2</sup> )	hip BMD (g/cm <sup>2</sup> )
CON001	1.09	1.003	0.610	0.979
CON002	1.23	0.985	0.654	1.093
CON006	1.23	1.065	0.593	1.093
CON009	1.23	1.06	0.650	0.986
CON010	1.12	0.791	0.595	0.731
CON004	1.25	1.16	0.684	1.105
CON005	1.18	1.044	0.679	1.091
CON008	1.19	1.038	0.719	0.988
<b>Mean</b>	<b>1.2</b>	<b>1.0</b>	<b>0.6</b>	<b>1.0</b>
<b>SD</b>	<b>0.1</b>	<b>0.1</b>	<b>0.1</b>	<b>0.1</b>
2	1.31	1.173	0.744	1.094
4	1.11	1.051	0.563	0.986
5	1.25	1.116	0.642	1.17
6	1.2	1.102	0.625	1.054
9	1.3	1.108	0.686	1.245
12	1.1	0.898	0.609	0.797
14		0.932	0.613	0.926
16	1.3	1.161	0.721	1.261
20	1.28	1.145	0.712	1.102
<b>Mean</b>	<b>1.2</b>	<b>1.1</b>	<b>0.7</b>	<b>1.1</b>
<b>SD</b>	<b>0.1</b>	<b>0.1</b>	<b>0.1</b>	<b>0.2</b>
3	1.36	1.048	0.737	1.282
7	1.24	1.107	0.617	1.169
8	1.21	1.047	0.634	1.135
11	1.04	0.923	0.599	0.904
13	1.28	1.146	0.647	1.118
17	1.15	0.942	0.606	1.029
18	1.43	1.149	0.827	1.138
21	1.16	0.932	0.656	1.114
<b>Mean</b>	<b>1.2</b>	<b>1.0</b>	<b>0.7</b>	<b>1.1</b>
<b>SD</b>	<b>0.1</b>	<b>0.1</b>	<b>0.1</b>	<b>0.1</b>

Table 5. Total and Site-specific Bone Mineral Content

	total BMC (g)	lumbar BMC (g)	Forearm BMC (g)	hip BMC (g)
CON001	2290.2	70.63	14.78	34.54
CON002	2702.7	59.44	16.46	51.56
CON006	2824.3	74.8	16.41	42.37
CON009	2704.1	68.19	16.27	46.32
CON010	2446.3	55.58	15.09	30.34
CON004	2643.8	78.17	17.50	36.4
CON005	2715.8	73.61	16.77	47.06
CON008	2702.3	70.18	20.07	40.59
<b>Mean</b>	<b>2628.7</b>	<b>68.8</b>	<b>16.7</b>	<b>41.1</b>
<b>SD</b>	<b>173.4</b>	<b>7.7</b>	<b>1.6</b>	<b>7.1</b>
2	2738.4	78.48	17.38	41.19
4	2593.2	67.12	15.76	41.92
5	3006.7	71.06	18.29	53.49
6	3094	75.32	20.98	45.77
9	3119.1	65.05	18.30	48.65
12	2622.4	69.73	15.76	36.71
14	.	68.87	15.74	39.69
16	3180.2	75.36	19.53	52.33
20	3087.2	71.72	20.86	44.72
<b>Mean</b>	<b>2930.2</b>	<b>71.4</b>	<b>18.1</b>	<b>44.9</b>
<b>SD</b>	<b>239.2</b>	<b>4.3</b>	<b>2.1</b>	<b>5.7</b>
3	3277.5	65.68	20.85	51.43
7	2817	69.83	17.01	42.54
8	3069.4	77.94	18.26	60.73
11	2385.9	65.56	16.92	43.72
13	3014.3	75.84	18.75	46.56
17	2206.5	50.73	14.20	35.53
18	3489.6	73.15	23.01	46.66
21	2641.8	61.96	7.15	45.93
<b>Mean</b>	<b>2862.8</b>	<b>67.6</b>	<b>17.0</b>	<b>46.6</b>
<b>SD</b>	<b>437.5</b>	<b>8.7</b>	<b>4.8</b>	<b>7.3</b>

APPENDIX C  
Figure



**Figure 1.** Schematic presentation of leptin action in energy balance and appetite control. When the size or total number of white adipose cells decreases, less leptin is released into the circulation and food intake is stimulated. With increased size and number, leptin release is increased, resulting in decrease food intake. However, in obesity, satiety does not occur. Obesity status is marked by such elevated leptin levels that the cytokine fails to exert its effect on appetite and energy balance.

APPENDIX D  
Protocol for Investigation Involving Human Subjects  
Informed Consent for Participants in Investigative Project

## **Protocol for Investigation Involving Human Subjects**

**Project Title:** Cardiovascular and Metabolic Dysfunction in Adolescents and Young Adults at Risk for Sleep Apnea Syndrome

**Investigators:** William G. Herbert, Ph.D., Principal Investigator, Sharon M. (Shelly) Nickols-Richardson, Ph.D., R.D., Co-Principal Investigator, Don Zedalis, M.D., John M. Gregg, DDS, Ph.D., Carol Haskell, M.D.

**Protocol:**

### **Justification of Project**

Excess body weight markedly increases the risks for high blood pressure, endothelial dysfunction, type 2 diabetes mellitus, dyslipidemia, increased cardiovascular morbidity and mortality. While the prevalence of “overweight” adults population is alarmingly high (65%), the problem is dramatically increasing in the children as well. The American Heart Association estimates that 8.8 million children in the United States are now overweight. Estimates from the Centers for Disease Control and Prevention and recent community studies indicate that 17-24% of U.S. adolescents are overweight or have high blood pressure.<sup>7</sup> In addition, there is growing evidence from prospective studies that individuals with increased levels of abdominal visceral body fat have abnormal more atherogenic blood lipid profiles that result in a greatly exaggerated risk for ischemic heart disease.<sup>12,10</sup> Adipose cells, particularly prevalent in those with increased abdominal visceral, tend to produce excess pro-inflammatory cytokines and this has been suggested as a mechanism that may further exacerbate the development of atherosclerosis and adult-onset diabetes.<sup>8, 138, 11</sup> Finally, The preponderance of evidence suggests that these conditions are inextricably linked to and exacerbated by Sleep Apnea Syndrome (SAS).

SAS is a disorder in which there is partial or complete obstruction of the upper airway during sleep. SAS is characterized during sleep periods by repetitive partial and/or total collapse of the upper airway. These events create hypoxia, sudden repeated stressful arousals to re-establish breathing, and excessive sympathetic nervous system activation. SAS has been widely reported to afflict 1-4% of the overall population. However more, with recent estimates using lower threshold scores for SAS to characterize long-term risk for development of comorbid cardiovascular disease indicate that as many as high 19% as 19% in of men and 15% in women in the United States are affected.<sup>5</sup> One other recent report further suggests that one in five adults in the United States have mild SAS, while one in fifteen has at least moderate SAS. Data on prevalence of SAS in children and young adults is scarce, yet some suggest that the health hazards of this disease are more potent in those under age 45. The limited data now available suggest that the prevalence of SAS in children and adults is similar at 2-4%, but with very few studies have attempted to assess prevalence for this segment of the population.

Among Italian children 9-15 years old, the incidence of snoring was estimated at 5-11%;

rates were twice as high in those with age- and gender-adjusted body mass index (BMI) scores above the 95<sup>th</sup> percentile, a history of problems with adenotonsillary enlargement, and elevated hemoglobin levels, the latter being which are suggestive of possible nocturnal repetitive hypoxemia related to SAS.<sup>4</sup> In 12-16 year old children of Seville, Spain, 29% were snorers, 14% had excessive daytime sleepiness, and 3% had SAS, as estimated by overnight portable polysomnography.<sup>119</sup>

Thus, there is a clear need for early identification and intervention for the extremely large proportion of individuals who go undiagnosed for SAS, especially among the rapidly growing fraction of our youth that is overweight and at higher risk for additional major metabolic and cardiovascular diseases by mid-life. This study will identify the presence or absence of at least mild SAS in the younger overweight subset and determine if the condition is associated with increased risk factors for atherosclerotic cardiovascular disease. The experience and findings of this project will provide a blueprint to guide the development of a larger prospective study to examine the effects that physical activity, nutritional and behavioral interventions may have on slowing this disturbing trend.

### ***Research Aims***

- Describe core Assess physiologic, biochemical, and clinical features associated with long-term risk for cardiovascular disease in overweight in adolescents and young adults who are with vs. without evidence of at risk for SAS vs. those who are not.
- Assess the extent to which SAS risk factors for hypertension and cardiovascular diseases are exacerbated these risk factors in overweight adolescents and young adults the overweight individuals in these age groups by SAS.
- Analyze and use these findings to establish predictive accuracy of a clinical algorithm to cost-effectively screen young people at high risk for SAS and who should be provided diagnostic testing with full-night polysomnography diagnostic testing (PSG).

### ***Procedures***

#### ***Subject Recruitment:***

Recruitment for the adolescents and young adult subjects will be accomplished through communications with a variety of campus and community organizations, some of which areas indicated below. The primary text to be used in these announcements will be slightly different for the adolescent vs. young adult classes of subjects (see Appendix A for statements). ):



- E-mail notices distributed through various university list serves.;
- Flyers posted on public notice boards throughout campus, including Schiffert Health Center, as well distributed by project personnel to as at local physician's offices.;
- Public service announcements and advertisements in local newspapers, cable network, and local television stations, e.g. New River Valley *Current*.;
- Public service announcements and advertisements through cable network and local television
- Other community organizations, (e.g., churches, recreation centers)
- Blacksburg Electronic Village website

*Pre-screening Procedures:*

Candidates may respond to the recruitment information by telephone or by linking to a via a project website, the URL for which is provided in the text of public announcements that are disseminated to recruit subjects (Appendix A). The website will provide details concerning the purpose, procedures, and requirements of the study and assist them candidates and parents/guardians (for children) in determining if they are interested, may be eligible (basic inclusion and exclusion criteria), and should in further discussion participation with the investigators about participating and if they are appropriate candidates (inclusion and exclusion criteria). If interested candidates do not have access to the Internet, same resources will be provided to them by telephone exchange with project personnel interview and mail out materials will be provided by postal service, after they have contacted one of the investigators. The website or the mail-out materials will provide additional descriptive information serve as an additional outlet for information about the research study, as well as to serve as an initial screening tool. These Website materials will be developed during fall semester pilot testing and will be a simple information resource on study requirements for participation in the study.

*Inclusion Criteria:*

- Males and females aged 13-26 years old will participate in this study. ;
- $\geq 85^{\text{th}}$  percentile for Body Mass Index ( $\text{kg}/\text{m}^2$ ), using age/gender-specific pediatric reference standards or adult clinical standards, as appropriate. ;

*Exclusion Criteria:*

- No Acute respiratory infections during previous 6 weeks, including tonsillitis and adenoiditis.
- No diagnosed or medically-treated cardiovascular, pulmonary, or metabolic disorders;
- Not receiving any prescribed vasoactive medications, hypnotics, sedatives, analgesics, psychotropic, steroids, and sympathomimetics;
- No Musculoskeletal conditions that would preclude maximal aerobic exercise testing;
- Habitual use of tobacco products within the past year;
- Failure to establish stable blood pressure;
- Sleep problems potentially related to emotional health (from PSQI, assessed by responses to sleep survey items that affect sleep status)

*Informed Consent Administration and Advanced Screening (up to 3 visits)*

Eligibility will be determined based on website submissions and mail-out versions for those with no Internet access. For adolescent subjects, initial communication will be with their parents or legal guardians. Subjects satisfying preliminary inclusion/exclusion criteria will be provided a copy of the informed consent document (Appendix C), including the assent document in the case of adolescent subjects via mail or e-mail, so subjects that adult and child (parent) candidates may thoroughly review the document prior to attending session 1.

Trent Hargens, MS and/or Stephen Guill, MS (Research Assistants) will schedule the screening sessions with potential subjects. This session will take approximately 80 minutes total. Subjects will be encouraged to ask questions on anything that is not explicitly clear to them in the informed consent/assent documents. If all questions and concerns are addressed adequately, subjects will be invited to initial each page of the informed consent/assent documents, thereby indicating that they understand everything on that page. Subjects will also be invited to sign, date, and provide their permanent address on the signature page of the informed consent. After signing, subjects will be provided a copy of the informed consent/assent documents, with the original filed in the research office.

**Preliminary Survey and Questionnaires**

Health History Form

Subjects will be administered a detailed health history form, through self-report and interview by Trent Hargens and/or Stephen Guill. Carol Haskell, MD (Research Coordinator) will conduct the

meetings if considered necessary to clarify eligibility special cases arise. The health history form will provide information on basic demographic and health variables. Parents and guardians will be present and will assist adolescent candidates who need clarification of key issues in the health history.

### Sleep Questionnaires

Subjects will complete two a standardized sleep questionnaires, the Epworth Sleepiness Scale (ESS) and the Pittsburgh Sleep Quality Index (PSQI).

- The ESS is a simple tool to assess patterns of daytime sleepiness, a strong predictor of SAS.
- The PSQI provides ratings of sleep quality, daytime sleep-related problems, and consumption of sleep-modifying drugs and foods.<sup>9, 10</sup> The survey PSQI responses will be used to evaluate sleep characteristics for each patient during the night of the affecting eligibility for the candidate's further participation in the study initial PSG test.

In addition to the PSQI, they will also complete the Epworth Sleepiness Scale questionnaire. It is a common screening tool used by clinicians to identify daytime sleepiness and possible etiologies. In this study the ESS and PSQI responses will help exclude individuals if there may be any emotional basis responsible for self-reported sleep problems. Secondly, their responses on the questionnaires will aid in selecting candidates who are more likely to satisfy study needs for group classifications of SAS or no-SAS., a strong indicator for the presence of SAS Any candidate with ESS and PSQI responses suggesting an emotional issue will be excluded from the study at this point and encouraged to consult with an appropriate health care professional to further address their sleep quality problem. In the case of adolescents, this suggestion will be provided to both the child and parent/guardian. A second sleep questionnaire will be completed to establish sleeping habits and sleeping quality of the study participants. A third sleep questionnaire, with selected relevant questions asked of all subjects, will be completed to assess general sleeping habits. Items will be drawn from this and incorporated into the study website as a pre-screening resource.

### Quality of Life Questionnaire

Subjects will also complete an SF-36 quality of life questionnaire. It is the best-known questionnaire for measuring health status by individuals.

### Dietary Analysis Record

Subjects will complete dietary forms to be inserted by Dr. N-R. Please insert here. Each participant will be instructed on the procedure for maintaining a 4-Day Diet Record at home

and for returning this record to the investigator at the specified intervals (10 minutes).

### Physical Activity Assessment Questionnaires

- Subjects will complete a physical activity history questionnaire to determine the level of activity they have participated in previously. See Appendix D for all questionnaires. Subjects will complete the Veteran's Specific Activity Questionnaire (VSAQ), which provides a quick and simple assessment of the participant's perception of their own physical fitness level.
- Subjects will also complete the Modifiable Activity Questionnaire for Adolescents. It is a simple assessment tool for quantifying the amount of physical activity the subject has participated in over the previous 12 months.<sup>1</sup>

If no further exclusions contraindications for participation in the study are identified, initial blood pressure screen will be performed. Height, weight, neck, hip and waist circumference measures will also be obtained at this time. Blood pressure readings will be taken on successive visits to establish a stable resting blood pressure reading.

#### *Session 1: Embletta Home Sleep Evaluation*

This will consist of:

- An overnight home sleep evaluation with an Embletta device. This device is harmless and painless for the subject, and is worn at night while the subject sleeps. An illustration picture of the device is included in Appendix D.
- A sleep technician will interpret Embletta data, with the results verified by the physician investigator who is a sleep specialist;.
- Data will be transposed into an Apnea-Hypopnea Index (AHI) scores, and subjects will be grouped into 4 classifications to establish the likelihood of SAS. These classifications will be utilized for statistical analysis.;
- Trent Hargens and/or Stephen Guill will instruct the college-age subjects on the setup and use of the Embletta. Carol Haskell, MD, will instruct the adolescent subjects and their parents on its use. Carol Haskell, MD, will implement conduct home visits with the child subjects/parents, if the need arises.

The goal is to obtain 10 subjects for each age group with No-SAS, and 20 subjects in each age group with some level of SAS (goal is total of 60 subjects for the study).

*Session 2: Blood Sampling/ Dual x-ray absorptiometry (DXA) test*

Session will consist of a 60 minute lab visit as follows:

- Fasting blood sample ( $\leq 75$ (50 ml) for comprehensive blood lipid profile, glucose analysis, and analysis of biomarkers for inflammatory and oxidative stress signaling for body mass regulation including, but not limited to:
  - Vascular endothelial growth factor protein (VEGF)
  - VEGF-Receptor 2 (VEGF-R2; mononuclear reporter cells)
  - Leptin
  - Interleukin-6 (IL-6)
  - C-Reactive Protein (CRP)
  
- DXA scan for total body/visceral adiposity and bone density, plus site-specific scans of the spine and hip for bone density. Bone density measures will provide useful information on baseline bone health. All DXA tests will be conducted in the BONE Laboratory, Room 229 Wallace Hall, on the Virginia Polytechnic Institute and State University campus. Each subject will lie on or sit next to the DXA (Hologic QDR 4500A) for bone mineral density and soft tissue mass measurements (20 minutes). Each subject will have total body bone mineral density and body composition, non-dominant total proximal femur bone mineral density, lumbar spine bone mineral density, and non-dominant forearm bone mineral density measured by DXA. All DXA scans will be conducted in the BONE Laboratory (Room 299 Wallace Hall) by Dr. Nickols-Richardson who has nearly 10 years of experience in conduction research studies with participants aged 4 to 94 using related equipment (specifically the QDR 100W and QDR 4500A DXA models). In addition, this investigator is a Licensed Radiologic Technologist-Limited in the Commonwealth of Virginia, and has passed an in-house radiation safety examination at Virginia Tech.

*Session 3: Arterial Stiffness/Maximal Ramping Exercise Test*

Session will consist of a 90 minute lab visit as follows:

- Arterial stiffness test via venous occlusion plethysmography;<sup>3, 6, 143, 6, 12</sup>

- Maximal ramping exercise test on an electronically braked cycle ergometer, with measurements for heart rate (via ECG) and blood pressure in exercise and recovery, and gas exchange for determination of ventilatory equivalent for CO<sub>2</sub>.

### ***Risks/Procedures to Minimize***

#### *Fasting Blood Sample*

There is a slight risk associated with the fasting blood sample collected through venipuncture. The specific risk, while difficult to quantify, can be estimated as the following and include:

- bleeding from the puncture sight (1/10)
- fainting or feeling lightheaded
- hematoma (1/10)
- fainting or feeling lightheaded (Difficult to precisely estimate, it is probably <1/20)
- infection (1/1,000)

Whenever blood is drawn, there is a small risk of bruising. Through this procedure, the risk for perforation of the vein is minimized. To minimize the risk of light-headedness or dizziness, each subject will have blood drawn in a seated position. Although infection is a risk with venipuncture, this is minimized by use of alcohol to cleanse the area prior to the blood draw, as well as the use safety (latex) gloves by the phlebotomist, in accordance with the blood borne pathogens standard of OSHA. All blood draws will be performed by a physician or a certified phlebotomist.

#### *DXA Scan*

The risk of harm arising from exposure to radiation during the DXA scans is minimal. The total exposure is 20 mR. This amount of radiation poses minimal risk, compared to radiation doses from dental bite-wing films (334 mR) and environmental background exposure (4 mR per week or 208 mR per year) that is expected to occur in one 12-month period. Dr. Nickols-Richardson, Licensed Radiologic Technologist, will perform the test on all subjects.

Exposure to radiation will occur during DXA scans for bone mineral density measurement. The combined total of exposure to each subject is 20 mR. This dose is very small, as radiation doses from a dental bit-wing film are 334 mR, environmental background is 4

mR per week, and chest X-ray films are 40 mR for 2 standard films. All subjects will be informed of this risk, will provide informed consent of this risk, and may choose not to complete any one, combination, or all of the DXA scans. If in the event that any scan is unreadable or unusable, a replacement scan will not be conducted to avoid further exposure.

### *Maximal Ramping 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. These risks decrease further given the current study population of younger adults and adolescents.<sup>2</sup> All subjects will be monitored via 12-lead ECG for any ischemic changes and testing will be stopped if any of the ACSM guidelines for test termination should occur. Some examples of test termination criteria are:

- Drop in SBP of  $\geq 10$  mm Hg from baseline blood pressure despite and increase in workload.
- ECG changes such as excessive ST segment depression ( $>2$  mm horizontal or downsloping depression)
- Sustained ventricular arrhythmias (e.g., ventricular tachycardia)
- Signs of poor perfusion (e.g., cyanosis or pallor)
- Development of clinical symptoms (e.g., angina, excessive fatigue, shortness of breath, dizziness, or near syncope)

All tests will be conducted and supervised by staff certified by the American College of Sports Medicine (ACSM) as Exercise Specialists. According to the ACSM's Guidelines for Exercise Testing and Prescription, 6<sup>th</sup> edition, given the young age of the study subjects, physician supervision is not required for maximal exercise testing.<sup>2</sup> The researchers present during the exercise test will have current certification from the American Heart Association in Basic Cardiopulmonary Life Support (BCLS) or the equivalent. At least 2 BCLS certified researchers will be present for each test, and will provide CPR in the event of an emergency, in addition to calling the EMS Rescue Squad. Researchers will provide CPR until the arrival of the EMS Rescue Squad.

The Virginia Tech campus is serviced by the Virginia Tech EMS Rescue Squad in the event of serious complications. In the past, the Virginia Tech Rescue personnel have communicated that their Average response time for arrival by the Rescue Squad is approximately five minutes campus wide, although this may vary depending on time of day and campus traffic. The Virginia Tech Rescue Squad is familiar with the Laboratory for Health and Exercise Science (231 War Memorial Hall), where testing will occur, as they have provided EMS services for the "TECH Center" program, a community-based exercise program that also operates in conjunction with run out of this Laboratory for the past (28 years).

Neither the researchers nor the university have money set aside to pay for medical

treatment that would be necessary if injured as a result a subject's participation in this study. Any expenses, including emergencies and long-term expenses will be the subject's responsibility. This information is communicated to study candidates in the informed consent/assent documents and the parental informed consent for minors.

### *Benefits*

Subjects may benefit from participation in this research by receiving personal information that may be useful for future changes in status that may be of benefit to health care professionals who may assess their health. The specific benefits of this type include the following regarding:

- SAS risk and information that may lead them to seek advice from their personal physician; for those with AHI scores >15, we will notify you and strongly encourage follow-up with appropriate sleep specialist. This is particularly important if the subject drives or operates heavy equipment, as excessive daytime sleepiness is associated with these higher scores.
- Body composition including body fat percentage, fat-free mass, and bone mineral density that may affect decisions on weight control;
- Comprehensive blood profile including full lipid profile with total cholesterol, triglyceride, LDL and HDL cholesterol, and blood glucose level that may affect assessment of risk for metabolic diseases;
- Aerobic fitness exercise capacity based on maximal exercise cycle ergometer test. In conjunction with this test result, study subjects will be provided with an individualized exercise prescription based on their results.

Follow-up to appropriate health care professionals will be recommended to the study subject, if deemed necessary, any test finding indicates a score outside the range for "typical values" as indicated by our current based on published guidelines,<sup>2</sup> (e.g., fasting blood glucose >109 mg/dL, fasting total cholesterol >200 mg/dL). In the case of the adolescent subject, information will be provided both to the subject and the subject's parents or legal guardian. The study subject and/or their guardian will then have the option to seek medical follow-up if they so choose. Any and all costs related to such medical follow-up will be borne by the subject and not by Virginia Tech.

### *Compensation*

In acknowledgement of their time commitment and participation in this study, subjects will also be provided stipends of \$15 for Session 1 of the research, \$15 for Session 2, and \$15 for Session



3, for a total of \$45 upon completion of the entire protocol.

### ***Confidentiality/Anonymity***

Individual subject data from this study will be kept strictly confidential. At no time will the researchers release individual subject data in a manner that it could be identified with a subject to anyone other than the individuals working on the project without written consent. Results will be kept in an electronic database which is password protected. Paper files will be stored in a locked cabinet in 213 War Memorial Hall. The information provided will have names removed and only a subject number (excluding social security numbers) will identify subjects during analyses and written reports of this research. Principal Investigator will keep a list of subject's identifications, with corresponding coding to properly identify individual subject data.

### ***Informed Consent / Assent Forms***

Subjects will be fully informed about this study, including risks and benefits involved and will agree to participate in writing by signing an informed consent in the case of the adult subject, and an assent document with corresponding consent by legal guardian in the case of the adolescent subject. Consent/Assent documents will be provided upon initial contact with the subject candidate so that each subject can review the forms prior to signing and participating in this study. Each subject (and legal guardian) will be allowed to ask questions. After all questions have been answered, the participant (and legal guardian) will be invited to sign the assent and consent forms. In the case of the adolescent subject, their legal guardian will provide their consent first, followed by the subject giving their assent.

### ***Biographical Sketch***

#### **William G. Herbert, Ph.D.**

Dr. Herbert is Professor and Director of the Laboratory for Health and Exercise Sciences at Virginia Tech, and will be the Principal Investigator of this research study. He administers cardiac rehabilitation and health-fitness programs based at the Virginia Tech campus and directs clinically related graduate student research in exercise science. His research activities center on effects of physical activity on health and cardiorespiratory functions in chronic disease population.

#### **Sharon M. (Shelly) Nickols-Richardson, Ph.D., R.D.**

Dr. Nickols-Richardson is Associate Professor and Co-Principal Investigator of this research study. She is a Registered Dietitian as well as a Licensed Radiologic Technologist. She will be responsible for nutritional assessments, DXA studies, and quality assurance with certain bioassays.

Donald Zedalis, M.D.

Dr. Zedalis is Medical Director of the Southwest Virginia Sleep Disorders Center, located near Christiansburg, VA. He is an Internist, with specialization in allergies and asthma. He has training and qualifications to direct a center devoted to diagnosis and treatment of sleep disorders patients.

John M. Gregg, DDS, Ph.D.

Dr. Gregg is an oral and maxillofacial surgeon in Blacksburg, VA, with training and expertise in neuroscience. He has a private surgical practice that, in part, involves interventions for select groups of SAS patients. He has published on outcomes with these patients. He also holds an appointment as Professor of Basic Sciences at the Via College of Osteopathic Medicine.

Carol Haskell, M.D.

Dr. Haskell is the clinical research coordinator, and has experience in recruiting, testing, and managing subjects with SAS. She has invaluable clinical skills and experiences that support our studies.

Trent A Hargens, M.S.

Trent is a doctoral student and research assistant, and has 9 years experience in clinical exercise testing and rehabilitation in cardiovascular, pulmonary, and metabolic diseases. He is certified by the American College of Sports Medicine (ACSM) as an Exercise Specialist.

Stephen G. Guill, M.S.

Stephen is a doctoral student and research assistant, and has undergraduate and graduate research experience in protocol design, subject recruitment, and supervision of exercise training testing. He also is and ACSM certified Exercise Specialist.

Nadine Guignell, B.S.

Nadine is a master's degree student and research assistant.

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04-370  
David



**Institutional Review Board**

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

DATE: December 22, 2004

MEMORANDUM

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

FROM: David Moore 

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

This memo is regarding the above referenced protocol which was previously granted approval by the IRB on August 9, 2004. You subsequently requested permission to amend your approved protocol to include the addition of the listed changes. Since the requested amendment is nonsubstantive in nature, I, as Chair of the Virginia Tech Institutional Review Board, have granted approval for requested protocol amendment, effective as of December 22, 2004. The anniversary date will remain the same as the original approval 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

# VIRGINIA POLYTECHNIC INSTITUTE AND STATE UNIVERSITY

## **Informed Consent for Participants in Investigative Project**

(For 18-26 year old subjects)

**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.

### **I. 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 within the past 12 months ;

- Any problem affecting your breathing (cold, sinus infection, etc.) during the previous 6 weeks;

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 past or 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 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 blood sample that is about the volume in 5 tablespoons of fluid (~75ml); this will be taken by needle 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 pre-test 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 millirads (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.(sentence deleted) In this study, the total amount of exposure is 20 mR (whole body = 1 mR, lumbar spine = 7 mR, hip = 7 mR, forearm = 5 mR), as shown in the right side of the table below. This represents ~2% 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.

<b>NIH-OPRR Radiation Limits for an Adult Research Participant per Year</b>	<b>Exposure During Participation in this Research Study</b>
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
<b>CUMULATIVE EXPOSURE = 18,000 mR</b>	<b>CUMULATIVE EXPOSURE = 20 mR</b>

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. 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 with adhesive collars directly on your skin across your chest and stomach. The preparation for the electrodes may cause slight skin irritation, but this does not

persist for more than a few minutes after removal. 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. 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 8-14 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**

#### *a) 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 be paid by you and not by Virginia Tech, nor any of its agents, including the researchers.

## **V. 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.

## **VI. 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.

## **VII. 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). It is important to the scientific outcomes of the study that you complete all tests described in this informed consent, including the body fat and bone health test. However, 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 your 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: \_\_\_\_\_

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Signature

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Date

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Witness (Research Coordinator)

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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. 231-4991  
Chair, IRB, Research Division

Trent Hargens, M.S. 231-6374

Kevin Davy, Ph.D. 231-3487

Investigator

Departmental Reviewer

Nadine Guignel, B.S. 231-6375

Investigator

## Vita

Nadine J. Guignel was born on September 13<sup>th</sup>, 1979 in Schoelcher, Martinique F.W.I. She did most of her education in her native island until high-school. Nadine continued her college years in the University of Montpellier II (Montpellier, France) where she obtained a minor in Biology in 1999. Nadine started her Bachelor of Science in Nutrition, Foods, and Exercise at EAI Tech (Sophia-Antipolis, France). She was transferred in 2001 Virginia Polytechnic Institute & State University (VPI&SU) in Human Nutrition, Foods & Exercise (option Clinical Exercise Physiology) where she received her B.S. in 2003. While in the United States, Nadine obtained lots of experience in the various field studies hours she spent in the cardio-pulmonary department of the regional hospital and the local recreational sport center. During the last two years, Nadine was the weight room clinical coordinator of the cardiac rehabilitation program offered at Virginia Tech. Nadine gained strong knowledge of the different steps and requirements associated with research as she was involved with both undergraduate and graduate research. After leaving VPI&SU, Nadine will head back to Martinique where she plans to be an exercise physiologist in a hospital-based rehabilitation program.