

**The Influence of Obstructive Sleep Apnea Syndrome on Insulin Resistance,
Metabolic Syndrome, and Endothelial Dysfunction in Young Men**

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(ABSTRACT)

Obstructive sleep apnea syndrome (OSAS), a chronic respiratory disorder affecting as many as 1 in 5 adults, is associated with repetitive collapse of the upper airway during sleep and results in fragmented sleep and intermittent periods of hypoxia and hypercapnia. If left untreated, OSAS increases the risk for hypertension, insulin resistance, metabolic syndrome (MetS) in a manner that is independent of obesity in mid-adulthood. However, it is still unknown if evidence of these relationships is apparent in young adults with OSAS who are otherwise healthy and free of other chronic comorbidities. **Objectives:** To determine if functional and biochemical evidence of insulin resistance, MetS, and vascular endothelial dysfunction (VED) exists in young, overweight men with OSAS and if the combined effects of obesity and OSAS augments the evidence of chronic disease pathogenesis beyond the effects of obesity alone. **Subjects:** Subjects were 12 overweight men with OSAS (age = 22.8 ± 0.8 ; BMI = 32.4 ± 1.0 ; apnea-hypopnea index (AHI) = 25.4 ± 5.4), 17 overweight men without OSAS (age = 22.5 ± 0.7 ; BMI = 31.6 ± 1.1 ; AHI = 2.2 ± 0.3), and 18 normal weight men without OSAS (age = 21.1 ± 0.5 ; BMI = 22.4 ± 0.4 ; AHI = 1.9 ± 0.3). **Methods:** Subjects were evaluated for OSAS using an unsupervised, portable polysomnography test. Total fat and central abdominal fat (CAF) were assessed using dual energy x-ray absorptiometry (DEXA). Fasting blood samples were used to quantify biochemical markers for insulin resistance (glucose, insulin, adiponectin, IL-6, and TNF- α) and endothelial dysfunction (CRP, VEGF, and VEGFR2) using ELISA, RIA, and flow cytometry. MetS was defined according to Adult Treatment Panel III (ATP III) clinical standards. Triglycerides, HDL cholesterol, and glucose were measured using a commercial lipid panel. Resting blood pressure was obtained manually via auscultation. VED was measured via strain gauge plethysmography, with endothelium-dependent vasodilatation being assessed from forearm reactive hyperemia after a 5-minute period of upper arm occlusion. **Statistics:** One-way ANOVA was used to determine group differences in variables. Two-way ANOVA was used to evaluate group x time interactions during the 2-minute recovery period following upper arm occlusion. Pearson partial correlation was used to assess relationships between continuous variables, with analyses being controlled for CAF or OSAS severity. Spearman correlation was used to assess relationships between number of MetS components present and both indices of adiposity and OSAS severity. Stepwise multiple linear regression analysis was used to determine significant predictors of OSAS severity, insulin resistance, components of the MetS, and endothelial dysfunction. **Results:** Overweight subjects with OSAS had more CAF, higher fasting triglycerides, and lower serum adiponectin concentrations than both overweight and normal weight non-apneic controls. Furthermore, fasting triglycerides were directly correlated to OSAS

severity, even after the influence of central abdominal fat was removed. OSAS severity was an independent predictor of triglyceride levels, and vice versa. Insulin resistance, leptin, insulin, and CRP were all higher in overweight subjects than controls, but no further differences were attributable to severity of OSAS. No differences in IL-6, TNF- α , ADMA, and expression of VEGFR2 were noted between any groups. No group or group x time interaction differences existed in regards to postocclusive reactive hyperemia responses. **Conclusions:** Young men with OSAS exhibit several unique anthropometric and biochemical abnormalities that may indicate early pathogenesis of or increased risk for future development for cardiovascular and metabolic disorders. Identification and treatment of OSAS at this age may be critical to prevent the onset and progression of these chronic disorders.

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Chapter 1

INTRODUCTION

Current estimates suggest over 170 million Americans, nearly 60% of the adult population, are either overweight or obese [1]. The dangers of excess body weight are well established, and are further supported by a recent prospective study from the National Institutes of Health –AARP cohort. These researchers found a 40% higher risk of early death in overweight middle aged adults than those of normal body weight [2]. Obese individuals in this study were nearly 3 times as likely as normal weight adults to die early. Recent data suggest that obesity is rapidly approaching smoking as the leading preventable cause of death in the United States [2]. In addition to increased risk for early mortality, obesity is associated with over 100 discrete metabolic, cardiovascular, immunologic, and respiratory disorders. The strongest relationships appear between obesity and a myriad of cardiovascular and metabolic disorders, particularly metabolic syndrome (MetS) and diabetes.

Recent media attention has increased public awareness of obstructive sleep apnea syndrome (OSAS), a chronic sleep disorder strongly associated with obesity. OSAS is characterized by repetitive bouts of upper airway collapse during sleep, resulting in intermittent periods of hypoxia, hypercapnia, and fragmented sleep [3]. OSAS is present in individuals who have over 5 apneic and hypopneic events per hour of sleep (Apnea/Hypopnea Index, AHI), with each event resulting in surges of sympathetic nervous system activity to re-establish breathing. Evidence from the Wisconsin Sleep Cohort suggests that OSAS is present in as many as 1 in 4 middle aged males and 1 in 10

middle aged females, with many cases going undiagnosed. Although typically considered to be an adult problem, recent evidence also confirms the presence of OSAS in children and adolescents, with as many as 4% of those populations being affected [4]. The global increase in prevalence of obesity, both in adults and adolescents, suggests the incidence of OSAS will continue to steadily rise across the entire population. Several prominent risk factors exaggerate the prevalence of this OSAS, including age, craniofacial abnormalities, and obesity [5].

Obesity is the primary risk factor for OSAS and is reported in over 40% of OSAS cases [6]. Furthermore, over 80% of individuals with OSAS are obese. Data from the Wisconsin Sleep Cohort [7] suggest that as little as a 10% increase in body weight increases risk for OSAS 6-fold. Excess adipose tissue around the upper airway increases neck circumference and presents mechanical challenges for the lumen of the pharynx to stay patent during sleep. An earlier study of radiographic features of the upper airway in OSAS found that neck circumference was a better indicator of OSAS severity than actual anatomical features of the pharynx, hyoid, and palate, suggesting the presence of obesity is likely more important than potential anatomical variations that could lead to OSAS [8]. In addition to adiposity around the neck circumference, central fat limits mechanical function of the lungs and diaphragm in a manner that may reduce breathing effort [9, 10]. These effects are amplified when a person becomes supine, having obvious implications for disorders due to abnormalities in breathing during sleep, such as OSAS [9].

Individuals suffering from OSAS usually experience excessive daytime sleepiness (EDS), due primarily to fragmented sleep caused by repetitive arousals over the course of the night associated with apneic events. EDS is associated with decrements in cognitive

and physical function, presenting challenges for a variety of occupations and other daily activities that necessitate cognitive performance. A recent meta-analysis of studies examining neuropsychological effects of OSAS found that untreated OSAS was associated with declines in coordination, vigilance, and executive functioning [11]. Decreases in cognitive function have also been demonstrated in children with latent OSAS, resulting in poorer performance in school and a lower IQ, on average [12, 13]. Furthermore, EDS presents as a public health hazard and is the reported cause of as many as 1 in 5 automobile accidents [14].

Beyond these cognitive deficits, OSAS predisposes an individual to a number of chronic cardiovascular and metabolic disorders, including hypertension and the metabolic syndrome (MetS). Increased sympathetic nervous system activity, intermittent hypoxia and hypercapnia, and fragmented sleep all likely contribute to exaggerated chronic disease morbidity in this population. Considerable evidence suggests an independent relationship between OSAS and hypertension [15, 16]. Peppard and colleagues [16] found a dose-response relationship between severity of OSAS and prevalence of hypertension, with individuals with mild and moderate OSAS having twice and three times the risk of hypertension, respectively, as non-apneic controls [16]. Furthermore, OSAS has recently been identified as a primary underlying cause of hypertension that is resistant to pharmacologic treatment [17]. Additional research suggests that OSAS is also independently associated with both insulin resistance and the MetS. Ip *et al* [18] demonstrated a 2-fold higher mean insulin resistance levels in overweight individuals with OSAS than controls (3.0 ± 5.4 vs. 1.6 ± 1.1 , respectively), and found severity of OSAS was an independent predictor of insulin resistance. In addition, this study found

that an increase of 2 apneas or hypopneas per hour of sleep was associated with a 1% increase in insulin resistance. Coughlin and colleagues [19] found the prevalence of MetS greater in individuals with OSAS than non-apneic controls (87% vs. 35%, respectively). Results of this study further suggested that MetS was over 9 times as likely to occur in individuals with OSAS. Furthermore, OSAS may increase risk for nocturnal cardiac arrhythmias, heart failure, and coronary artery disease [20-22].

The relationship between OSAS and chronic disease morbidity is well established in middle aged and older adults, as the majority of experimental data to date has been obtained from adults over the age of 45 years old. However, it is unknown at which age latent OSAS promotes the pathogenesis of chronic diseases such as cardiovascular diseases and diabetes. The purpose of this project was to examine anthropometric, biochemical, and functional characteristics of young men with OSAS to determine if evidence of chronic disease pathogenesis already exists in this population. These findings would have obvious implications for earlier identification and treatment of OSAS.

RESEARCH AIMS

Research Aim 1: To determine if OSAS is associated with biochemical evidence of insulin resistance in young overweight men.

Insulin resistance was calculated using the Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) method, a non-invasive estimation using fasting insulin and glucose. This method was used to determine if overweight subjects with OSAS had

elevated insulin resistance, as compared to overweight and normal weight subjects without clinical evidence of OSAS. Additionally, adipokines that have been consistently demonstrated to affect insulin resistance, namely proinflammatory hormones leptin, tumor necrosis factor – alpha (TNF- α) and interleukin 6 (IL-6) and the anti-inflammatory adipokine adiponectin, were quantified to determine if there was a proinflammatory phenotypic pattern evident in overweight young men with OSAS that may further explain insulin resistance. Finally, these data were analyzed to determine if insulin resistance and levels of these hormones were directly related to variables that characterize OSAS severity, overall and regional distribution of adiposity, and physical activity patterns.

Research Aim 2: To determine if there is biochemical or physiological evidence of endothelial dysfunction in young overweight men with OSAS.

Endothelial function was quantified via venous occlusion plethysmography (VOP), which estimates resting blood inflow to the periphery and endothelium-dependent vasodilatory capacity after brief ischemia [23]. These measures were used to determine if overweight young men with OSAS had VOP evidence of endothelial dysfunction that is greater than overweight subjects without OSAS; overweight subjects were expected to show some VOP evidence of impaired endothelial function, in comparison to the normal weight sedentary but otherwise healthy subjects. Vascular endothelial growth factor (VEGF) and vascular endothelial growth factor receptor 2 (VEGFR2) were analyzed to examine if biochemical indicators of angiogenesis were elevated due to latent OSAS. C-reactive protein (CRP) was quantified to assess the possibility of vascular inflammation associated with the early stages of vascular endothelial damage. Finally, analyses were

performed to determine if endothelial function and levels of these hormones were directly related to variables that characterize OSAS severity.

Research Aim 3: To determine whether OSAS is associated with a greater incidence of MetS than is obesity alone in young men.

Components of the MetS, as defined by the NCEP [24], were analyzed using a standard fasting lipid panel with glucose analysis, anthropometric measurement of waist circumference, and blood pressure assessed by auscultation. Raw data from these measurements were used to determine if mean values of each of the individual components of the MetS were elevated in those young overweight men with latent OSAS. Furthermore, the number of components of the MetS were counted in each study group and analyzed to determine if incidence of the MetS (at least 3 components) or the total number of components was higher in overweight men with OSAS than overweight and normal weight controls. Finally, analyses were performed to determine if MetS components were directly related to variables that characterize severity of OSAS and regional distribution of adiposity.

BACKGROUND

Insulin Resistance

Insulin resistance is a condition characterized by the inability of insulin to facilitate blood glucose, uptake, and transport in muscle, fat, and hepatic tissue [25]. The result of insulin resistance is elevated plasma levels of insulin and glucose, which is an intermediate step in the pathophysiology of chronic metabolic disorders, such as type II

diabetes and MetS. The gold standard for quantification of insulin resistance is the hyperinsulinemic euglycemic clamp, an invasive technique measuring how much glucose is needed to effectively respond to an increase in insulin without inducing hypoglycemia [26]. As this measurement is invasive and potentially dangerous, very few studies employ this technique and existent studies often lack statistical power due to minimal sample sizes. However, non-invasive estimations such as the HOMA-IR method have been developed to simplify the measurement. HOMA-IR is based on a model that quantifies interactions between plasma glucose and insulin that suggest whether or not beta cells are deficient, and is calculated by dividing the product of fasting insulin and glucose by a constant value. The HOMA-IR correlates well to the euglycemic clamp ($r=0.88$, $p < 0.01$), and may be particularly useful for approximating the changes in insulin resistance that precede diabetes [27].

Considerable evidence from studies using both invasive and non-invasive techniques has demonstrated a direct relationship between obesity and insulin resistance. Recent evidence suggests that OSAS is also associated with insulin resistance. Vgontzas *et al* [28] found higher fasting glucose (106.2 ± 4.1 vs. 85.4 ± 4.4 mg/dL) and insulin (25.7 ± 4.2 vs. 14.6 μ U/L ± 2.5) in overweight middle aged subjects than BMI and age matched controls. A clinic sample of 270 middle aged adults demonstrated higher insulin resistance, as assessed by the HOMA method, in individuals with OSAS (1.8 vs. 1.3) [18]. In this study, AHI was directly associated with HOMA values independently of obesity, and insulin resistance was present even in lean individuals with OSAS. Results from a community based study showed that individuals with at least mild OSAS had over twice the relative risk of insulin resistance than their non-apneic counterparts [29].

Furthermore, multiple linear regression analysis revealed that AHI was positively associated with insulin resistance in a manner that was independent of obesity. Interestingly, a study examining the benefits of just 2 nights of nasal continuous airway pressure (nCPAP) usage demonstrated an improvement in insulin sensitivity [30]. The short term nature of this study removes potential confounding effects of fluctuations in body weight. Although these studies suggest the relationship between OSAS and insulin resistance to be independent of obesity, the fact that obesity is the primary risk factor for both conditions suggests that they have pathophysiological similarities and obesity likely further attenuates the insulin resistance.

Beyond obesity, many of the mechanisms linking OSAS and insulin resistance remain unclear. One potential mechanism may be the effect of catecholamines and glucocorticoids, stress hormones associated with elevations in blood glucose, that are released and chronically elevated due to heightened sympathetic drive in individuals with OSAS [31, 32]. Cortisol, in particular, may lead to increased body weight and deposition of adipose tissue in the abdomen, face, and neck [33, 34]. An additional likely mechanism linking OSAS and insulin resistance is altered production of adipokines, hormones produced by adipose tissue with potent effects on a variety of physiological processes. Leptin, IL-6, TNF- α , and adiponectin are adipokines that directly and indirectly mediate glucose homeostasis and related effects of insulin [35, 36]. Disruptions in these hormones in latent OSAS may help explain the independent relationship between OSAS and insulin resistance.

Leptin, a byproduct of the obesity gene *ob*, helps regulate energy expenditure and appetite control via hypothalamic inhibition of the appetite-regulating hormone

neuropeptide γ . Limited evidence also suggests leptin may directly affect insulin resistance by promoting fatty acid oxidation [37, 38]. Leptin levels are typically higher in obese individuals, suggesting a hyperphagic state in which leptin satiety signaling effects fail to limit body mass, i.e., leptin resistance [39, 40]. Leptin may be of particular interest in OSAS, as the sympathetic nervous system, catecholamines, and glucocorticoids all alter leptin production and release [41-43]. Ip *et al* [44] found that fasting leptin was nearly 30% higher in overweight middle aged adults with OSAS than BMI matched controls (9.2 ± 4.2 vs. 6.5 ± 3.8 ng/ml). An additional study demonstrated that leptin levels are also higher in middle aged adults of normal body weight with OSAS (6.0 ± 0.4 ng/ml) than non-apneic counterparts (3.8 ± 0.2 ng/ml) [45]. In addition to finding elevated leptin in individuals with OSAS, several studies also report associations between OSAS severity, as assessed by AHI, and serum leptin that are independent of body weight [28, 46, 47]. Correlations in these studies ranged from $r = 0.38$ to 0.55 .

IL-6 is a pro-inflammatory adipokine that exaggerates insulin resistance primarily by causing deficiencies in insulin signaling at the cell membrane [35, 36]. IL-6 is also associated with a downregulation of peroxisome proliferator-activated receptor gamma [48] an anti-inflammatory nuclear receptor which promotes insulin sensitivity, when activated [49]. Plasma IL-6 is also an independent predictor of future type 2 diabetes, and IL-6 is positively associated with insulin resistance, even after adjustment for BMI [50, 51]. IL-6 is typically elevated in obesity, and plasma levels are positively correlated with both BMI and total body fat [52]. Several studies have demonstrated elevations in plasma IL-6 of obese adults with OSAS, as compared to obese controls. In a study of obese middle aged males, subjects with OSAS had nearly twice the plasma IL-6 levels as

controls matched for BMI (11.7 ± 9.8 vs. 6.1 ± 4.4 pg/ml) [53]. An additional study demonstrated that plasma IL-6 concentrations were nearly 4 times higher in obese subjects with OSAS, as compared to obese controls (8.7 ± 0.3 vs. 2.1 ± 0.2 pg/ml), despite the fact that the control subjects had a higher BMI [54].

TNF- α is another pro-inflammatory adipokine that appears to modulate insulin resistance, and further complicates the condition by upregulating other proinflammatory cytokines [55-57]. Obese individuals have elevated messenger ribonucleic acid (mRNA) expression and protein levels of TNF- α , with TNF- α concentrations being strongly correlated with body fat and BMI [58-61]. A limited number of studies have examined TNF- α concentrations in individuals with OSAS. Minoguchi *et al* [62] stratified middle aged OSAS subjects into severe or mild disease categories, finding higher fasting serum TNF- α in moderate to severe OSAS (2.3 ± 0.5 pg/ml) than mild OSAS (1.8 ± 0.5 pg/ml), obese controls (1.6 ± 0.3 pg/ml), or normal weight controls (1.1 ± 0.4 pg/ml). In this study, serum TNF- α was correlated to AHI in a manner that was independent of obesity. Another study of middle aged adults also demonstrated higher TNF- α concentrations in obese subjects with OSAS than obese controls (4.6 ± 3.4 vs. 3.3 ± 2.1 pg/ml).

Adiponectin, a recently discovered anti-inflammatory adipokine produced exclusively by white adipose tissue, is a peroxisome proliferator-activated receptor gamma responsive hormone that has potent insulin sensitizing effects [63]. An early study of adiponectin in obesity found the protein concentrations nearly 60% lower in obese subjects than normal weight controls [64]. Studies of adiponectin in OSAS have been limited and produced conflicting results. Zhang and colleagues [65] found that adiponectin was nearly 30% lower in overweight subjects with OSAS than overweight

controls (5.0 ± 1.0 mg/L vs. 7.1 ± 1.3 mg/L). In contrast, however, Wolk *et al* [66] examined adiponectin levels in obese middle aged adults with OSAS and BMI-matched controls, finding 25% higher adiponectin concentrations in the OSAS group (8.5 ± 0.9 vs. 6.3 ± 0.6 ug/ml). The contradictory results of studies examining adiponectin in OSAS necessitate further study of this adipokine.

Endothelial Dysfunction

The initial step in the pathophysiology of vascular disease is damage to the endothelium, which can occur due to a variety of intrinsic or extrinsic stimuli [67]. Endothelial damage is associated with impairments in endothelium-dependent vasodilatation, the expansion of blood vessels supplying peripheral tissue with blood and nutrients to support normal physiological function. Endothelial dysfunction can be measured by a variety of experimental techniques, including ultrasound, pulse wave velocity, and VOP [68]. VOP measures resting inflow to peripheral tissues and vasodilatory reaction of blood vessels to brief periods of ischemia. This measure is lauded for its reproducibility and accuracy, and is considered by many to be the gold standard for in vivo assessment of endothelial function [23]. Many studies use VOP invasively in conjunction with venous administration of pharmacologic agents inducing endothelial-dependent vasodilatation. Higashi and colleagues [69], however, have demonstrated strong correlations ($r = 0.91$) between forearm blood flow values obtained after invasive administration of vasoactive drugs and non-invasive quantification via reactive hyperemia, the vascular blood flow response to a brief period of ischemia.

Endothelial dysfunction has been demonstrated in individuals with major vascular disease risk factors, such as smoking and hypertension [70, 71]. In addition to being a primary step in the pathophysiology of atherosclerosis and coronary artery disease, endothelial dysfunction is associated with a number of chronic metabolic disorders, including diabetes and the MetS [72]. Decrements in endothelial function have also been demonstrated in uncomplicated obesity. Perticone *et al* [73] found that maximum blood flow was lower in obese subjects (6.5 ± 1.8 mL/[min x 100 mL]) than both overweight and normal weight controls (10.8 ± 2.7 and 19.8 ± 2.8 mL/[min x 100 mL], respectively). Endothelial dysfunction has also been identified in individuals with OSAS. A recent study of middle-aged adults demonstrated 40% lower flow-mediated dilation values in subjects with OSAS than non-apneic controls ($10.9\% \pm 2.6$ vs. $6.0\% \pm 3.2$) [74]. An additional study examined endothelial function via reactive hyperemia in overweight middle aged adults with untreated OSAS and controls, and found that peak blood flow after a 10-minute period of ischemia was blunted nearly 30% in individuals with OSAS. After subjects with OSAS were treated for an average of 4.6 months with nCPAP therapy, peak blood flow after reactive hyperemia increased over 20% (40.8 ± 3.2 vs. 49.4 mL/[min x 100 mL] ± 4.6) [75]. The association between OSAS and endothelial dysfunction is likely due in part to increased sympathetic nervous system activity and nocturnal surges in blood pressure associated with OSAS, and may help explain the increased incidence of OSAS in coronary heart disease, atherosclerosis, and stroke.

In addition to measures obtained using VOP, biochemical markers of vascular function may help further identify processes that lead to deficits in vascular status. CRP is an emerging cardiovascular disease risk factor that is a stable serum biomarker of

inflammation, and is thought to be directly related to vascular inflammation associated with endothelial damage and atherosclerotic plaque formation [67]. Several studies have demonstrated elevated serum CRP levels in obesity. A population study from the Third National Health and Nutrition Survey found that both obese middle-aged males were over twice as likely as their normal weight counterparts to have clinically elevated CRP levels (>1.00 mg/dl) [76]. This relationship was considerably exaggerated in females, with obese individuals being over 6 times as likely to have elevated CRP levels. This pattern of elevated CRP in overweight and obese individuals held true despite statistical adjustments for smoking status and presence of chronic disease. CRP levels were also elevated in the youngest age cohort studied (17-39 years old). Additional data from a younger cohort of subjects (children and adolescents ages 6-18 years old) found an odds ratio of elevated CRP levels of 2.2 in children who had BMI's in the 85th to 95th percentile according to age and sex [77]. These findings are alarming, and suggest that overweight may be associated with vascular inflammation that precedes endothelial dysfunction even in individuals during childhood.

Additional studies suggest that CRP may be further exaggerated in individuals with OSAS. Shamsuzzaman and colleagues [78] demonstrated nearly a three-fold greater plasma CRP levels in middle-aged subjects with OSAS than age and BMI matched controls (0.81 ± 0.15 vs. 0.28 ± 0.12 mg/dL, respectively). In this study, CRP levels were directly correlated with OSAS severity, as assessed by AHI ($r = 0.55$). A second study compared serum CRP concentrations in individuals with OSAS and cardiovascular disease, a condition known to be associated with elevations in CRP. Kokturk *et al* [79] found that mean CRP levels were over twice as high in individuals with OSAS ($2.44 \pm$

5.11 mg/dL) than individuals with cardiovascular disease (1.16 ± 5.8 mg/dL). These researchers also found a direct correlation between AHI and CRP levels ($r = 0.61$).

Additional cytokines further quantify endothelial dysfunction by estimating their effects on growth of new blood vessels, a process induced in chronic conditions associated with hypoxia, such as myocardial ischemia and OSAS [80]. VEGF is a protein hormone that acts in this manner, primarily upon the vascular endothelium facilitating angiogenesis [81]. Although it is difficult to invasively quantify production of VEGF and related hormones from the endothelium, blood monocytes act as reporters for endothelial cell function, in essence mimicking their activity [82]. Under normal conditions, VEGF is produced by a variety of tissue types, including adipocytes [48]. Although it is plausible that obesity also augments production of the VEGF hormone, current experimental evidence does not support this hypothesis. However, research does support the hypothesis that hypoxia associated with the clinical presentation of OSAS increases production of VEGF. A recent study found that serum VEGF levels were, on average, nearly three times higher in adults with severe OSAS than their non-apneic counterparts (150 ± 111 pg/mL vs. 450 ± 339 pg/mL) [83]. An additional study sought to determine whether the presence of severe hypoxia in OSAS was associated with increased plasma concentrations of VEGF. Schulz and colleagues [84] demonstrated higher VEGF levels in severe OSAS with severe nocturnal hypoxia (410 ± 77 pg/mL) than either subjects with severe OSAS and moderate hypoxia (224 ± 38 pg/mL) or non-apneic controls (245 ± 61 pg/mL).

Metabolic Syndrome (MetS)

MetS is defined as a clustering of traditional cardiovascular disease risk factors, and is present in individuals who have at least three of the following: waist circumference ≥ 102 cm in males and ≥ 88 cm in females, blood pressure (BP) levels $\geq 130/ \geq 85$ mmHg, serum triglyceride (TG) levels ≥ 150 mg/dl, fasting blood glucose ≥ 100 mg/dl, and serum high density lipoprotein cholesterol (HDL-C) levels < 35 mg/dl in males and < 40 mg/dl in females [85]. Obesity is a core component of the MetS, and is one likely reason for the unusually high incidence of MetS and other metabolic disorders in OSAS. However, recent evidence suggests that OSAS and MetS are linked in a manner that is independent of obesity. In fact, individuals with OSAS are over twice as likely to have MetS as compared to obese controls [19]. Potential mechanisms explaining the exaggerated MetS state in OSAS include fragmented sleep patterns leading to some unidentified endocrine dysfunction, increased sympathetic drive and amplified catecholamine response during sleep that persists into waking hours, and endothelial dysfunction [22]. Numerous studies have examined the relationship between OSAS and individual components of the MetS.

The strongest relationship between OSAS and MetS components appears with hypertension. Evidence from the Wisconsin Sleep Cohort demonstrated a dose response relationship between risk for hypertension and severity of OSAS [16]. In this study, middle aged individuals with mild and moderate OSAS had BMI-adjusted relative risks for hypertension of 2.0 and 2.89, respectively. Further data from the Wisconsin Sleep Cohort demonstrated elevations in mean BPs in those individuals with at least mild OSAS (131/80 vs. 122/75 mmHg for controls) [15]. The Seventh Report of the Joint National Committee on the Prevention, Detection, Evaluation, and Treatment of

hypertension reiterates the importance of OSAS in the pathology of hypertension [17]. This report identifies OSAS as a primary cause of hypertension that should be evaluated by physicians whose patients are resistant to full doses of pharmacological treatment. Although substantial evidence demonstrates a direct relationship between OSAS and hypertension, adequately controlled clinical trials in younger adults are still lacking.

The inverse relationship between obesity and HDL-C, another component of MetS, is well established. Limited evidence also suggests a similar relationship between OSAS and HDL-C that is independent of obesity. Results from a recent trial examining middle aged adults found that overweight subjects with OSAS had lower mean HDL-C levels than their overweight non-apneic counterparts (50.7 vs. 42.9 mg/dl, respectively) [19]. Although Tan *et al* [86] did not find mean differences in HDL-C, they did find evidence that latent OSAS may disrupt the functional ability of HDL to transfer oxidized LDL, as was determined by a series of functional fluorescence assays. Substantial evidence from physical activity studies with apparently healthy adults has confirmed the direct relationship between physical activity and serum HDL-C concentrations [87, 88]. As a primary symptom of OSAS is excessive daytime sleepiness, it is appealing to speculate that OSAS may be associated with low HDL-C, in part, due to very low levels of physical activity.

Although high triglycerides are largely dependent on the presence of obesity and/or dyslipidemia, several studies have indicated an independent relationship between OSAS and TG. A recent study of middle aged adults demonstrated over 20% higher TG levels in overweight adults with OSAS than BMI matched controls, with mean TG levels meeting criteria for a MetS risk factor in the OSAS group (177.2 mg/dl) but not controls

(141.8 mg/dl) [19]. An additional study comprised primarily of overweight middle aged males demonstrated similar differences, reporting TG levels of 141.8 mg/dl in subjects with OSAS and 106.3 mg/dl in controls [44]. In this study, 5 times as many subjects with OSAS had extreme TG values (≥ 178 mg/dl) than BMI matched controls. One night of treatment with nasal continuous airway pressure (nCPAP) lowered TG levels in those individuals with OSAS, further suggesting the role of untreated OSAS in elevated TGs. Evidence regarding the relationship between OSAS and TG in younger adults is also still lacking.

Elevations in blood glucose are often reported in overweight individuals, and are the direct result of impairments in insulin's ability to assist glucose transport and uptake. Although the high incidence of diabetes in individuals with OSAS is largely due to the comorbid presence of obesity, several recent trials have shown that elevations in fasting blood glucose in OSAS are independent of obesity. Ip and colleagues [44] demonstrated higher levels of fasting blood glucose in individuals with OSAS (95.4 mg/dl) than controls (91.8 mg/dl). Although individuals with OSAS were heavier, on average, than controls, AHI remained a significant predictor of fasting insulin levels and insulin resistance when data were controlled for adiposity. In a study of obese middle aged adults, Vgontzas et al [28] found that fasting blood glucose levels were over 20% higher in obese individuals with OSAS (106.2 ± 4.1 mg/dl) than BMI matched controls (85.4 ± 4.4 mg/dl). The relationship between OSAS and impaired glucose uptake remains controversial, as several studies have also reported no difference in fasting blood glucose levels between subjects with OSAS and BMI matched controls. However, the results of a recent systematic review of 24 studies exploring the relationship between OSAS and

metabolic disorders suggests that current data supports the hypothesis that disruptions in glucose tolerance in OSAS are independent of obesity. Further research, including studies of younger adults, is needed provide additional experimental evidence of this relationship.

Individuals with at least 3 of the MetS risk factors have heightened risk of cardiovascular disease morbidity and mortality. Results of the Kuopio Ischaemic Heart Disease Risk Factor Study demonstrated that men meeting criteria for MetS had over 4 times the relative risk of cardiovascular disease mortality as controls [89]. However, a number of MetS components are related to modifiable lifestyle choices. Physical inactivity, for example, is associated with an increased incidence of MetS [90]. Regular participation in exercise has beneficial effects on all components of the MetS, and is likely a main reason exercise is associated with a decrease in cardiovascular and all cause mortality [91].

Limitations of Previous Research & Significance of Proposed Study

The relationship between OSAS and chronic disease morbidity, particularly hypertension, metabolic syndrome, and diabetes, is well established in middle aged and older adults. Considerable evidence suggests the presence of functional and biochemical endothelial dysfunction in these adults with OSAS and there is sufficient data to suggest an independent relationship between OSAS and both insulin resistance and MetS. Although these relationships have been established, current research is limited in that the vast majority of studies have been examined middle-aged and older adults. Thus, it is unknown at what age latent OSAS may promote pathogenesis of vascular and metabolic

disorders. Furthermore, many of the previous longitudinal and cross-sectional studies lack a normal weight and overweight control group, both of which are necessary to determine if changes in vascular function and insulin resistance are due primarily to the presence of latent OSAS or concomitant obesity. The proposed study addressed these limitations in previous research by examining anthropometric, biochemical, and functional characteristics of young men with OSAS to determine if evidence of endothelial dysfunction, metabolic syndrome, and dyscytokinemia exists in this population. These deleterious changes, if present, may promote early development of diabetes and cardiovascular disease, thus having obvious implications for earlier identification and treatment of OSAS.

METHODS

Subject recruitment occurred through communications with a variety of campus and community organizations, including e-mail notices distributed through various university listserves, flyers posted on public notice boards throughout campus, and public service announcements and advertisements in local newspapers. Upon expressing interest, potential subjects were screened via telephone or email to determine eligibility.

To participate, potential subjects must have met the following criteria:

- ◆ Age 18 to 28 years old
- ◆ Had not used tobacco products within the past year
- ◆ No acute respiratory infection over the past 3 weeks
- ◆ Not taking medications with vasoactive properties
- ◆ No diagnosed cardiovascular, musculoskeletal, and metabolic disorders

Eligible subjects initially completed questionnaires assessing health history, quality of life via medical outcomes short form - 36 (MOS SF-36), dietary intake (4-day diet record), perceived sleepiness during routine daily activities (Epworth Sleepiness Scale, ESS), and perceived functional capacity via the Veteran's Specific Activity Questionnaire (VSAQ). Subjects then were evaluated for OSAS risk via a condensed, take home polysomnography exam. After being given detailed written and oral setup instructions, subjects were given the digital recording device (Embletta – Reykjavik, Iceland) to wear during one weeknight of typical sleep. The Embletta recorded respiratory inflow, peripheral oxygen saturation, heart rate, snoring frequency and magnitude, and thoracic and abdominal breathing effort. A combination of data from these measurements was used by a certified sleep technician to determine likely severity of OSAS (AHI). AHI, in addition to BMI and physical activity pattern data obtained from the initial eligibility assessment, were used to place subjects into the appropriate study group (Table 1). All subjects were sedentary, and reported no structured physical activity over the previous 6 months. After being placed into an experimental group, subjects underwent 4 additional measurements. The Virginia Tech Institutional Review Board determined that this research is in compliance with federal guidelines governing the Protection of Human Subjects.

Table 1 – Criteria for Subject Placement into Groups

Group	BMI (kg/m²)	AHI (events/hr)
Overweight with OSAS (owOSAS)	> 25.0	> 5
Overweight without OSAS (owNOSAS)	> 25.0	< 5
Normal weight without OSAS (nwNOSAS)	< 25.0	< 5

Fasting blood measurement

Subjects had ~50 mL of blood drawn via cubital venipuncture by a board certified physician and presented for testing between 6:00 and 10:00 AM having had at least 6 hours of sleep, no food for the prior 8 hours, and no alcohol, caffeine, or exercise reported for the prior 24 hours. A small sample of whole blood was used immediately to analyze the following: glucose, triglycerides, total cholesterol, HDL-C, and LDL-cholesterol (LDL-C). Blood samples were centrifuged following venipuncture to separate plasma and serum, which was frozen at -80°C for batch analysis of biochemical markers after completion of the study (See Table 2). Whole blood was also used to quantify monocyte expression of VEGFR2 via cell staining and fluorescence-activated cell sorting (FACS) analysis. Briefly, whole blood was suspended in blocking buffer and stained using a primary antibody to bind VEGFR2 (Flk-1 mouse monoclonal IgG₁, Santa Cruz Biotech, Santa Cruz CA). The optimal dilution for the primary antibody was 1:100. An additional fluorescent tagging protein was added to bind the VEGFR2: primary antibody complex (Goat F(ab)₂ anti-mouse IgG₁ FITC, Southern Biotech, Birmingham AL). The optimal dilution for the secondary antibody was also 1:100. Finally, red blood cells were lysed using a commercial kit (Immunolyse whole blood lysing reagent kit, Beckman

Coulter, Fullerton, CA). Peripheral blood mononuclear cells were suspended in phosphate buffered saline, refrigerated, and analyzed within 24 hours. Percentage of monocytes expressing VEGFR2 was determined using fluorescent-activated cell sorting analysis, with the viable cell gate being narrowed to identify expression of VEGFR2 in monocytes. White blood cell count was also measured using Coulter Counter (Becton, Fullerton CA), and was multiplied by percentage of cells expressing VEGFR2 to generate absolute expression of the receptor.

Table 2 – Methods of analysis and coefficient of variations of biomarkers

Biomarker	Method of Analysis	Coefficient of Variation
Adiponectin	ELISA	9.2%
C-reactive protein	ELISA	10.4%
Insulin	RIA	9.8%
Interleukin-6	ELISA	9.2%
Leptin	RIA	7.2%
Tumor necrosis factor alpha	hs-ELISA	8.6%
Vascular endothelial growth factor	ELISA	11.4%

Venous Occlusion Plethysmography (VOP)

Subjects also reported for forearm VOP testing (Hokanson EC-10, Bellevue, WA) in identical fasting conditions described in the previous section. After 10 minutes of quiet rest, 10 consecutive measurements of resting blood inflow were obtained via a strain gauge that quantified volume change in the forearm. The median 5 values were

averaged to generate a mean inflow score. After this rest period, forearm blood flow was occluded for 5 minutes to assess the endothelium-dependent vasodilatory capacity to return blood flow to ischemic tissue. Reactive hyperemia scores were quantified 0, 30, 60, and 120 seconds after reperfusion.

Dual energy x-ray absorptiometry (DXA) scan

Each subject reported to 229 Wallace Hall for the DXA scan, which was used to assess total body and regional distribution of fat and fat-free mass. Site specific measures of adiposity included abdomen, limbs, and the head. Bone mineral density and content measures, which were collected concurrently with this device, also provided useful information on baseline bone health in young men with OSAS for future publications. Subjects were positioned supine on the DXA platform (Hologic QDR 4500A, Bedford, MA) for these scans.

Statistical Analysis

Data were stored electronically in a password protected SPSS® (SPSS, Inc., Chicago, IL) database (Version 14.0). Statistical significance was determined *a priori* for all experimental analyses as a p value < 0.05.

Research Aim # 1

Group comparisons of independent variables were made using one-way analysis of variance (ANOVA). The sources of error and the degrees of freedom for the one-way ANOVA are depicted in Table 3 (analysis based on total subject number of 47).

Relationships between variables characterizing insulin resistance and both OSAS severity and adiposity were assessed using Pearson product moment of correlation. Multiple linear regression analysis was used to determine which variables were independent predictors of OSAS severity and insulin resistance.

Table 3 – Sources of Error and Degrees of Freedom for One-Way ANOVA

Source	Degrees of Freedom
Between	Groups – 1; $3-1 = 2$
Within	Subjects – Groups; $47 - 3 = 44$
Total	Subjects – 1; $47 - 1 = 46$

Research Aim #2

Group comparisons were made using one-way analysis of variance. Relationships between MetS components and variables denoting OSAS severity and adiposity were assessed using Pearson product moment of correlation. Relationships between number of MetS components and the same variables were calculated using Spearman correlation. Stepwise linear regression analysis was used to determine if OSAS severity and/or indices of adiposity were independent predictors of each MetS component, and vice versa.

Research Aim # 3

Group comparisons of VEGF and CRP were made using one-way analysis of variance (ANOVA). Repeated-measures, two-way ANOVA was used to determine the

effects of group, time, and interactions on the reactive hyperemia blood flow responses. The sources of error and the degrees of freedom for the two-way ANOVA are depicted in Table 4 (analysis based on 47 total subjects; n = 12 owOSAS, n = 17 owNOSAS, and n = 18 nwNOSAS). Relationships between endothelial dysfunction and both OSAS severity and adiposity were assessed using Pearson product moment of correlation. Multiple linear regression analysis was used to determine which variables were best predictors of OSAS severity (independent variables included initial blood flow response to reactive hyperemia, BMI, CAF, and neck circumference) and initial blood flow response to reactive hyperemia (independent variables included OSAS severity, BMI, CAF, and neck circumference).

Table 4 – Sources of Error and Degrees of Freedom for Two-Way ANOVA

Source	Degrees of Freedom
Group	Number of groups – 1; 3-1 = 2
Time	Number of times – 1; 13-1 = 12
Group x Time	(groups – 1)*(times – 1); (2)*(12) = 24
Within	Time (# of owOSAS – 1) + Time (# of owNOSAS -1) + Time (# of nwNOSAS – 1); 13 (12 – 1) + 13 (17-1) + 13(18-1) = 572
Total	(Groups * Times * Subjects) – 1 = (3 * 13 * 15.67) – 1 = 610

ASSUMPTIONS

1. Subjects provided correct information regarding the presence of acute and chronic illness, activity history, and medication usage in the self-reported health history questionnaire.
2. Subjects provided accurate information for the remaining study questionnaires, including the MOS SF-36, VSAQ, and ESS.
3. Sedentary subjects refrained from regular physical activity over the past 6 months, as noted in the initial qualification and health history questionnaires.
4. All laboratory equipment had been properly calibrated and maintained.
5. Subjects reported to fasting measurements without food 8 hours prior to the tests, had refrained from alcohol and caffeine for 24 hours prior, and had received at least 6 hours of sleep before reporting.
6. Subjects did not alter activity patterns over the course of their inclusion in the study.

LIMITATIONS

1. Degree of OSAS was determined using the Embletta home polysomnography device, which is less accurate and reliable than in-lab, physician supervised polysomnography.
2. LDL levels were not measured directly, but were calculated using a standard predictive equation.
3. Funding constraints did not allow for the inclusion of an additional group of normal weight apneics to adequately complement the sedentary control group.

4. Study subjects were primarily Caucasian college students, and reported on a voluntary basis.
5. Three subjects failed to complete fasting blood sampling due to adverse reactions to phlebotomy.

CAPACITY TO CONDUCT THE STUDY

Over the past 6 years, our laboratory has developed into an effective research team and gained considerable experience in developing experimental protocols, conducting clinical trials, presenting data at national and international research meetings, and publishing data in peer reviewed journals. Our research team is comprised of basic and applied research scientists specializing in immunology, endocrinology, nutrition, adiposity and clinical physiology. Our research team also includes clinicians trained in the identification and treatment of OSAS. Much of our work has been aimed at identifying biochemical and physiological markers of cardiovascular dysfunction and risk of hypertension associated with OSAS and to understanding how these may be improved in patients who comply with CPAP therapy. Our research team has examined health related consequences of OSAS in two clinical trials, supported in part by ResMed's Clinical Affairs Office and the ResMed Sleep Disordered Breathing Foundation.

The first clinical trial demonstrated that short-term CPAP therapy (4-weeks) for moderate-severe OSAS can be effective in improving cardiopulmonary markers of exercise performance and is accompanied by enhanced aerobic exercise capacity [92]. This study also established that following CPAP therapy, individuals with OSAS perceived less physical exertion during all stages of a graded exercise test, demonstrating

enhanced aerobic exercise tolerance. These results suggest that effectively treated OSAS patients have an improved capacity to participate comfortably in aerobic exercise, which may have implications on disease management and overall chronic disease morbidity.

The second clinical trial examined if biochemical and physiological markers may be improved in middle-aged patients with pre-hypertension who comply with 12 weeks of CPAP therapy in addition to a low-intensity exercise training program. This study demonstrated that untreated individuals with OSAS exhibit unique blood pressure and ventilatory responses that strongly suggest autonomic dysfunction. This study also showed that 12 weeks of CPAP treatment had no beneficial effects in lowering either the resting or exaggerated blood pressure responses in exercise among these pre-hypertensive patients. Although sample sizes in this study were too small for statistical evaluation of treatment subgroups, those patients in the mild exercise program did not lose weight, improve cardiovascular fitness, or demonstrate any lowering blood pressures after treatment. Our study was the first to prospectively evaluate this question of blood pressure outcomes after CPAP in patients not receiving any antihypertensive medications. Data from both clinical trials of middle-aged study patients, with full night polysomnography-confirmed OSAS, demonstrate that selected biomarkers and functional parameters sensitive to vascular and cardiovascular dysregulation, including some clearly revealed by clinical exercise testing, may uniquely discriminate between individuals with vs. without OSAS and who are of similar Body Mass Index (BMI).

We believe it was important to extend this work of screening and risk assessment for OSAS to asymptomatic overweight young men. The previous research conducted in our laboratories confirms our ability to successfully undertake this proposed research

project. Our research colleagues have substantial experience in conducting and interpreting GXTs and DXA scans, sampling and processing plasma and serum from whole blood, and analyzing biochemical adipokine and receptor levels via colorimetric assay, ELISA, RIA, and FACS analysis.

DEFINITIONS

Adipokine – a hormone produced by white adipose tissue [93]

Adiponectin – an anti-inflammatory adipokine which improves insulin sensitivity and is negatively correlated with body weight [94]

Angiogenesis – formation of new blood vessels [67]

Asymmetric Dimethylarginine (ADMA) - An inhibitor of nitric oxide (NO) synthesis that has been demonstrated to promote endothelial dysfunction, increase vascular resistance, and increase progression of atherosclerosis [95]

Apnea – complete cessation of airflow for a period of 10 seconds [96]

Apnea/Hypopnea Index (AHI) – index of OSAS severity based on total number of apneic and hypopneic events per hour of sleep [96]

C-reactive protein (CRP) - An acute biomarker of systemic inflammation produced by the liver which denotes increased risk for hypertension and cardiovascular disease [97]

Cytokine – a chemical messenger that can induce or inhibit a variety of local and systemic physiological processes, e.g. inflammation [98]

Enzyme Linked Immunosorbent Assay (ELISA) – a method of measuring levels of antibodies in a plasma or serum solution [99]

Fluorescence-Activated Cell Sorter (FACS) – a system designed to quantify biochemical markers and cell surface receptors based on the intensity and quantity of specific fluorescent antibodies bound to molecules of interest [100]

Homeostasis Model of Assessment (HOMA) – a calculated index of insulin resistance typically used in non-invasive studies, defined as the product of fasting insulin and glucose divided by a constant (22.5) [27]

Hypercapnia – increased levels of arterial blood carbon dioxide [101]

Hypothalamic-Pituitary-Adrenal Axis (HPA axis) – an axis of endocrine organs that are inter-related in the production, storage, and secretion of a vast array of hormones [31]

Hypopnea – significant reduction in airflow characterized by one of three criteria: 50% reduction in airflow, <50% reduction in airflow coupled with a >3% reduction in oxygen saturation, or <50% reduction in airflow coupled with EEG evidence of arousal [96]

Hypoxia – reduced levels of arterial blood oxygen [101]

Insulin Resistance – impaired biological response to insulin, causing a subsequent decrease in insulin mediated disposal of glucose and desensitization of peripheral tissues to insulin [102]

Interleukin-6 (IL-6) – a 28kDa pro-inflammatory adipokine that is associated with impairments in insulin signaling and glucose metabolism [36]

Leptin – a 40kDa hormone product of the obesity gene *ob* that helps regulate energy expenditure and appetite control via hypothalamic inhibition of the appetite-regulating hormone neuropeptide Y [36]

Metabolic Syndrome (MetS) – clustering of traditional cardiovascular risk factors which increases risk for cardiovascular morbidity and mortality, and is present in individuals

who have three of the following: waist circumference of ≥ 102 cm in males and ≥ 88 cm in females, BP levels of $\geq 130/\geq 85$ mmHg, TG levels of ≥ 150 mg/dl, fasting blood glucose of ≥ 100 mg/dl, and HDL-C levels < 35 mg/dl in males and < 40 mg/dl in females [90]

nasal Continuous Positive Airway Pressure – treatment modality for OSAS that employs pressurized air to create a pneumatic splint in the upper airway during sleep [103]

Obstructive Sleep Apnea Syndrome (OSAS) – condition characterized by repetitive collapse of the upper airway during sleep, and is associated with intermittent periods of hypoxia, hypercapnia, and fragmented sleep [96]

Peroxisome Proliferator-Activated Receptor Gamma (PPAR γ) – a nuclear receptor that is involved in the regulation of insulin sensitivity [104]

Reactive Hyperemia – Vasodilatory response of the endothelium to a brief period of ischemia [23]

Tumor Necrosis Factor-alpha (TNF- α) – a multifunctional 26kDa pro-inflammatory adipokine that effects lipid metabolism and upregulates the production of other pro-inflammatory cytokines [36]

Vascular Endothelial Growth Factor (VEGF) - A signaling protein involved in both vasculogenesis and angiogenesis [81]

Vascular Endothelial Growth Factor Receptor 2 (VEGFR2) – One of several receptors for the VEGF hormone that is involved exclusively with angiogenesis [81]

Venous Occlusion Plethysmography (VOP) – technique using mercury filled silastic strain gauges and blood pressure cuffs placed over the upper arm and wrist to estimate

resting inflow to periphery and endothelium-dependent vasodilatation following a period of ischemia [23]

LIST OF ABBREVIATIONS

ADMA – Asymmetric Dimethylarginine

AHI – Apnea/Hypopnea Index

ANOVA – Analysis of Variance

BMI – Body Mass Index

CRP – C-Reactive Protein

DXA – Dual Energy X-Ray Absorptiometry

EDS – Excessive Daytime Sleepiness

ESS – Epworth Sleepiness Scale

FACS – Fluorescence Activated Cell Sorter

GXT – Graded Exercise Test

HDL-C – High Density Lipoprotein – Cholesterol

HOMA – Homeostasis Model of Assessment

HTN – Hypertension

IL-6 – Interleukin-6

LDL-C – Low Density Lipoprotein – Cholesterol

MOSF-36 – Medical Outcomes Short Form- 36

mRNA – messenger Ribonucleic Acid

MetS – Metabolic Syndrome

nCPAP – nasal Continuous Positive Airway Pressure

NO – Nitric Oxide

NWA-NoOSAS – Normal Weight Active No Obstructive Sleep Apnea Syndrome

NWS-NoOSAS – Normal Weight Sedentary No Obstructive Sleep Apnea Syndrome

OSAS – Obstructive Sleep Apnea Syndrome

OWS-NoOSAS – Overweight Sedentary No Obstructive Sleep Apnea Syndrome

OWS-OSAS – Overweight Obstructive Sleep Apnea Syndrome

PPAR γ – Peroxisome Proliferator-Activated Receptor Gamma

PTG – Plethysmography

TC – Total Cholesterol

TG - Triglyceride

TNF- α – Tumor Necrosis Factor Alpha

VEGF – Vascular Endothelial Growth Factor

VEGFR2 – Vascular Endothelial Growth Factor Receptor 2

VOP – Venous Occlusion Plethysmography

VSAQ – Veterans Specific Activity Questionnaire

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Chapter 2

Central Abdominal Fat, Adipokines, and Insulin Resistance in Young Men with Obstructive Sleep Apnea Syndrome

Abstract

Background: Obstructive sleep apnea syndrome (OSAS), an obesity-related chronic disorder affecting as many as 1 in 5 adults, is associated with increased risk for chronic cardiovascular and metabolic disorders, such as insulin resistance. However, with respect to insulin resistance, it is unclear whether this increased risk is attributable to OSAS or its most frequent and prominent comorbidity obesity. **Objective:** To determine if insulin resistance exists in young men with preclinical OSAS independently of obesity. **Subjects:** Forty-six sedentary young men, age 18-26 years old: 12 overweight with OSAS (BMI = 32.4 ± 1.0 kg/m², AHI = 25.4 ± 5.4); 18 overweight without OSAS (BMI = 31.6 ± 1.1 kg/m², AHI = 2.2 ± 0.3); and 16 normal weight without OSAS (BMI = 22.4 ± 0.4 kg/m², AHI = 1.9 ± 0.3). **Measurements:** The presence and severity of OSAS were assessed using portable, unsupervised polysomnography. Total fat and CAF were quantified using dual energy x-ray absorptiometry (DEXA). Fasting serum leptin and insulin concentrations were measured using RIA, while serum adiponectin, leptin, TNF- α , and IL-6 were measured using ELISA. Fasting blood glucose concentration was obtained using whole blood and rapid commercial reflectance photometry. Insulin resistance was defined using the HOMA-IR. **Results:** Subjects with OSAS had more CAF and lower adiponectin than both overweight and normal weight controls (CAF: 9.1 ± 0.7 , 7.3 ± 0.5 , and 3.6 ± 0.3 kg, respectively, $p < 0.05$; adiponectin: 9.9 ± 0.8 ng/ml vs.

13.1 ± 1.4 and 13.1 ± 1.4 ng/ml, respectively, $p < 0.05$) Both overweight groups had significantly higher insulin, insulin resistance and leptin than normal weight controls ($p < 0.05$). However, these biomarkers were not higher in overweight subjects with OSAS than overweight controls. Serum TNF- α and IL-6 did not differ between any groups. Insulin resistance directly correlated with CAF ($r = 0.65$, $p < 0.001$) and total fat ($r = 0.68$, $p < 0.001$, but not OSAS severity. Insulin resistance was not an independent predictor of OSAS severity. **Conclusion:** Although young overweight men with OSAS did not demonstrate increased insulin resistance as defined by HOMA-IR, their greater CAF and lower adiponectin may put them on a trajectory for metabolic disturbances that increase early development of insulin resistance and type II diabetes.

Keywords: obstructive sleep apnea syndrome, insulin resistance, adipokines, central abdominal fat

INTRODUCTION

Obesity is the primary risk factor for obstructive sleep apnea syndrome (OSAS), a chronic sleep disorder that may affect as many as 1 in 5 adults¹. OSAS is characterized by repetitive bouts of upper airway collapse during sleep, resulting in intermittent periods of hypoxia, hypercapnia, fragmented sleep, and heightened sympathetic nervous system activity². If left untreated, OSAS increases risk for chronic cardiovascular and metabolic disorders, such as hypertension,^{3, 4} diabetes,⁵ and metabolic syndrome^{6, 7}. Although the influence of obesity likely exaggerates this risk, considerable evidence suggests an independent relationship exists between OSAS and many of these conditions.

Studies attempting to determine if the relationship between OSAS and insulin resistance is independent of obesity have been conflicting. Early studies, in particular, have produced equivocal results⁵⁻¹¹. Many of these studies appear to share common methodical limitations, such as inadequate sample size and poor study design or statistical control to eliminate the effect of confounding variables (e.g., regional distribution of adiposity and regular participation in physical activity)¹²⁻¹⁴. However, several well-controlled investigations demonstrated higher insulin resistance in middle-aged, obese subjects with OSAS than controls of similar age and body habitus. Perhaps the most convincing evidence comes for the large, multi-center Sleep Heart Health Study⁵, which found that OSAS severity was associated with elevated fasting glucose and insulin resistance independently of obesity. Additional studies have employed multiple regression analysis to demonstrate that OSAS severity is indeed an independent predictor of insulin resistance^{9, 15}.

Many mechanisms linking OSAS and insulin resistance remain unclear. Hypothesized links include an increase in catecholamines secondary to repetitive stimulation of the sympathetic nervous system¹⁴ and fragmented sleep patterns that disrupt the circadian rhythm of hormones that facilitate glucose metabolism¹². An additional link between OSAS and insulin resistance may be the presence of low grade systemic inflammation, which is supported by evidence of elevated serum C-reactive protein¹⁶. In addition, middle-aged adults with OSAS exhibited a pro-inflammatory phenotype of adipose tissue-derived hormones (adipokines) known to alter glucose regulation and insulin sensitivity^{17, 18}, namely an upregulation of interleukin-6 (IL-6)¹⁹, leptin²⁰, and tumor necrosis factor-alpha (TNF- α)¹⁹ and a suppression of the anti-inflammatory adiponectin²¹. These adipokines are of particular relevance to OSAS, as IL-6 and TNF- α have somnogenic properties²² and leptin contributes to ventilatory control during sleep.²³ Studies examining the insulin sensitizing effects of adiponectin in OSAS have been limited and conflicting^{21, 24} and warrant further research. Previous studies of adipokines in OSAS are limited in that they typically fail to account for the presence of visceral or central abdominal fat (CAF), an important depot of adipose tissue in the pathogenesis of chronic metabolic disorders which is also suggested as the main anthropometric variable that determines the presence of OSAS in obese individuals.

Thus, the purpose of the current study was to determine if insulin resistance and/or a pro-inflammatory phenotypic expression of adipokines are due primarily to the presence of OSAS or obesity, or if an additive effect is evident when obesity is coupled with OSAS. This study design is unique, as the potential confounding effects of regular participation in physical activity, use of medications, and presence of chronic

cardiovascular and metabolic disorders are controlled. Furthermore, no studies to date have examined evidence of insulin resistance in young, preclinical men, and it is still uncertain if the pathogenesis of insulin resistance has begun in this age cohort. Thus, these findings could have implications for early prevention, diagnosis, and treatment of both OSAS and type II diabetes. We hypothesize that insulin resistance will occur in young overweight men with OSAS, and that the combination of OSAS and obesity will produce an increase in insulin resistance that is additive beyond the effects of obesity alone.

METHODS

Subjects

Subjects were 18-26 year old males, who responded to flyers, newspaper advertisements, and e-mails. Exclusion criteria were: (a) habitual use of tobacco products within the past year; (b) regular participation in physical activity during the previous six months; (c) diagnosed or medically treated cardiovascular or metabolic disease; and (d) regular use of medications known to alter vascular function or inflammation. The initial assessment of subjects included measurement of height and weight to generate body mass index (BMI), and determination of OSAS via an overnight, portable polysomnography (PSG) test. Accordingly, subjects who qualified for the study were placed into one of three groups: 1) overweight with OSAS (BMI > 25.0 kg/m², AHI > 5.0); 2) overweight without OSAS (BMI > 25.0 kg/m², AHI < 5.0); and 3) normal weight without OSAS

(BMI < 25.0 kg/m², AHI < 5.0). The research protocol and procedures were approved by the Institutional Review Board within both the department and university.

Procedures

Subjects were evaluated for OSAS by a 5-channel, portable PSG device (Embletta PDS, Reykjavik, Iceland). The Embletta recorded the following measures during sleep: snoring, airflow, oxygen saturation, heart rate, thoracic breathing effort, and abdominal breathing effort. Subjects were given written and oral setup instructions, and wore the device for at least 6 hours during one night of sleep. Data were manually scored by a registered sleep technologist and confirmed by a sleep physician. OSAS severity was assessed by the apnea/hypopnea index (average number of apneas and hypopneas per hour of sleep), with apneas and hypopneas being scored according to recommendations set forth by the American Society for Sleep Medicine²⁵.

All anthropometric measures were obtained while subjects were in lightweight clothing and socks. BMI was defined as body weight in kilograms divided by height in meters squared. Neck circumference was measured at the laryngeal prominence, between the midanterior neck and midcervical spine. Total body fat was quantified using dual energy x-ray absorptiometry (DEXA) (Hologic QDR 4500A, Bedford, MA). Central abdominal fat (CAF) was estimated using the DEXA as described previously²⁶. To eliminate inter-observer variability, all measurements from the DEXA were obtained and analyzed by one investigator. Weekly scans of an external soft tissue bar were used to insure quality of the DEXA measurements, and test-retest reliability of this unit in our laboratory has been previously reported²⁷.

Subjects reported between 0600 and 1000 hours to have fasting blood drawn via cubital venipuncture. Testing conditions included: (a) at least 6 hours of sleep; (b) no food for the previous 8 hours, and (c) no alcohol or caffeine for the previous 24 hours. Whole blood was centrifuged for 10 minutes at 2500 RPM to isolate serum, which was immediately frozen at -80°C for later batch analysis. Leptin was measured via radioimmunoassay (Linco Research, St. Charles, MO; CV = 7.2%). IL-6 and adiponectin were quantified via a custom, multi-plex sandwich ELISA (SearchLight, Pierce Biotechnology, Rockford, Ill.; CV = 9.2%). Serum TNF- α was measured using a high sensitivity ELISA kit (Quantikine HS, R&D Systems, Minneapolis, MN; CV = 8.6%). Fasting insulin was measured using radioimmunoassay (Diagnostic Products Corporation, Los Angeles, CA; CV = 9.8%). Fasting blood glucose was assessed using a reflectance photometry (Cholestech LDX, Hayward, CA). Total error for glucose using this method reported by the manufacturer is $< \pm 11\%$. The homeostasis model of assessment for insulin resistance (HOMA-IR) method was used to quantify insulin resistance, and is a calculation based on fasting insulin and glucose²⁸ (insulin resistance = [fasting insulin x fasting glucose]/22.5).

Statistics

Statistical analyses were performed using SPSS version 15.0 (SPSS Inc., Chicago, IL). One-way analysis of variance (ANOVA) was used to make group comparisons between variables. Relationships between variables characterizing insulin resistance and both OSAS severity and adiposity were assessed using Pearson product moment of correlation. Multiple linear regression analysis was used to determine which variables

were best predictors of OSAS severity and insulin resistance. Statistical significance was determined *a priori* for all experimental analyses as a p value < 0.05.

RESULTS

Subject characteristics are presented in Table 1. Both control groups had AHI values < 5.0. The mean AHI in subjects with OSAS was indicative of moderately severe disease²⁵. There were no differences between groups in regards to age and excessive daytime sleepiness. Overweight subjects had higher BMI, neck circumferences, and overall fat mass than the normal weight controls (p < 0.05). Although there were no differences in these measurements between overweight groups, the overweight group with OSAS had a higher percent body fat and CAF than both the overweight and normal weight controls (Figure 1, p < 0.05). Severity of OSAS was directly correlated with BMI (r = 0.40, p < 0.01), total fat mass (r = 0.37, p < 0.01), and CAF (r = 0.44, p < 0.01).

Biochemical markers of insulin resistance are displayed in Table 2. There were no differences between any groups in TNF- α and IL-6. Fasting blood glucose was higher only in overweight subjects without OSAS (p < 0.05). HOMA-IR (3.6 and 3.0 vs. 1.6) and leptin (11.9 and 9.7 vs. 4.7 ng/ml) were higher in both overweight groups than normal weight controls. However, these values were not higher in the group with OSAS than overweight controls. Serum adiponectin was lower only in the group with OSAS (Figure 2, p < 0.05). When controlled for the potential confounding effects of CAF, AHI was not correlated to HOMA-IR, glucose, insulin, IL-6, TNF- α , leptin, or adiponectin. Across all subjects, CAF showed moderate to high correlations with HOMA-IR (r = 0.65,

$p < 0.001$), insulin ($r = 0.67$, $p < 0.001$), and leptin ($r = 0.81$, $p < 0.001$). Similar correlations existed between total fat mass and these variables (HOMA-IR $r = 0.68$, $p < 0.001$; insulin $r = 0.69$, $p < 0.001$; and leptin $r = 0.81$, $p < 0.001$).

Stepwise multiple linear regression analysis was used to determine which variables, if any, were independent predictors of either HOMA-IR or AHI. Three sets of variables were analyzed to determine if independent predictors of AHI existed across all subjects: (1) HOMA-IR, TNF- α , leptin, adiponectin, and IL-6; (2) HOMA-IR, insulin, fat mass, and CAF; and (3) fat mass, CAF, neck circumference, and percent body fat. No variables in model 1 were significant predictors of AHI. Fat mass and CAF were both independent predictors in model 2 (Fat mass $\beta = -1.24$, $p = 0.04$; CAF $\beta = 1.65$, $p = 0.008$) and model 2 (Fat mass $\beta = -1.22$, $p = 0.05$; CAF $\beta = 1.65$, $p = 0.009$). Three sets of variables were analyzed to determine if independent predictors of HOMA-IR existed across all subjects: (1) AHI, neck circumference, and Epworth Sleepiness Score; (2) TNF- α , leptin, adiponectin, and IL-6; and (3) fat mass, CAF, neck circumference, and percent body fat. No variables in model 1 were significant predictors of HOMA-IR. In model 2, leptin ($\beta = 0.77$, $p < 0.001$) and IL-6 ($\beta = -0.23$, $p < 0.05$) were independent predictors. In model 3, only fat mass was an independent predictor ($\beta = 0.68$, $p < 0.001$).

DISCUSSION

This study is the first, to our knowledge, to demonstrate early evidence of insulin resistance in young, sedentary, overweight men with occult OSAS. Although there were no differences between overweight groups in regards to age, BMI, or fat mass,

overweight subjects with OSAS had more CAF than controls (\uparrow 20%, $p < 0.05$). Furthermore, subjects with OSAS had lower serum adiponectin than either control group (\downarrow 30%, $p < 0.05$). These findings suggest that young men with OSAS exhibit unique anthropometric and biochemical features that may be indicative of early steps in the pathogenesis of insulin resistance.

Previous studies examining insulin resistance in middle-aged, overweight adults with OSAS have been conflicting. Recently, however, several well-controlled studies have demonstrated evidence of insulin resistance in OSAS that is independent of the effects of obesity. Investigations by Ip⁹ and Vgontzas²⁹ both found higher insulin resistance (HOMA-IR) in overweight, middle-aged adults with OSAS than controls. The current study demonstrated higher insulin resistance only in overweight vs. normal weight groups. Although not significant, higher insulin (\uparrow 35 %, $p = 0.14$) and HOMA-IR (\uparrow 20%, $p = 0.51$) were found in subjects with OSAS than overweight controls. These differences are likely not statistically significant due to small sample size. However, the magnitude of difference between groups may suggest clinical significance. In a longitudinal study of the Pima Indians, Weyer et al³⁰ demonstrated that as little as a 20% increase in fasting insulin in otherwise healthy, overweight adults over 5 years was associated with the progression from normoglycemia to impaired glucose tolerance.

Obesity is a known factor in the development and progression of both insulin resistance and OSAS. Distribution of adiposity, particularly around the abdomen, has implications for the development of insulin resistance^{31, 32}. Relatively few studies have sought to examine central or visceral fat in overweight subjects with OSAS. Vgontzas et al²⁹ found visceral fat 50% higher in middle-aged, overweight subjects with OSAS

than controls matched for BMI and subcutaneous fat area. Findings from Ogretmenolu and colleagues³³ further support the importance of visceral fat in OSAS, as differences in visceral fat area were able to correctly distinguish individuals with OSAS from simple snorers of similar body habitus with 100% sensitivity. Findings from the current study support those of Vgontzas²⁹, and may help partially explain the increased incidence of metabolic disorders, such as insulin resistance, that are commonly seen in individuals with OSAS.

Obesity likely contributes to the development of concomitant disorders within OSAS. Previous investigations attempted to determine if the relationship between OSAS and insulin resistance is independent of obesity in middle-aged adults. Using multiple linear regression analysis, Punjabi¹⁵ and Ip⁹ reported that indices of adiposity and severity of OSAS were both independent predictors of insulin resistance in overweight, middle-aged adults. Conversely, Gruber⁷ and Onat³⁴ argue that adiposity and the presence of the metabolic syndrome, not insulin resistance, were independently associated with OSAS. The current study supports these findings, as indices of fat, not OSAS severity, were independent predictors of insulin resistance, and vice versa. A key finding is that CAF was the strongest predictor of OSAS. This may be particularly important for future development of insulin resistance and metabolic disorders, as adipose tissue in this area is well vascularized and is a primary endocrine organ with implications for the production and release of potent hormones affecting glucose and insulin metabolism³⁵.

The current study sought to examine serum concentrations of several of these hormones derived from adipose tissue. Elevated pro-inflammatory biomarkers of insulin resistance have been commonly reported in OSAS. Ciftci and colleagues¹⁹ reported

higher serum concentrations of both IL-6 and TNF- α in overweight, middle-aged subject with OSAS than controls matched for age, BMI, and daytime sleepiness. The findings of the current study disagree and are potentially due to younger age of our subjects and absence of daytime sleepiness, the cardinal symptom of OSAS which itself is associated with an upregulation of these hormones. Leptin, a hormone which regulates appetite and energy expenditure, is consistently elevated in OSAS and represents a state of leptin resistance. Leptin concentrations are nearly 40% higher in overweight subjects with OSAS than overweight controls^{20, 29, 36-38}. In the current study, serum leptin was not different between these groups, though mean values were 20% higher in subjects with OSAS. The lack of statistical significance in this biomarker may be due to small sample size.

Studies of adiponectin in OSAS have been limited and conflicting. Zhang and colleagues²¹ found that adiponectin was nearly 30% lower in middle-aged, overweight subjects with OSAS than overweight controls. In contrast, Wolk *et al*²⁴ examined adiponectin in similar subjects with OSAS and controls, finding 25% higher adiponectin concentrations in the OSAS group. The findings from the current study demonstrate 20% lower serum adiponectin in subjects with OSAS than both control groups. These findings concur with those of Zhang, suggesting that a suppression of the insulin-sensitizing adiponectin may be an initial step in the pathogenesis of insulin resistance within OSAS.

Many mechanisms linking OSAS and insulin resistance remain unclear. One likely mechanism is a reaction to the repetitive activation of the sympathetic nervous system via stimulation from hypoxia and hypercapnia. These events are associated with an increase in the release of catecholamines that causes an acute increase in blood

glucose. As OSAS becomes more severe and sympathetic drive occurs more frequently, the acute increases in blood glucose become sustained and may lead to hyperinsulinemia and the development of insulin resistance¹⁴. Recent research suggests the physiological activity of adipose tissue may be another mechanism linking OSAS and insulin resistance. Adiponectin is produced solely by adipose tissue, and directly improves insulin sensitivity by improving glucose uptake in the muscle, decreasing hepatic gluconeogenesis, improving fatty acid oxidation in muscle and the liver, and regulating key steps in the signal transduction cascade of insulin^{17, 18}. Adiponectin concentrations are of particular interest in OSAS. Fasshauer and colleagues³⁹ recently demonstrated that injection of the β -adrenergic receptor agonist isoproterenol inhibited adipocyte expression of adiponectin mRNA nearly 75% in a dose-dependent fashion. β -adrenergic receptors are targets of those catecholamines released due to repetitive activation of the sympathetic nervous system. Taken together, this suggests that clinical features of OSAS may effectively inhibit the production of adiponectin, thus decreasing insulin sensitivity. Our results demonstrate reduced serum adiponectin in OSAS, suggesting that impairments in the function of adiponectin may be an initial biological step in the pathogenesis of insulin resistance in these individuals.

The design of the current study was unique beyond the fact that it explores insulin resistance in a cohort of young men with OSAS that had previously been unstudied. The exclusion criteria prohibited subjects from participating who were physically active, taking anti-inflammatory medications, smokers, and living with diagnosed cardiovascular and metabolic disorders. Taken together, these criteria provided extraordinary experimental control of factors that typically confound similar studies conducted in

middle-aged adults with OSAS. We would, however, be remiss if not mentioning potential limitations to the current study. First, the presence of OSAS in this cohort was determined by an unsupervised portable PSG test. Portable PSG devices have been validated against the gold standard, in-lab PSG⁴⁰. Second, this study is limited by small sample sizes. Retrospective power analysis of the HOMA-IR, insulin, and leptin revealed that as little as 3 more subjects per group would have yielded adequate power to detect statistically significant differences.

In conclusion, young men with occult OSAS exhibit early signs of altered insulin metabolism, which is yet to manifest insulin resistance. These men demonstrated higher CAF and lower serum adiponectin than both overweight and normal weight controls. These differences may represent initial steps in the pathogenesis of insulin resistance within OSAS. Currently, the majority of OSAS cases are first diagnosed and treated when individuals are in their mid-forties. Our findings reinforce the need for early detection and treatment of OSAS to prevent the onset and progression of chronic metabolic disorders, such as insulin resistance, in these individuals.

TABLES

Table 1 - Subjects Characteristics

Measure	Overweight with OSAS (n=12)	Overweight without OSAS (n=18)	Normal Weight without OSAS (n=15)
Age (yr)	22.8 ± 0.8	22.5 ± 0.7	21.1 ± 0.5
BMI (kg/m ²)	32.4 ± 1.0	31.6 ± 1.1	22.4 ± 0.4 ^a
AHI (events/hr)	25.4 ± 5.4 ^b	2.2 ± 0.3	1.9 ± 0.3
ESS (x/24)	7.8 ± 1.1	8.3 ± 0.8	6.3 ± 0.6
Neck (cm)	40.7 ± 0.6	40.8 ± 0.7	36.4 ± 0.4 ^a
Body Fat (%)	29.8 ± 1.0 ^b	26.2 ± 1.2	18.9 ± 1.2 ^a
Total Fat Mass (kg)	30.6 ± 2.0	26.7 ± 2.1	13.5 ± 1.0 ^a
CAF (kg)	9.1 ± 0.7 ^b	7.3 ± 0.5	3.6 ± 0.3 ^a

Data are presented as mean ± SEM. BMI = Body Mass Index; AHI = Apnea/Hypopnea Index; ESS = Epworth Sleepiness Score; CAF = Central Abdominal Fat ^a = different from both overweight groups (p < 0.05) and ^b = different from both control groups (p < 0.05).

Table 2 – Biochemical Markers of Insulin Resistance

Measure	Overweight with OSAS (n=12)	Overweight without OSAS (n=18)	Normal Weight without OSAS (n=15)
Glucose (mg/dl)	86.8 ± 2.6	93.1 ± 2.3 ^c	85.8 ± 2.1
Insulin (µU/L)	16.9 ± 1.8	12.5 ± 2.3	7.6 ± 1.4 ^d
HOMA-IR	3.6 ± 0.4	3.0 ± 0.6	1.6 ± 0.3 ^a
TNF-α (ng/ml)	1.0 ± 0.1	0.9 ± 0.1	0.9 ± 0.1
IL-6 (ng/ml)	6.5 ± 2.4	6.7 ± 2.2	7.2 ± 1.9
Leptin (ng/ml)	11.9 ± 1.0	9.7 ± 1.4	4.7 ± 0.8 ^a
Adiponectin (µg/ml)	9.9 ± 0.8 ^b	13.9 ± 1.4	13.9 ± 1.4

Data are presented as mean ± SEM. ^a = different from both overweight groups (p < 0.05), ^b = different from both control groups (p < 0.05), and ^c = different from normal weight control group (p < 0.05), ^d = different from overweight with OSAS (p < 0.05) and a trend towards different from overweight control (p = 0.06).

FIGURES

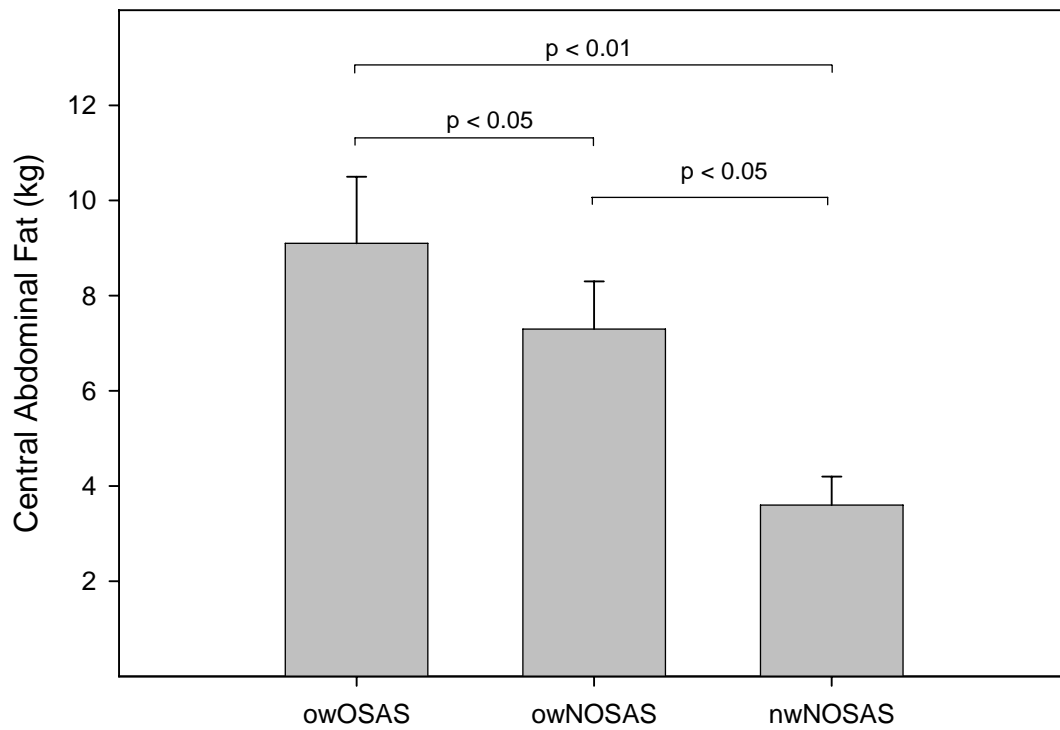


Figure 1 – Central Abdominal Fat, by Group. Overweight individuals with OSAS (owOSAS) had greater amounts of central abdominal fat than both overweight subjects without OSAS (owNOSAS) and normal weight subjects without OSAS (nwNOSAS).

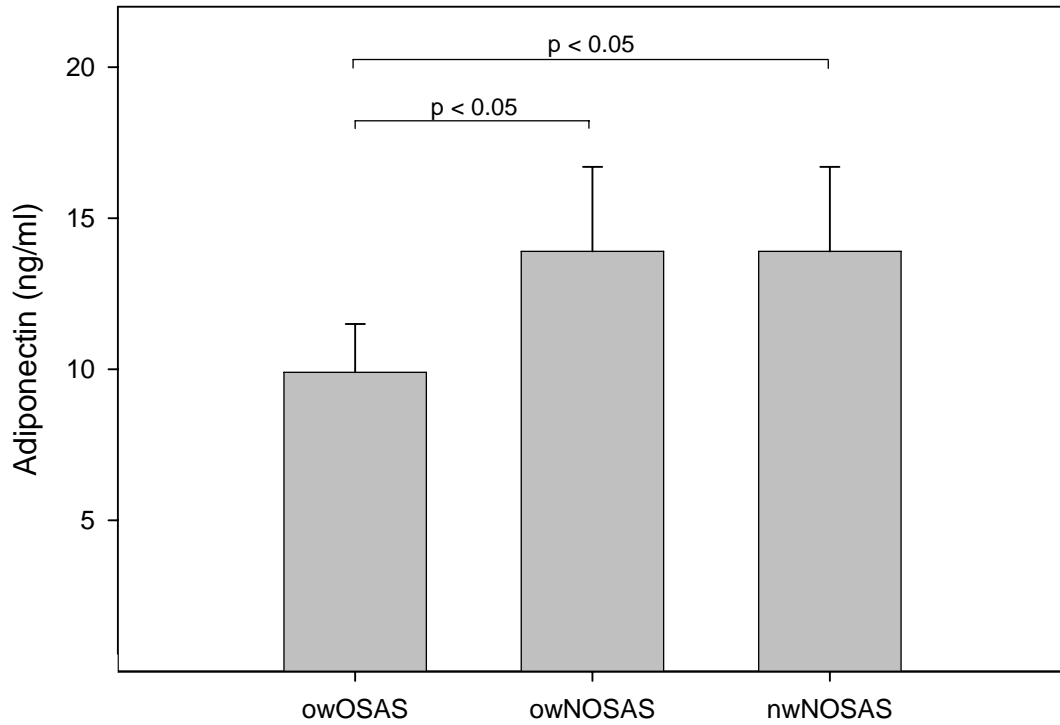


Figure 2 – Serum Adiponectin, by Group. Overweight subjects with OSAS (owOSAS) had lower serum levels of adiponectin than both overweight subjects without OSAS (owNOSAS) and normal weight subjects without OSAS (nwNOSAS).

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Chapter 3

The Influence of Obstructive Sleep Apnea Syndrome on Metabolic Syndrome in Young Men

Abstract

Background: Obstructive sleep apnea syndrome (OSAS) is an obesity-related disorder that may affect as many as 1 in 5 adults. Untreated OSAS is associated with an increased risk for metabolic syndrome (MetS), though it is unclear whether the relationship is independent of central abdominal fat (CAF) and at what age evidence of the development of MetS begins to appear. **Objective:** To determine if young men with occult OSAS exhibit a greater prevalence of MetS and exaggerated levels of individual MetS components in a manner that is independent of obesity. **Subjects:** Forty-seven sedentary young men, age 18-26 years old: 12 overweight with OSAS (BMI = 32.4 ± 1.0 kg/m², AHI = 25.4 ± 5.4 events/hr), 18 overweight without OSAS (BMI = 31.6 ± 1.1 , AHI = 2.2 ± 0.3), and 17 normal weight without OSAS (BMI = 22.4 ± 0.4 , AHI = 1.9 ± 0.3). **Measurements:** Presence and severity of OSAS was determined using an unsupervised, portable polysomnography device. Total and central abdominal fat (CAF) were quantified using dual energy x-ray absorptiometry. Blood pressure was obtained manually via auscultation. Triglycerides, glucose, and HDL-cholesterol were analyzed as part of a commercial lipid profile kit after an overnight fast. **Results:** Subjects with OSAS had 25% more CAF than overweight controls ($p < 0.05$) and higher triglycerides (136.7 ± 21.3 mg/dl vs. 92.9 ± 8.9 , $p = 0.06$). Severity of OSAS was directly related to fasting triglycerides ($r = 0.32$, $p < 0.05$) after partial correlation to remove confounding

effects of CAF. Waist circumference, blood pressure, HDL-cholesterol, glucose, and number of MetS components were similar between both overweight groups. Number of MetS components was directly correlated to indices of adiposity, but not OSAS severity. Using multiple linear regression analysis, waist circumference, triglycerides, and glucose were all independent predictors of OSAS severity. **Conclusion:** Although risk factors have yet to cluster as clinical MetS in young men with OSAS, several unique physiological and anthropometric abnormalities exist in this cohort beyond what is seen in uncomplicated obesity that may indicate early pathogenesis of MetS.

Keywords: obstructive sleep apnea syndrome, metabolic syndrome, central abdominal fat

INTRODUCTION

Obstructive sleep apnea syndrome (OSAS) is a chronic disorder characterized by repetitive collapse of the upper airway during sleep, and may affect as many as 1 in 5 adults¹. OSAS is associated with intermittent periods of hypoxia, hypercapnia, and fragmented sleep, events which increase sympathetic drive. If left untreated, OSAS increases risk for hypertension²⁻⁴, vascular disorders⁵, and metabolic syndrome (MetS)^{6, 7}. MetS is the constellation of traditional cardiovascular risk factors, and definitions of the disorder vary depending on geographical location and threshold for criteria⁸⁻¹¹. The National Cholesterol Education Panel (NCEP)⁸ classification of MetS includes obesity, hypertension, hyperglycemia, hypertriglyceridemia, and dyslipidemia. The NCEP criterion is standard regardless of location, and has been endorsed by the American College of Cardiology and the American Heart Association. The presence of MetS appears to increase cardiovascular disease risk beyond the incidence of individual risk factors alone. Lakka and colleagues reported that men with MetS had as much as a four-fold greater risk of coronary heart disease related mortality, after adjustment for cardiovascular disease risk factors¹².

Previous studies reported a higher prevalence of MetS in middle-aged, overweight adults with OSAS than non-apneic adults of similar age and body habitus. Coughlin et al⁶ reported a prevalence of MetS of 87% in overweight, middle-aged adults with OSAS, as compared to 35% of non-apneic controls of similar body habitus. Furthermore, adults with OSAS were 9 times as likely to have MetS as controls. In a similar study, Gruber et al⁷ demonstrated a higher prevalence of MetS in obese middle-aged adults with OSAS.

In this cohort, 74% of subjects with OSAS and 24% of controls had MetS. Adults with OSAS had a nearly 6-fold higher likelihood of having MetS.

Considerable evidence suggests that OSAS and the development of individual risk factors comprising the MetS are closely related. Obesity presents mechanical challenges to the upper airway, lungs, and diaphragm¹³⁻¹⁵, particularly when the body is supine during sleep¹⁶. Production of fat derived hormones (adipokines) increases in obesity, with many of these adipokines having potent effects on energy expenditure and body weight regulation¹⁷, insulin resistance^{18, 19}, and regulation of the sleep/wake cycle^{20, 21}. Previous studies reported close associations between OSAS and hypertension³, hypertriglyceridemia⁶, hyperglycemia^{22,23}, and dyslipidemia²⁴. However, previous studies are limited in that they typically fail to account for confounding factors (e.g., use of medications and regular participation in physical activity) which are known to alter the MetS risk profile²⁵. Furthermore, no studies to date have examined MetS in a cohort of young men with preclinical OSAS to determine if evidence of MetS exists even at a young age.

Thus, the purpose of this study was to determine if young men with OSAS demonstrated evidence of MetS, and if this relationship is independent of the effects of obesity and physical activity. We hypothesized that sedentary young men with OSAS will have a greater prevalence of MetS and higher levels of MetS components than both overweight and normal weight sedentary controls. Furthermore, we predicted the pattern of MetS risk to be additive within these groups, with the greatest evidence of MetS appearing in overweight subjects with OSAS and the least evidence in normal weight controls. Demonstrating MetS risk in a young cohort of males would have major

implications for early identification and treatment of OSAS, as first diagnosis and treatment of OSAS typically occurs in the fifth decade of life.

METHODS

Participants

Subjects were male volunteers, ages 18-26 years old, who expressed interest by responding to flyers posted in the community, mass electronic mails sent to various university organizations, and a promotional website. Exclusion criteria were: tobacco use within the past 12 months, regular participation in physical activity within the previous six months, diagnosed cardiovascular or metabolic disease, or use of vasoactive, anti-inflammatory, or sympathomimetic medications. Subjects who qualified were placed into one of three groups, based on body mass index (BMI) and presence of OSAS: 1) overweight with OSAS (BMI > 25.0 and AHI > 5.0); 2) overweight without OSAS (BMI > 25.0 and AHI < 5.0); and 3) normal weight without OSAS (BMI < 25.0 and AHI < 5.0). Research protocols and procedures were approved by the Institutional Review Board at Virginia Polytechnic and State University.

OSAS and Daytime Sleepiness

OSAS was assessed using a 5-channel, digital portable polysomnography device (Embletta PDS, Reykjavik, Iceland). Setup, operation, and data generated from this device were described previously²⁶. The device was worn at least 6 hours during one night of sleep. Sleep tests were manually scored by a registered sleep technologist and

confirmed by a sleep physician. OSAS severity was assessed by the apnea/hypopnea index (AHI), defined as the total number of apneas and hypopneas by hours of sleep. Events were scored according to guidelines set forth by the American Society for Sleep Medicine²⁷. Daytime sleepiness, a cardinal symptom of OSAS, was evaluated using the Epworth Sleepiness Scale, an eight-item questionnaire that seeks to determine how sleepy an individual becomes while performing routine daily tasks²⁸.

MetS

MetS was defined using the National Cholesterol Education Panel (NCEP) criteria⁸, which is the presence of at least three of the following risk factors: waist circumference of >102 cm, blood pressure \geq 130/85 mmHg, fasting triglycerides \geq 150 mg/dL, fasting blood glucose \geq 110 mg/dL, and HDL cholesterol < 40 mg/dL. Waist circumference was measured at the smallest point between the iliac crest and the lowest rib, and was taken while the subject was standing after exhalation. Blood pressure was obtained manually via auscultation, after subjects had been seated quietly for at least 5 minutes. A fasting blood sample was obtained between 0600 and 1000 hours using venipuncture at the inner cubital vein. Testing conditions included no food intake for the previous eight hours, at least six hours of sleep on the previous night, and no alcohol intake for the previous 24 hours. Fasting triglycerides, blood glucose, and HDL cholesterol were measured directly using a commercial kit (Cholestech LDX) that employs a combination of standardized enzymatic reactions and solid phase technology.^{29, 30}

Measures of Body Composition

All measures of body composition were obtained while subjects were wearing lightweight clothing and no shoes. Height and weight were measured to determine body mass index (BMI). Neck circumference was measured at the laryngeal prominence, between the midanterior neck and midcervical spine. Total body fat mass was determined using dual energy x-ray absorptiometry (DEXA, Hologic QDR 4500A). Central abdominal fat (CAF) was obtained using the DEXA as described previously³¹. DEXA measurements were obtained and analyzed by one investigator to eliminate inter-observer variability. Weekly scans of an external soft tissue bar were used to insure quality of the DEXA measurements. Test-retest reliability of this unit was previously reported³².

Statistical Analyses

Statistical analyses were performed using SPSS version 15.0 (SPSS Inc., Chicago, IL). Group comparisons were made using one-way analysis of variance. Relationships between MetS components and variables denoting OSAS severity and adiposity were assessed using Pearson product moment of correlation. Relationships between number of MetS components and the same variables were calculated using Spearman correlation. Stepwise linear regression analysis was used to determine if OSAS severity and/or indices of adiposity were independent predictors of each MetS component, and vice versa. Statistical significance was determined *a priori* for all experimental analyses as a p value < 0.05.

RESULTS

Subject Characteristics

Subject characteristics are displayed in Table 1. The AHI for both control groups was < 5.0 , denoting the absence of OSAS. The mean AHI of 25.4 ± 5.4 events/hr in overweight subjects with OSAS was indicative of moderately severe OSAS²⁷. Age and excessive daytime sleepiness scores did not differ between any groups. Neck circumference, BMI, and total fat mass were higher in overweight subjects than normal weight controls ($p < 0.05$); no further differences in these variables were noted between overweight subjects with and without OSAS. However, percent body fat and CAF were higher, on average, in overweight subjects with OSAS than both control groups ($p < 0.05$).

Individual MetS Components

MetS components are displayed in Table 1. There were no differences between groups with regards to HDL cholesterol, systolic blood pressure, and diastolic blood pressure. Fasting blood glucose levels were higher only in the overweight subjects without OSAS ($p < 0.05$). Waist circumference was higher in overweight subjects than normal weight controls ($p < 0.05$), but was not different between overweight subjects with and without OSAS. Triglycerides were higher in subjects with OSAS than both control groups ($p < 0.05$, Figure 1). After controlling for the influence of CAF, OSAS severity was correlated only with triglyceride levels ($r = 0.37$, $p < 0.05$). CAF was

correlated with triglycerides ($r = 0.34$, $p < 0.05$). However, after adjustment for the severity of OSAS, this relationship dissipated.

Presence of MetS

Both overweight groups met more criteria of the MetS than normal weight controls ($p < 0.05$). No further differences in the average number of components were noted between the overweight subjects with and without OSAS. OSAS severity did not correlate with overall number of MetS components. However, CAF was correlated with number of MetS components ($r = 0.42$, $p < 0.01$). In all, 33% of overweight subjects with OSAS met the NCEP criteria for MetS, as compared to 28% of overweight controls and 12% of normal weight controls. Furthermore, 25% of subjects with OSAS exceeded the threshold for 4 MetS components, as compared to 11% of the overweight controls. None of the normal weight group exhibited four MetS risk factors.

Independent Predictors of MetS Components and OSAS Severity

Stepwise multiple linear regression analysis was used to determine which MetS components were independent predictors of OSAS severity. Variables entered into the model were: waist circumference, systolic blood pressure, diastolic blood pressure, triglycerides, HDL cholesterol, and glucose. Triglycerides ($\beta = 0.46$, $p < 0.01$) were the only independent predictor of AHI. An additional model was created to determine if AHI and/or indices of adiposity were independent predictors of any of the individual MetS components. Waist circumference was excluded, as it is considered an index of adiposity itself. Variables entered into this model were AHI, BMI, CAF, and neck circumference.

None of the variables were significant predictors of systolic blood pressure or diastolic blood pressure. AHI was the only independent predictor of triglycerides ($\beta = 0.46$, $p < 0.01$). Neck circumference ($\beta = 0.86$, $p < 0.05$) and CAF ($\beta = - 0.61$, $p < 0.01$) were predictors of glucose. CAF was the only independent predictor of HDL cholesterol ($\beta = - 0.36$, $p < 0.05$)

DISCUSSION

The current study is the first to examine the presence and components of MetS in a younger, sedentary cohort of men with preclinical OSAS. As previously reported by our laboratory³³, subjects with OSAS had more CAF than either control groups. Although both overweight groups had similar BMI, neck circumference, and overall fat mass, subjects with OSAS had nearly 25% more CAF than controls ($p < 0.05$). Fasting triglycerides were only higher in subjects with OSAS, and OSAS severity was the only independent predictor of triglyceride levels. Taken together, these findings suggest that young, overweight men with occult OSAS exhibit several unique characteristics that likely increase future risk for MetS.

Several previous investigations reported a greater distribution of fat within the abdomen in middle-aged, overweight adults with OSAS than controls of similar body weight. Vgontzas²² and Shinohara¹⁴ both found visceral fat over 50% higher in overweight subjects with OSAS than controls matched for total and subcutaneous fat. Furthermore, these studies demonstrated correlations between OSAS severity and central fat. The findings from the present study support Vgontzas and Shinohara, and reports

both elevated CAF in subjects with OSAS (\uparrow 25% vs. overweight controls, $p < 0.05$) and correlations between CAF and the severity of OSAS. Increased CAF in OSAS is likely the end result of chronically heightened sympathetic drive³⁴, which causes the production and release of stress hormones, such as cortisol, that stimulate the storage of fat in the abdominal region³⁵. This depot of fat storage likely contributes to the development and progression of chronic cardiovascular and metabolic disorders within OSAS.

Beyond the association between excessive central adiposity and OSAS, previous research reported evidence of other risk factors exceeding the clinical threshold for MetS in middle-aged adults with OSAS. Considerable evidence suggests an independent relationship between hypertension and OSAS. Results from the Wisconsin Sleep Cohort demonstrated that middle-aged adults with moderate OSAS had nearly three fold the risk of having hypertension as their non-apneic counterparts³. Middle-aged adults with OSAS have as much as 20% higher levels of fasting blood glucose than controls of similar body weight²². Additional studies have found both reductions in circulating HDL cholesterol in OSAS and an inverse relationship between OSAS severity and HDL cholesterol levels⁶. The current study did not report mean group differences in blood pressure, HDL cholesterol, fasting glucose between the overweight groups with vs. without OSAS. These results do not support previous findings in middle-aged adults. Future studies should be conducted to determine at what age abnormalities in these MetS components typically begin to appear.

Although these blood pressure, HDL cholesterol, and glucose were not affected by the presence of OSAS, triglycerides were higher only in the group with OSAS. These findings support Ip and colleagues³⁶, who reported triglycerides 40% higher in

overweight, middle-aged adults with OSAS than overweight controls. Furthermore, the current study demonstrated that triglycerides were directly correlated with the severity of OSAS, even after adjustment for CAF. A similar correlation existed between triglycerides and CAF. Once adjusted for the severity of OSAS, however, the relationship dissipated. Taken together, these findings suggest that the presence of OSAS is directly related to elevations in triglyceride levels in a manner that is independent of obesity. Many mechanisms linking OSAS and hypertriglyceridemia remain unclear. However, Li and colleagues³⁷ recently used animal models to demonstrate that intermittent hypoxia increases triglyceride synthesis via an upregulation of both a transcription factor needed for the synthesis of lipids (sterol regulatory element binding protein) and an enzyme necessary for the hepatic production of triglycerides (stearyl coenzyme A desaturase). Upregulation of these factors resulted in increased synthesis and hepatic release of triglycerides.

In addition to alterations in individual components of the MetS, previous research demonstrated the constellation of risk factors indicative of clinical MetS within middle-aged adults with OSAS. Coughlin⁶ and Gruber⁷ reported the prevalence of MetS exceeded 70%, with overweight adults with OSAS having as much as a nine-fold greater risk of having MetS as overweight controls. In the present study, similar percentages of overweight subjects with and without OSAS had MetS (33% and 28%, respectively). Our findings do not support those of Coughlin and Gruber, both in regards to the magnitude of the prevalence as well as higher prevalence in subjects with OSAS. The differences between our findings and previous investigations are likely a reflection of the younger age of subjects in the current study. However, the higher CAF and triglycerides

suggest that increased risk for future cardiometabolic disorders exists in these young men, though it is yet to project in the clustering pattern indicative of MetS.

Many mechanisms responsible for the relationship between OSAS and MetS in middle-aged adults remain unclear. A likely scenario, as previously suggested by Chasens³⁴, involves the multifaceted effects of repetitive activation of the sympathetic nervous system which occurs during OSAS in an attempt to re-establish normal breathing. One consequence of this response is activation of the hypothalamic-pituitary-adrenal axis, which initiates release of stress hormones norepinephrine, epinephrine, and cortisol³⁸. Cortisol stimulates storage of adipose tissue around the abdomen³⁵, itself a component of the MetS, which contributes to the development of other MetS components. These stress hormones have potent systemic effects, namely causing elevations in blood pressure and glucose. As sympathetic drive continues over time, particularly as severity of OSAS increases, acute elevations in glucose may become chronic and induce insulin resistance³⁴. Insulin resistance contributes to the development of hypertension³⁹, and coupled with the autonomic dysfunction resulting from increased sympathetic drive, may help partially explain the increasing body of evidence that suggests an independent relationship between OSAS and hypertension. Taken together, the numerous regional and systemic consequences of increased sympathetic drive likely contribute to the constellation of MetS components often seen in middle-aged adults with OSAS.

The present study does not report elevations in either blood pressure or glucose in young men with latent OSAS. Triglycerides and CAF, however, were both higher in those subjects with OSAS, and triglycerides were independent predictors of OSAS

severity. The temporal etiology of MetS is still largely unknown. The findings from this study may represent initial steps in the pathogenesis of MetS that precede clinical presentation of the disorder. This hypothesis is supported by Morrison and colleagues⁴⁰, who found that central obesity and triglyceride levels in adolescents were predictors of early adulthood onset of MetS. Additional evidence examining the early development of MetS was reported by Fukuchi and colleagues⁴¹, who used basic animal models to observe metabolic changes associated with dietary-induced obesity. Their findings demonstrated that increases in visceral fat and triglycerides preceded any changes in either insulin or glucose, further supporting the role of central fat and triglycerides in the early development of metabolic disorders. Additional longitudinal research should be conducted to better understand the temporal etiology of MetS, particularly within the domain of OSAS.

There were several potential limitations for this study. OSAS was diagnosed using a portable, unsupervised device. Although portable devices lack certain parameters of full polysomnography (e.g. electroencephalograph), estimates of OSAS severity between the two modalities correlate moderately well ($r = 0.62, p < 0.001$)⁴² and portable devices are able to consistently identify those individuals with moderate OSAS. Also, CAF was quantified using DEXA, a two dimensional analysis which is unable to distinguish visceral from subcutaneous fat. However, previous studies demonstrated high correlations between CAF quantified via DEXA and visceral fat measured using computed tomography⁴³.

Overall, the findings of this study suggest that several unique physiological and anthropometric changes indicative of MetS risk exist in young men. Although these

abnormalities likely represent the early stages of MetS pathogenesis, the changes in this population have yet to cluster in a pattern typical of MetS beyond what is seen in uncomplicated obesity. Thus, identification and treatment of OSAS at this time in life may be critical to prevent the further development of the MetS.

TABLES

Table 1 – Subject Characteristics

Measure	Overweight with OSAS (n = 12)	Overweight without OSAS (n = 15)	Normal Weight without OSAS (n = 15)
Age (yr)	22.8 ± 0.8	22.2 ± 0.8	21.1 ± 0.6
AHI (events/hr)	25.4 ± 5.4 ^a	2.0 ± 0.3	2.0 ± 0.3
BMI (kg/m²)	32.4 ± 1.0	32.3 ± 1.3	22.4 ± 0.3 ^b
CAF (kg)	9.1 ± 0.7 ^a	7.6 ± 0.6	3.4 ± 0.3 ^b
Waist Circumference (cm)	101.9 ± 2.2	97.9 ± 3.0	78.3 ± 1.6 ^b
Triglycerides (mg/dL)	136.7 ± 21.3 ^a	92.9 ± 8.8	79.3 ± 11.7
Systolic BP (mmHg)	124.2 ± 2.6	122.8 ± 3.3	120.9 ± 2.5
Diastolic BP (mmHg)	84.7 ± 1.5	86.8 ± 2.4	80.4 ± 1.5
HDL Cholesterol (mg/dL)	34.1 ± 2.8	37.7 ± 3.1	40.5 ± 3.6
Glucose (mg/dL)	86.8 ± 2.6	94.5 ± 2.4 ^c	85.3 ± 2.9

Data are presented as mean ± SEM. AHI = Apnea/hypopnea Index; CAF = Central Abdominal Fat. ^a = different from both control groups (p < 0.05), ^b = different from both overweight groups (p < 0.05), ^c = different from normal weight control group (p < 0.05).

FIGURES

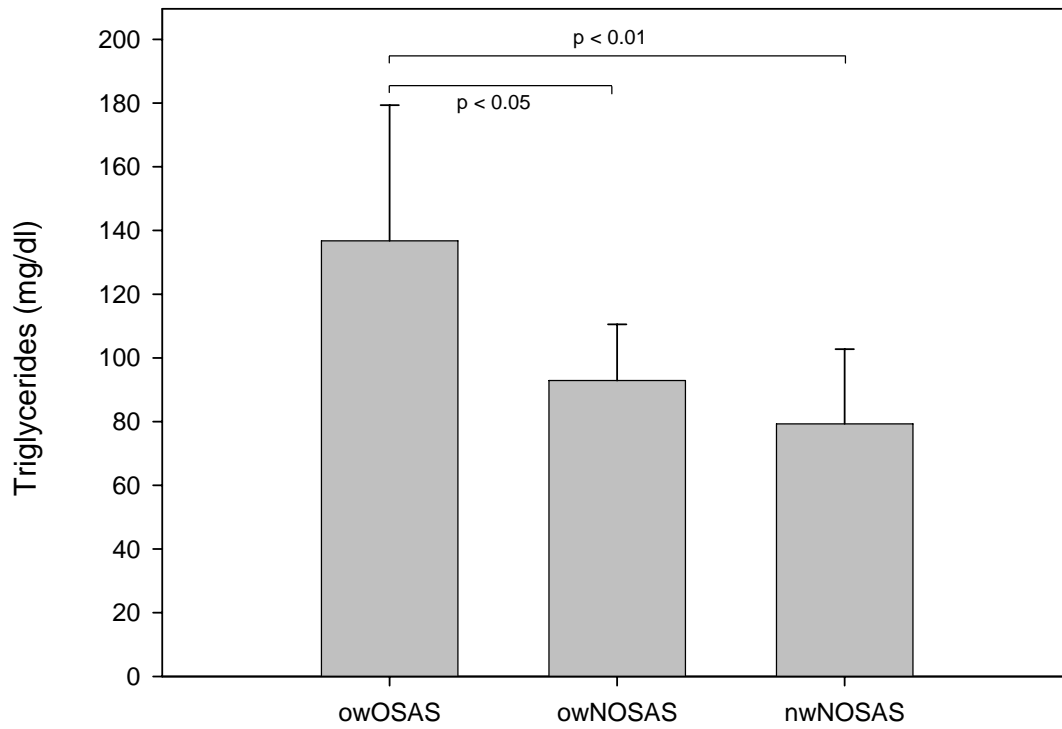


Figure 1 – Fasting Triglycerides, by Group. Overweight subjects with OSAS (owOSAS) had higher fasting triglycerides than both overweight subjects without OSAS (owNOSAS) and normal weight subjects without OSAS (nwNOSAS).

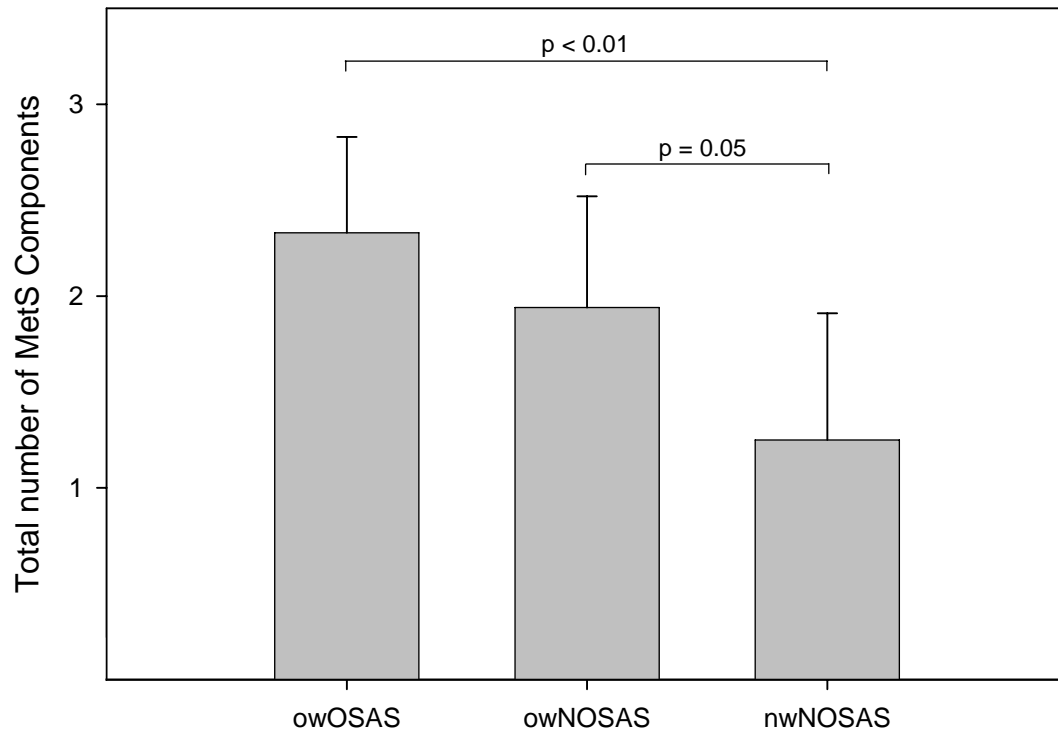


Figure 2 – Total Number of MetS Components, by Group. The total number of MetS components, on average, was higher in both overweight groups than normal weight controls.

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Chapter 4

Moderate Obstructive Sleep Apnea Syndrome and Endothelial Function in Young

Men

Abstract

Background: Obstructive sleep apnea syndrome (OSAS), an obesity-related chronic disorder affecting as many as 1 in 5 adults, heralds increased risk for hypertension. However, it is unclear how early in life evidence of vascular endothelial dysfunction (VED), a key initial step in the pathogenesis of hypertension, begins to appear and to what degree this relationship may depend on adiposity vs. OSAS. **Objective:** To determine if young men with OSAS exhibit impaired endothelium-dependent vasodilatation and/or an upregulation of cytokines indicative of VED beyond what is seen in obesity alone. **Subjects:** 12 overweight men with OSAS (BMI = 32.4 ± 1.0 kg/m², AHI = 25.4 ± 5.4), 18 overweight without OSAS (BMI = 31.6 ± 1.1 kg/m², AHI = 2.2 ± 0.3), and 16 normal weight without OSAS (BMI = 22.4 ± 0.4 kg/m², AHI = 1.9 ± 0.3). **Measurements:** Severity of OSAS was measured using portable polysomnography. Endothelium-dependent vasodilatation was assessed by venous occlusion plethysmography, which quantifies forearm reactive hyperemia after 5 minutes of upper arm occlusion. Total and central abdominal fat (CAF) were measured using dual energy x-ray absorptiometry. Serum vascular endothelial growth factor (VEGF), asymmetric dimethylarginine (ADMA), and C - reactive protein (CRP) were analyzed after a 10-hour overnight fast. Expression of VEGF Receptor 2 (VEGFR2) was assessed using cell staining from whole blood and flow cytometry gated to examine the monocyte

population. **Results:** Reactive hyperemia did not differ between groups at any time during the 2-minute recovery period following ischemia. VEGF was higher in both overweight groups than the normal weight group ($p < 0.05$). CRP was higher in overweight subjects without OSAS ($p < 0.05$) and tended to be higher in overweight subjects with OSAS ($p = 0.08$) than normal weight controls. However, neither CRP nor VEGF was further elevated in subjects with OSAS than overweight controls. ADMA was similar in all study groups. **Conclusion:** Young men with OSAS do not exhibit functional or biochemical evidence of VED beyond what is seen in uncomplicated obesity. Future studies should be conducted to determine at which age evidence of VED begins to appear in OSAS.

Keywords: obstructive sleep apnea syndrome, vascular endothelial dysfunction, reactive hyperemia, VEGF, C-reactive protein, ADMA

INTRODUCTION

Obstructive sleep apnea syndrome (OSAS), a chronic disorder affecting as many as 1 in 5 adults, is characterized by repetitive collapse of the upper airway during sleep¹. OSAS is associated with intermittent periods of hypoxia, hypercapnia, and fragmented sleep, with these events occurring repetitively during the night to activate the sympathetic nervous system and re-establish normal breathing^{1, 2}. Recent evidence suggests that untreated OSAS increases risk for chronic cardiovascular diseases, such as hypertension. Peppard and colleagues³ reported a dose-response relationship between OSAS severity and risk ratio for hypertension, with moderate OSAS conferring a nearly 3-fold higher risk of hypertension. Likely mechanisms linking OSAS and hypertension include functional alterations in the vascular endothelium induced by intermittent hypoxia and an increase in sympathetic drive which, over time, may lead to autonomic and vascular endothelial dysfunction (VED)⁴.

VED represents an early stage in the etiology of hypertension, and typically precedes clinical presentation of structural changes in vessel walls⁵. Previous investigations demonstrated that VED has prognostic ability for the future risk of cardiovascular outcomes and hypertension^{6, 7}. VED is typically assessed by endothelium-dependent vasodilatation, commonly measured after either infusion of vasoactive drugs or occlusion of peripheral vessels^{8, 9}. Using these techniques, previous investigations demonstrated evidence of VED in middle-aged adults with OSAS. Oflaz¹⁰ employed drug infusion and brachial artery ultrasound to quantify endothelial function in OSAS patients vs. non-apneic controls; they reported 20% lower flow-mediated dilatation in

evening hours for the OSAS group. This group difference was markedly increased when measurements were obtained immediately upon waking (\downarrow 40% in OSAS), suggesting an important role for the physiological consequences of OSAS in impairing vascular function. Imadojemu¹¹ examined endothelial function in OSAS using postocclusive reactive hyperemia, reporting that peak blood flow after a 10-minute period of ischemia was blunted nearly 30% in individuals with OSAS. Peak blood flow increased over 20% after subjects with OSAS were treated with nasal continuous positive airway pressure (nCPAP) for more than two weeks, further supporting the role of latent OSAS on VED.

In addition to functional assessments, several biochemical markers may help further quantify early damage to the vascular endothelium. C-reactive protein (CRP) is an acute phase protein produced by the liver, and is a biomarker of inflammation⁵. Inflammation plays a key role in VED, and is initiated in response to the upregulation of adhesion molecules and prothrombotic cytokines¹². Elevations in CRP are typically reported in sustained hypoxia¹³. To this end, previous investigations sought to determine if CRP is elevated in middle-aged adults with OSAS, a condition characterized by intermittent bouts of hypoxia during sleep. Shamsuzzaman¹⁴ demonstrated that plasma CRP was directly correlated with severity of OSAS and that average values were nearly three-fold higher than in a group of controls. CRP concentrations were reported twice as high in OSAS patients compared to those with cardiovascular disease, a condition traditionally associated with elevations in CRP¹⁵. Although these studies suggest a close association between OSAS and CRP, other evidence indicates that the connection is fundamentally related to adiposity^{16, 17}.

Vascular endothelial growth factor (VEGF) is a signaling protein produced primarily by vascular smooth muscle cells that facilitates the formation of new blood vessels and contributes to the production of nitric oxide (NO)¹⁸. Elevations in VEGF were reported in atherosclerosis¹⁹, and are of particular interest in OSAS, as hypoxia is the major factor that induces production of VEGF²⁰. While some investigations yielded evidence of a close association between elevated VEGF and OSAS severity²¹⁻²⁵, others have not demonstrated such a relationship²⁶⁻²⁸. Previous studies of VEGF are limited in that they have failed to account for variations in VEGFR2, the receptor specific for signal transduction involving angiogenesis and regulation of NO²⁹⁻³¹. Quantification of this receptor may lend further insight on mechanisms by which OSAS and VED may be related.

Asymmetric dimethylarginine (ADMA) is an emerging biochemical marker of VED, and effectively inhibits the formation of NO from L-arginine³². Recent investigations demonstrated elevations in ADMA in diabetes, dyslipidemia, hypertension, and coronary artery disease³³. Elevated ADMA may be partially responsible for VED typical of OSAS, as previous studies demonstrated attenuated levels of endothelial nitric oxide synthase (eNOS) and NO in OSAS^{34, 35}. One study reported on plasma ADMA in middle-aged adults with OSAS, finding that 4 weeks of nCPAP treatment was able to significantly lower ADMA³⁶. However, this study is limited in that no additional study groups were included to control for the potential effects of confounding factors, such as obesity and blood lipids.

Previous investigations reported functional and biochemical evidence of VED in middle-aged adults with OSAS. However, no studies to date have sought to determine if

evidence of VED also holds true in younger adults with occult OSAS. Few studies have carefully controlled for factors known to affect vascular function and future risk for hypertension (e.g., obesity and regular participation in physical activity). Thus, the purpose of the current study was to address these limitations in previous research and determine if sedentary, young men with occult OSAS exhibit impaired endothelium-dependent vasodilatation and/or an upregulation of cytokines indicative of VED beyond what is seen in obesity alone. We hypothesized that subjects with OSAS would demonstrate a blunted postocclusive reactive hyperemia, in addition to an upregulation of cytokines that signify early evidence of VED.

METHODS

Subjects

Subjects were males, aged 18-26 years old, who volunteered in response to flyers posted in the community, newspaper articles, and mass electronic mails sent to various university organizations. Exclusion criteria were: (a) use of tobacco products within the past year; (b) participation in physical activity over the previous six months; (c) diagnosed or treated cardiovascular or metabolic disease; and (d) regular use of vasoactive, anti-inflammatory, or sympathomimetic prescription medications. Subjects who qualified for the study were placed into one of three groups, based on body mass index and presence of OSAS determined via portable, in-home polysomnography: 1) overweight with OSAS (BMI > 25.0 kg/m² and AHI > 5.0); 2) overweight without OSAS (BMI > 25.0 kg/m² and AHI < 5.0); and 3) normal weight without OSAS (BMI < 25.0

kg/m² and AHI < 5.0). Research procedures and protocols were approved by the Institutional Review Board for projects involving human subjects at Virginia Polytechnic and State University.

OSAS and daytime sleepiness

OSAS was assessed using a 5-channel, digital home polysomnography device (Embletta PDS, Reykjavik, Iceland). The device measures airflow, snoring, heart rate, oxygen saturation, and breathing effort by the thorax and abdomen. The setup and operation of this device has been described previously³⁷. Data were manually scored by a registered sleep technologist and confirmed by a certified sleep physician. OSAS severity was assessed using the apnea/hypopnea index (AHI), which was the total number of apneas and hypopneas per hour of sleep. Excessive daytime sleepiness, a cardinal symptom of OSAS, was quantified using the Epworth Sleepiness Scale, an 8-item questionnaire designed to determine how sleepy an individual becomes performing routine daily tasks.

Anthropometric measures

All anthropometric measurements were obtained while subjects were wearing lightweight exercise clothing and socks. Subjects were stratified into groups based on body mass index (BMI), which was body weight in kilograms divided by height in meters squared. Neck circumference was measured at the laryngeal prominence, between the midanterior neck and midcervical spine. Overall and central abdominal fat (CAF) were assessed using dual energy x-ray absorptiometry (DEXA) scans (Hologic QDR 4500A, Bedford, MA). All DEXA measurements were obtained and analyzed by one licensed investigator

to eliminate inter-observer variability, and weekly scans of a soft tissue bar were conducted to maintain sensitivity of the device. The reliability of this instrument has been previously reported³⁸.

Vascular function

Subjects reported for measurement of vascular endothelial function and biochemical markers having slept at least 6 hours, abstained from food for the previous 8 hours, and abstained from alcohol or caffeine for the prior 24 hours. Reactive hyperemia procedures have been described previously³⁹. Before testing, subjects rested quietly in a supine position for 10 minutes. After the quiet rest period, 10 consecutive measurements of resting forearm blood inflow were obtained via strain gauge plethysmography (Hokanson EC 10, Bellevue, WA). Slopes indicative of blood flow were scored manually using proprietary software (NIVP3 version 2.9, Hokanson, Bellevue, WA), with the median 5 values being averaged to generate resting inflow. After baseline testing, forearm blood flow was occluded for 5 minutes by an automated, pressurized cuff placed over the upper arm inflated to a suprasystolic pressure. Postocclusive reactive hyperemia, or return of blood flow to the ischemic tissue, was quantified 0, 30, 60, 90, and 120 seconds after release of the pressurized cuff.

Biochemical measures

Fasting blood samples were obtained via cubital venipuncture by a board certified physician between 6:00 and 10:00 AM. After blood sampling, plasma and serum were separated via centrifugation and frozen at -80°C for batch analysis after all subjects had

completed the experimental protocol. CRP and VEGF were quantified via ELISA (SearchLight, Pierce Biotechnology, Rockford, Ill.), with CVs being 10.4% and 11.4%, respectively. ADMA was measured using ELISA (DLD Diagnostika GmbH, Hamburg, Germany), with the CV for this biomarker being 8.7%.

VEGFR2 Expression

VEGFR2 was quantified by cell staining and flow cytometry procedures. Briefly, whole blood was suspended in blocking buffer and stained using a primary antibody to bind VEGFR2 (Flk-1 mouse anti-human monoclonal IgG₁, Santa Cruz Biotech, Santa Cruz CA). Optimal dilution of the primary antibody was 1:100. An additional fluorescent tagging protein was added to bind the VEGFR2: primary antibody complex (Goat F(ab)₂ anti-mouse IgG₁ FITC, Southern Biotech, Birmingham AL). Optimal dilution of the secondary antibody was 1:100. Finally, red blood cells were lysed using a commercial kit (Immunolyse whole blood lysing reagent kit, Beckman Coulter, Fullerton, CA). Peripheral blood mononuclear cells were suspended in phosphate buffered saline, refrigerated, and analyzed within 24 hours. Percentage of monocytes expressing VEGFR2 was determined using fluorescent-activated cell sorting analysis (Fisher Scientific, Pittsburgh, PA), with the viable cell gate being narrowed to identify expression of VEGFR2 in monocytes.

Statistical Analyses

Statistical analyses were performed using SPSS version 15.0 (SPSS Inc., Chicago, IL). One-way analysis of variance (ANOVA) was used to make group comparisons of VEGF,

CRP, and ADMA. Repeated-measures, two-way ANOVA was used to determine the effects of group, time, and interactions on the reactive hyperemia blood flow responses. Relationships between VED and both OSAS severity and adiposity were assessed using Pearson product moment of correlation. Multiple linear regression analysis was used to determine which variables were best predictors of OSAS severity (independent variables included initial blood flow response to reactive hyperemia, BMI, CAF, and neck circumference) and initial blood flow response to reactive hyperemia (independent variables included OSAS severity, BMI, CAF, and neck circumference). Statistical significance was determined *a priori* for all experimental analyses as a p value < 0.05.

RESULTS

Subject Characteristics

Subject characteristics are in Table 1. There were no between-group differences in age and excessive daytime sleepiness. Neither overweight nor normal weight controls had an AHI > 5.0. Subjects with OSAS had an AHI that was indicative of moderate disease severity, as defined by the American Society of Sleep Medicine⁴⁰. Overweight subjects with OSAS had higher CAF than overweight controls ($p < 0.05$), despite no differences in BMI and overall fat mass between these groups ($p = 0.32$ and $p = 0.23$, respectively).

Endothelial Function

Postocclusive reactive hyperemia responses did not differ between groups (Figure 1). Across all subjects, severity of OSAS was directly correlated to initial (0 sec; $r = 0.32$, p

< 0.05), 30 second ($r = 0.32$, $p < 0.05$), 60 second ($r = 0.36$, $p < 0.01$), and 90 second ($r = 0.38$, $p < 0.01$) blood flow after initial reperfusion. However, when partially correlated to remove the potential confounding effects of fat mass, only the relationship between AHI and 90 second blood flow remained significant ($r = 0.33$, $p < 0.05$). Reactive hyperemia was not correlated to total fat or CAF. Linear regression analysis revealed that OSAS severity was the only significant predictor of initial blood flow response to reactive hyperemia ($\beta = 0.32$, $p < 0.05$). Initial blood flow response to reactive hyperemia and CAF were both independent predictor of OSAS severity ($\beta = 0.30$, $p < 0.05$ and $\beta = 0.44$, $p < 0.01$, respectively).

Biochemical Measures

CRP was higher in overweight controls ($p < 0.01$, Figure 2) and tended to be higher in subjects with OSAS ($p = 0.08$) than normal weight controls. However, serum CRP was similar in overweight subjects with and without OSAS. CAF was moderately correlated to serum CRP when the potential confounding effects of OSAS severity were removed ($r = 0.46$, $p < 0.05$). Serum VEGF and the expression of VEGFR2 are displayed in Table 2. VEGF was higher in both overweight groups than the normal weight control group ($p < 0.05$, Figure 3). However, VEGF was not higher in overweight subjects with OSAS than overweight controls. ADMA was similar in subjects with OSAS ($0.53 \pm 0.03 \mu\text{mol/L}$), overweight controls ($0.50 \pm 0.04 \mu\text{mol/L}$), and normal weight controls ($0.53 \pm 0.03 \mu\text{mol/L}$). OSAS severity was not correlated to VEGF, VEGFR2, ADMA, or CRP.

DISCUSSION

The current study represents the first to examine both functional and biochemical indices of VED in young men with preclinical OSAS. The major finding of this study is that obesity, not severity of OSAS, is associated with increased evidence of VED. Although CAF, as previously reported⁴¹, was 25% higher in subjects with OSAS than overweight controls of similar BMI, no further differences existed between these groups in regards to postocclusive reactive hyperemia or biochemical markers of VED. However, serum VEGF was higher in both overweight groups than normal weight controls ($p < 0.05$). CRP was higher in overweight controls ($p < 0.01$) and tended to be higher in subjects with OSAS ($p = 0.08$) than in normal weight controls. Taken together, these findings suggest that obesity, not the presence of OSAS, increases evidence of VED in young men. The lack of further influence of OSAS may be due to the fact that these subjects are younger and have likely been living with OSAS for a shorter time than their older untreated apneic counterparts who, in previous investigations, have been reported to exhibit VED.

Only one study to date has employed reactive hyperemia without drug infusion to examine VED in OSAS. Imadojemu and colleagues¹¹ used this technique in middle-aged adults, finding that postocclusive blood flow response was blunted 30% in subjects with OSAS, as compared to non-apneic controls of similar age and body habitus. Blood flow remained significantly lower in subjects with OSAS than controls for the first 60 seconds after initial return of blood flow to the forearm. The findings from the present study do not agree, as blood flow was not different between any of the groups over the two minute recovery period following 5 minutes of upper arm occlusion. This is potentially due to

the fact that subjects in this study had less severe disease and were likely at an earlier stage in the pathogenesis of OSAS than those in previous investigations.

In addition to functional measurements, several biomarkers exist that further quantify VED. CRP, a systemic marker of inflammation, was identified as a risk factor for cardiovascular disease, as atherosclerosis is associated with chronic inflammation of the vascular wall. Studies examining CRP in middle-aged adults with OSAS have been equivocal. Shamsuzzaman¹⁴ reported CRP three-fold higher in OSAS than controls matched for age and body habitus. Furthermore, they found only moderate correlations between CRP and OSAS severity ($r = 0.55$, $p < 0.01$). Punjabi and colleagues⁴² recently reported similar findings, with a linear relationship between CRP and severity of OSAS, after findings were statistically adjusted for age and adiposity. However, Ryan et al¹⁷ presented evidence that this relationship may be largely attributed to the presence of obesity. The findings from the present study support those of Ryan¹⁷, as serum CRP was not different between overweight subjects with and without OSAS. Furthermore, CRP was directly related to the amount of total fat and CAF, but not the severity of OSAS. These findings suggest that obesity, not OSAS, is associated with chronic inflammation in young men.

Previous investigations of VEGF in middle-aged adults with OSAS have been conflicting. Recently, Pedel²⁷ reported that age, not presence and severity of OSAS, predicts VEGF in middle-aged and older adults. An investigation by Teramoto²⁵ suggested the contrary, as morning and evening serum concentrations of VEGF were nearly 3-fold higher in subjects with OSAS compared to non-apneic controls of similar age and body habitus. Nighttime administration of oxygen alone restored VEGF to

values comparable to non-apneic controls, suggesting a primary role of intermittent hypoxemia in the upregulation of VEGF in OSAS. The findings from the current study contrast previous studies that report a relationship between OSAS and VEGF, and may be due to the fact that subjects with OSAS did not exhibit differences in average or lowest oxygen saturation. Future investigations should compare VEGF in young men with OSAS that exhibit reductions in oxygen saturation to OSAS not complicated by hypoxia.

This study is the first to date to investigate the role of VEGFR2 in OSAS, a condition associated with intermittent bouts of hypoxia and hypercapnia during sleep. Previous investigations reported a downregulation of this receptor associated with hypoxia. Nilsson⁴³ used a basic animal model to demonstrate that hypoxia markedly decreased transcripts and expression of VEGFR2. The findings from the present study do not agree with those of Nilsson, and are likely due to limitations imposed by our small sample size. VEGFR2 analysis was only available for a subset of our subjects. Although expression of VEGFR2 in OSAS was numerically lower than both overweight and normal weight controls (20% and 40%, respectively), differences in expression were not statistically significant. Future investigations should seek to quantify the expression of VEGFR2 in OSAS that is accompanied by severe hypoxia to determine if variations in this receptor may contribute to VED in this disorder. In addition, more accurate assessments of VEGFR2 can be made if the receptor is analyzed to determine absolute expression by both monocytes and the whole white blood cell population.

ADMA, an inhibitor of the production of NO, was reported in one previous investigation of OSAS. Ohike and colleagues³⁶ demonstrated that 4 weeks treatment with nCPAP decreased plasma ADMA over 30% in middle-aged adults with OSAS.

However, their study was limited in that no control groups were included to determine if plasma ADMA was elevated above what would be seen in non-apneic, middle-aged adults with similar comorbidities. The current study improves on the design of Ohike's investigation, as groups were carefully controlled for activity levels, absence of chronic cardiovascular and metabolic disorders, and lack of regular use of vasoactive medications. The results of the current study indicate that serum ADMA in young men does not differ on the basis of either the presence of OSAS or obesity. Similar, well-controlled investigations should be conducted using middle-aged subjects to determine if ADMA is related to the presence of OSAS in later life.

Many mechanisms linking OSAS and VED remain unclear. However, considerable evidence suggests that the relationship is likely dependent, at least in part, on hypoxia. Hypoxia is the primary stimulus for the release of VEGF²⁰. The upregulation of VEGF has been suggested to promote the clustering of endothelial cells and formation of vascular lesions⁴⁴. Hypoxia upregulates the production of inflammatory cytokines, such as CRP, that act as chemoattractants for adhesion molecules¹³. Furthermore, hypoxia attenuates the production of NO and expression of eNOS⁴⁵, both of which are known to contribute to the development of VED¹². The design of the current study was ideal to detect evidence of VED. Postocclusive reactive hyperemia is an index of endothelium-dependent vasodilatation, the expansion of blood vessels that is directly influenced by the bioactivity of NO⁴⁶. The array of cytokines measured encompassed inflammatory processes (CRP), inhibition of NO production (ADMA), and endothelial cell clustering (VEGF). The findings of this study did not provide evidence of VED in young men with OSAS, and are likely due to either the absence of hypoxemia in subjects

with OSAS or that the clinical pathogenesis of hypertension has simply yet to begin at this age.

Several design and methodological features make the current study unique. In addition to examining a cohort of young men with OSAS that have been previously unstudied (18 – 26 years old), subjects were all sedentary, non-smokers who were free from chronic cardiovascular and metabolic disorders and who were not taking any vasoactive medications. Thus, this investigation represents a well controlled examination of both functional and biochemical evidence of endothelial dysfunction in young men with OSAS. However, there are a few specific limitations in this study that deserve clarification. Subjects with OSAS were not complicated by hypoxemia during the night. Hypoxia is known to upregulate production of VEGF and attenuate production of NO, suggesting hypoxia may be a critical component of OSAS that leads to VED. Also, measurement of endothelial-dependent vasodilatation was conducted using a 5-minute period of ischemia. While previous investigations have used blood flow response to this period of occlusion to examine VED^{47, 48}, recent evidence suggests that maximum blood flow continues to decrease as upper arm occlusion time approaches 10 minutes⁴⁹. However, a study conducted by Higashi and colleagues⁸ demonstrates that reactive hyperemia following a 5-minute period of occlusion correlates strongly with blood flow responses to intravenous injection of acetylcholine ($r = 0.91$, $p < 0.01$), a drug known to induce endothelial-dependent vasodilatation.

In conclusion, young men with OSAS did not exhibit evidence of VED. Obesity, however, was associated with elevated serum concentrations of VEGF and CRP. The findings of the current study suggest that the development of VED likely either occurs

after longer exposure to occult OSAS or exposure to OSAS that is further complicated by hypoxemia, which was notably absent in OSAS in this study. Future studies should employ similar experimental techniques to determine which of these scenarios is most likely accurate.

TABLES

Table 1 - Subjects Characteristics

Measure	Overweight with OSAS (n=12)	Overweight without OSAS (n=18)	Normal Weight without OSAS (n=17)
Age (yr)	22.8 ± 0.8	22.5 ± 0.7	21.1 ± 0.5
Height (cm)	170.0 ± 5.6	178.2 ± 1.3	176.7 ± 1.5
Weight (kg)	100.1 ± 4.1	100.1 ± 3.6	70.4 ± 1.8 ^a
BMI (kg/m ²)	32.4 ± 1.0	31.6 ± 1.1	22.4 ± 0.4 ^a
Systolic BP (mmHg)	124.2 ± 2.6	122.6 ± 2.9	121.8 ± 2.3
Diastolic BP (mmHg)	84.7 ± 1.5	86.0 ± 2.1	81.5 ± 1.6
Heart Rate (bpm)	91.8 ± 4.5	87.1 ± 3.8	85.2 ± 2.8
Total Fat (kg)	30.6 ± 2.0	26.7 ± 2.1	13.5 ± 1.0 ^a
Central Ab Fat (kg)	9.1 ± 0.7 ^b	7.3 ± 0.5	3.6 ± 0.3 ^a
AHI (events/hr)	25.4 ± 5.4 ^b	2.2 ± 0.3	1.9 ± 0.3
Average O ² Sat (%)	95.5 ± 1.8	95.5 ± 1.8	96.5 ± 0.5
Lowest O ² Sat (%)	86.8 ± 4.6	84.1 ± 8.6 ^c	89.7 ± 2.6
ESS (x/24)	7.8 ± 1.1	8.3 ± 0.8	6.3 ± 0.6

Data are presented as mean ± SEM. ^a Different from both overweight groups (p < 0.05); ^b Different from both control groups (p < 0.05); ^c Different from normal weight control group (p < 0.05).

Table 2 –Serum VEGF and Expression of VEGFR2

Measure	Overweight with OSAS (n = 9)	Overweight without OSAS (n = 12)	Normal Weight without OSAS (n = 11)
VEGF (pg/ml)*	183.2 ± 43.5	178.3 ± 33.1	88.2 ± 17.7 ^a
VEGFR2 (%)	3.9 ± 1.3	4.9 ± 1.1	6.6 ± 4.7

Data are presented as mean ± SEM. ^a Different from both overweight groups (p < 0.05). VEGFR2 = Percentage of monocytes expressing VEGFR2; * VEGF was reported in the same number of observations as measures in Table 1.

FIGURES

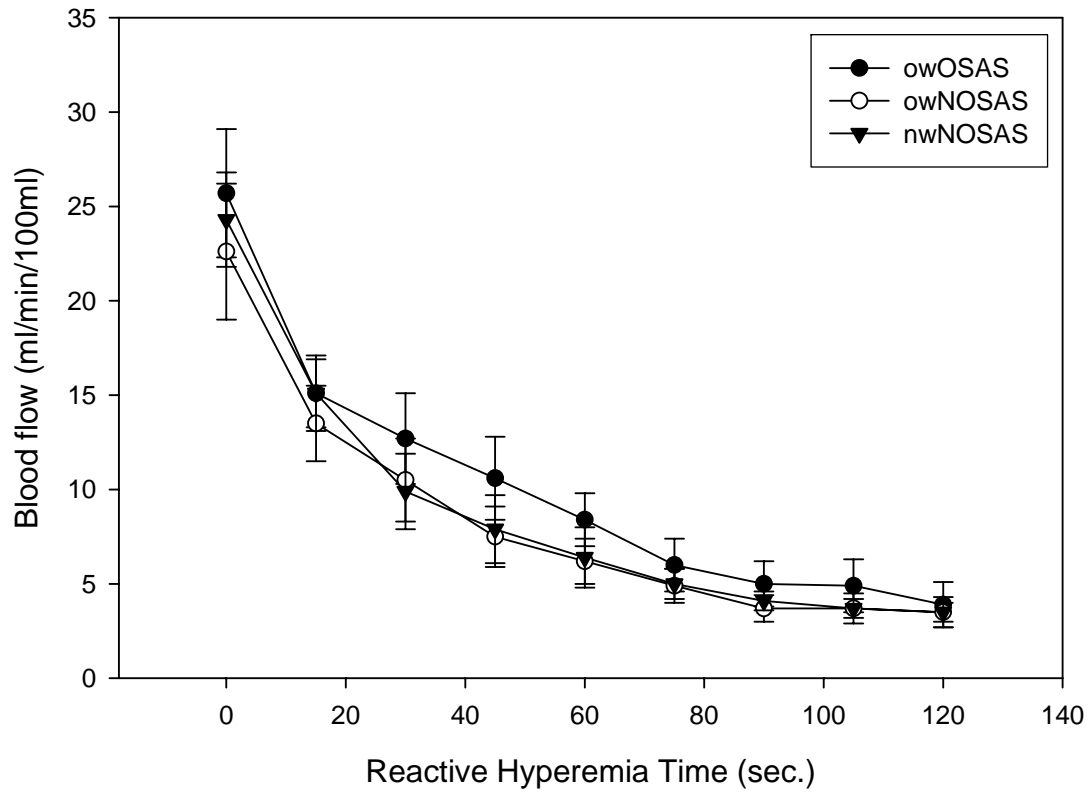


Figure 1 – Postocclusive Reactive Hyperemia, by group. There were no differences in reactive hyperemia at any time between overweight subjects with OSAS (owOSAS), overweight subjects without OSAS (owNOSAS), or normal weight subjects without OSAS (nwNOSAS)

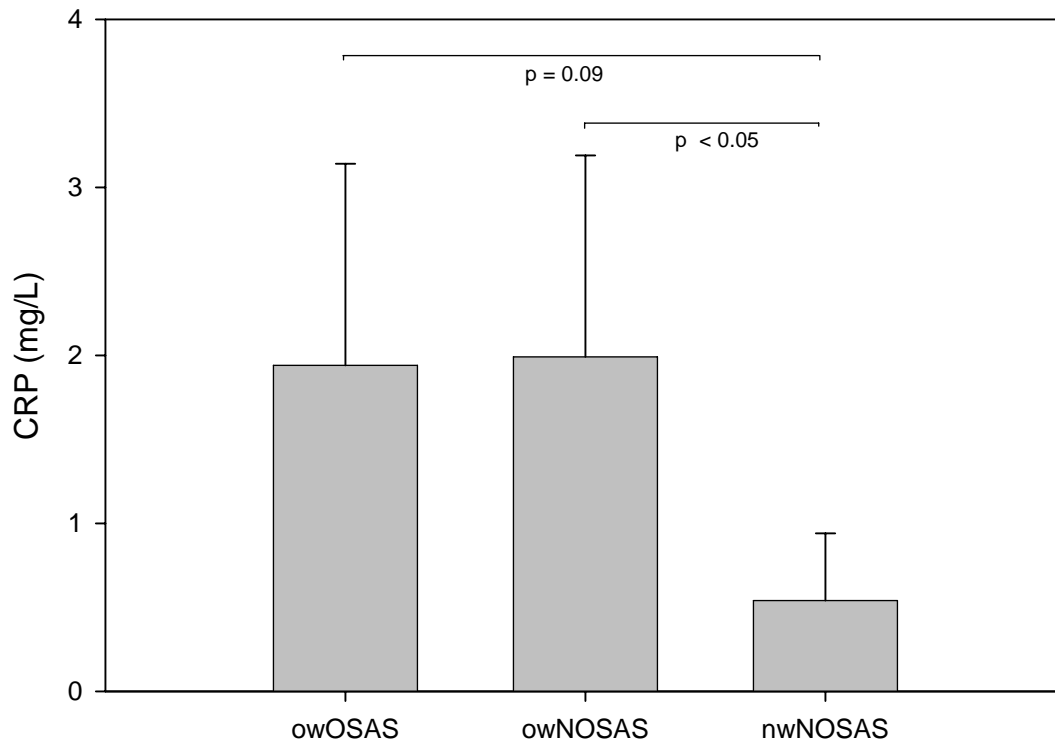


Figure 2 – Serum CRP, by Group. Serum CRP was higher in overweight controls (owNOSAS) than normal weight controls (nwNOSAS), and tended to be higher in overweight subjects with OSAS (owOSAS) than nwNOSAS.

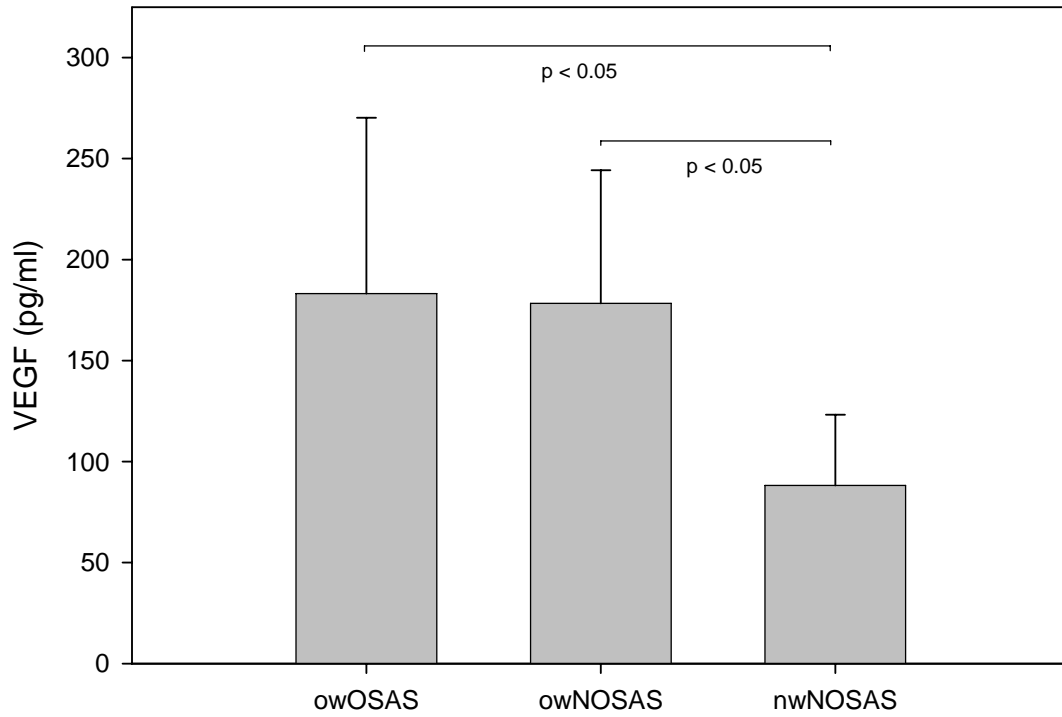


Figure 3 – Serum VEGF, by Group. Serum VEGF was higher in both overweight groups than normal weight controls.

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Chapter 5

Summary and Recommendations for Future Research

SUMMARY

OSAS, a chronic respiratory disorder affecting as many as 1 in 5 adults, is associated with repetitive collapse of the upper airway during sleep and results in fragmented sleep and intermittent periods of hypoxia and hypercapnia^{1, 2}. Though prevalence rates for this condition appear high, OSAS typically goes undiagnosed and overlooked clinically. As many as 90% of individuals living with moderate and severe OSAS remain undiagnosed and untreated³. The global rise in the prevalence of obesity, the primary risk factor for OSAS, suggests the incidence of OSAS will likely increase as well. Previous investigations demonstrated an increased risk for chronic cardiovascular and metabolic disorders in middle-aged adults with OSAS. Though obesity likely contributes to this exaggerated risk, considerable evidence suggests the relationships between OSAS and hypertension^{4, 5}, insulin resistance⁶⁻⁸, and MetS⁹⁻¹¹ are independent of the effects of obesity in middle-aged and older adults. However, it is still not known whether this relationship also holds true in young men who have been living with OSAS for a shorter period of time.

Thus, the purpose of the current study was to determine if evidence of the pathogenesis of chronic cardiovascular and metabolic disorders (i.e., insulin resistance, MetS, and VED) existed in young, overweight men with OSAS and if the combined effects of obesity and OSAS amplified the disease process more than obesity alone. Subjects were male volunteers aged 18-26 years old, who were sedentary and free of

chronic cardiovascular and metabolic disorders. Subjects were placed into one of the following three groups based on body weight (BMI) and OSAS severity (AHI): 1) overweight with OSAS (BMI > 25.0 kg/m² and AHI > 5.0), 2) overweight without OSAS (BMI > 25.0 kg/m² and AHI < 5.0), and 3) normal weight without OSAS (BMI < 25.0 kg/m² and AHI > 5.0). Measurements included a portable, unsupervised polysomnography exam to determine OSAS severity, dual energy x-ray absorptiometry (DEXA) to quantify total and central abdominal fat, strain gauge plethysmography assessment of forearm vascular endothelial function, and a fasting blood sample processed to examine biochemical markers of insulin resistance (glucose, insulin, adiponectin, interleukin-6 (IL-6), and tumor necrosis factor – alpha (TNF- α)), metabolic syndrome (HDL-cholesterol, triglycerides, and glucose), and endothelial dysfunction (vascular endothelial growth factor (VEGF), VEGF receptor 2 (VEGFR2), asymmetric dimethylarginine (ADMA), and C – reactive Protein (CRP)).

A primary finding of the current study was that overweight young men with OSAS had more CAF than overweight and normal weight, non-apneic controls, despite no differences in BMI or percent body fat. This altered distribution of fat is likely a product of the increased sympathetic nervous system activity associated with OSAS that triggers the release of stress hormones, such as cortisol, which stimulate the storage of fat in the abdominal region. Though higher CAF has been previously reported in middle-aged adults with OSAS^{12, 13}, this study is the first to demonstrate that this altered pattern of fat distribution occurs early in the course of OSAS pathology. Increased CAF has vast implications for the pathogenesis of both OSAS and chronic cardiometabolic disorders. Excessive amounts of fat stored within the abdomen places mechanical restrictions on the

respiratory process, impairing vital capacity and ventilation in a manner that is exaggerated when the body position is supine during sleep^{14, 15}. Furthermore, central obesity is associated with an increase in sympathetic nervous system activity that likely further contributes to the pathogenesis of OSAS¹⁶. CAF is known to be a metabolically active endocrine organ that augments the production and release of many proinflammatory hormones that alter insulin resistance and vascular endothelial function¹⁷. Thus, the increased CAF reported in young men with OSAS suggests that these individuals exhibit an increased risk for future cardiovascular and metabolic disorders.

Though increased CAF may be suggestive of future chronic disease risk in these young men, subjects were further analyzed to determine if evidence of the pathogenesis of insulin resistance already existed in this cohort. Insulin resistance was measured non-invasively and, though it was 20% higher, was not statistically higher in subjects with OSAS than controls matched for BMI. TNF- α and IL-6, two proinflammatory adipocytokines that are associated with insulin resistance and are typically elevated in middle-aged adults with OSAS, were not different in regards to both body weight and presence of OSAS. However, adiponectin, an anti-inflammatory hormone produced exclusively by adipose tissue, was lower only in subjects with OSAS. Adiponectin is of particular interest in OSAS, as β -adrenergic receptor agonists, such as the catecholamines released as a byproduct of repetitive sympathetic nervous system activation, inhibit the production of this insulin-sensitizing hormone¹⁸. Taken together, these findings suggest that the suppression of adiponectin may be particularly sensitive to clinical features of

OSAS and likely represent a key initial step in the pathogenesis of insulin resistance in young men with OSAS.

Data were further analyzed to determine if evidence of the MetS was present in these subjects, and if presence and/or constellation of traditional cardiometabolic risk factors was primarily due to the severity of OSAS or obesity. The prevalence of MetS was similar for overweight subjects with and without OSAS. No mean group differences were noted in regards to blood pressure, glucose, HDL cholesterol, or waist circumference, either. However, fasting triglycerides were elevated only in subjects with OSAS. Furthermore, triglycerides were directly related to the severity of OSAS, even after results were partial correlated to remove the effects of CAF. Although triglycerides were directly related to the amount of CAF, partial correlation to remove the effects of OSAS severity caused this relationship to dissipate. Coupled with increased CAF in subjects with OSAS, these findings suggest that elevations in triglycerides may represent an early stage in the pathogenesis of MetS, and appear in accordance with previous animal models that have demonstrated elevations in triglycerides and CAF precede the development of insulin resistance¹⁹.

The final aim of this study was to determine if young men with OSAS exhibited evidence of endothelial dysfunction. Postocclusive reactive hyperemia or the return of blood flow to the forearm after a 5-minute period of ischemia was not different between any of the study groups. VEGF and CRP, cytokines indicative of vascular endothelial dysfunction that are typically elevated in middle-aged subjects with OSAS, were higher in overweight subjects than normal weight controls. However, no further differences were attributable to the presence or severity of OSAS. Serum CRP was

directly correlated to the amount of CAF. Taken together, these findings suggest that obesity, not OSAS, is associated with early evidence of endothelial dysfunction in this cohort of subjects. Thus, the deleterious effects of OSAS likely take longer to translate into functional and biochemical evidence of endothelial dysfunction.

Overall, this study demonstrated that young men with latent OSAS exhibit several unique physiological and anthropometric features that may signify the early pathogenesis of chronic cardiovascular and metabolic disorders. These abnormalities, namely lower serum adiponectin and higher CAF and triglycerides, were evident in subjects with OSAS beyond the influence of obesity alone. Taken together, these findings may represent initial biochemical changes that precede clinical presentation of more traditional markers of chronic disease risk. .

CLINICAL APPLICATIONS

The current study did not demonstrate the clinical presentation of insulin resistance, MetS, and endothelial dysfunction in young men with OSAS beyond the effects of uncomplicated obesity. However, several unique anthropometric and biochemical features were identified in young men with OSAS that may represent early steps in the pathogenesis of insulin resistance and MetS. Identification and treatment of OSAS early in life may be critical to prevent the future development of these disorders, particularly as the rising prevalence of obesity suggests that the number of young men living with occult OSAS will also increase. Thus, the age range of subjects in this study (18 – 26 years old) may represent the ideal time to first identify and treat OSAS. Currently, OSAS is first diagnosed in the fifth decade of life, and is typically further

complicated at that time by the comorbid presence of other chronic cardiovascular and metabolic disorders. Excessive daytime sleepiness and a large neck circumference are often used to help risk stratify those adults with a high likelihood of having OSAS. Interestingly, neither excessive daytime sleepiness nor neck circumference was higher in overweight subjects with OSAS than overweight controls. Future studies should be conducted to help identify functional or biochemical features that are unique in young men with OSAS to aid clinicians in identifying those at high risk for OSAS who should undergo full polysomnography.

RECOMMENDATIONS FOR FUTURE RESEARCH

The following are areas of future research may be pertinent in expanding what is currently known about the role of OSAS in the pathogenesis of chronic disorders:

1. The current study did suggest that young men with OSAS exhibit unique anthropometric and biochemical features that may be indicative of early stages in the pathogenesis of insulin resistance and MetS. This study should be expanded in order to make better inferences about the role of OSAS in the development of chronic disease. First, sample size should be increased to improve the statistical power to detect significant differences, including more subjects with mild, moderate, and severe OSAS to better understand if chronic disease risk varies based on OSAS severity in this cohort. In addition, a fourth group of subjects that are of normal body weight, yet have OSAS, should be included. Evidence of chronic disease pathogenesis in these subjects would further support the independent role of OSAS in increasing chronic disease risk. Also, the current

study should be replicated in men aged 26 – 45 years old, as this age range represents an additional cohort of men that have yet to be studied. Examining men of this age would help better identify the age range where additional evidence of chronic disease pathogenesis begins to appear.

2. The results of the current study demonstrate that neck circumference and excessive daytime sleepiness, two indices typically used to assess risk for OSAS in middle-aged and older adults, may not be appropriate measurements to risk stratify younger adults. This finding is of particular interest, as the number of young adults living with occult OSAS is expected to increase with the concurrent global rise in obesity. Future studies should attempt to identify if there are unique and cost-effective variables, such as biomarkers in blood or responses to graded exercise testing, which can be used clinically to predict the presence of OSAS and identify those individuals who should undergo full polysomnography. More detailed analysis of blood samples could potentially be employed to identify if genetic markers, such as a specific gene variant, or alterations in monocyte expression exists due to the presence of OSAS. Additionally, peripheral quantitative computed tomography (pQCT) devices may help identify if relative muscular and adipose tissue content of the upper airway are better able to predict the presence of OSAS in individuals with similar neck circumferences.
3. Nasal continuous positive airway pressure (nCPAP) is the primary method for treating OSAS, and when worn properly each night of sleep, is able to effectively reverse the harmful consequences of this disorder. However, very little is known about the feasibility of and adherence to this treatment modality

in younger adults. Clinical trials should be conducted to address these limitations. Should nCPAP alone be an ineffective treatment modality in this population, additional studies would be needed to explore complimentary or alternative forms of treatment (e.g., dietary and/or exercise induced weight loss) in this cohort of young adults.

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APPENDIX A

SEARCHLIGHT MULTIPLEX ELISA

DETAILED PROCEUDRES

1. Dilute samples 1:4 (1 part sample to 3 parts diluent) before testing.
2. Add 50µl of standard or sample to each well in duplicate.
3. Cover with enclosed adhesive plate sealer and incubate for 60 minutes at room temperature (20-25°C) on an orbital shaker at 200rpm.
4. Wash thoroughly three times with prepared wash buffer and pat dry. Remove residual liquid by tapping the inverted plate on clean absorbent paper.
5. Add 50µl of Streptavidin-HRP Reagent to each well.
6. Cover with enclosed adhesive plate sealer and incubate for 30 minutes at room temperature (20-25°C) on an orbital shaker at 200rpm.
7. During Step 6, prepare CCD camera to read kit immediately after assay is done.
8. Wash thoroughly three times with prepared wash buffer and pat dry. Remove residual liquid by tapping the inverted plate on clean absorbent paper. Make sure all bubbles are removed before continuing.
9. Prepare SuperSignal[®] Substrate by adding 4ml of Stable Peroxide to 4ml of SuperSignal[®] Luminol Enhancer. Mix gently.
10. Add 50µl of SuperSignal[®] Substrate to each well. Read within 1-10 minutes.
11. Read the luminescence assay on a 12-bit, 14-bit, or 16-bit cooled CCD camera. Save images as 16-bit TIFF files.

APPENDIX B

INSULIN RIA

DETAILED PROCEDURES

1. Label four plain (uncoated) 12x75 mm polypropylene tubes T (total counts) and NSB (nonspecific binding) in duplicate.
2. Label fourteen Insulin Ab-Coated Tubes A (maximum binding) and B through G in duplicate. Label additional antibody-coated tubes, also in duplicate, for controls and patient samples.
3. Pipet directly to the bottom 200 μ L of the zero calibrator A into the NSB and A tubes, and 200 μ L of each remaining calibrator, control and patient sample into the tubes prepared.
4. Add 1.0 mL of 125 I Insulin to every tube. Vortex.
5. Incubate for 18–24 hours at room temperature (15–28°C).
6. Decant thoroughly.
7. Count for 1 minute on gamma counter.

APPENDIX C

LEPTIN RIA

DETAILED PROCEDURES

1. Pipet 300µl of Assay Buffer to the Non-Specific Binding tubes, 200µl to the reference tubes, and 100µl to the remainder of the tubes.
2. Pipet 100µl of Standards and Quality Controls in duplicate.
3. Pipet 100µl of each sample in duplicate.
4. Pipet 100µl of ¹²⁵I-Human Leptin to all tubes.
5. Pipet 100µl of Human Leptin antibody to all tubes except Total Count tubes and Non-Specific Binding tubes.
6. Vortex, cover, and incubate overnight (20-24 hours) at 4°C.
7. Add 1.0 ml of cold (4°C) Precipitating Reagent to all tubes (except Total Count tubes).
8. Vortex and incubate 20 minutes at 4°C.
9. Centrifuge, 4°C, all tubes [except Total Count tubes (1-2)] for 20 minutes at 2,000-3,000 xg.
10. Immediately decant the supernate of all tubes except Total Count tubes (1-2), drain tubes for at least 15-60 seconds.
11. Count all tubes in a gamma counter for 1 minute. Calculate the ng/ml of Human Leptin in unknown samples using automated data reduction procedures.

APPENDIX D

VEGFR2 CELL STAINING

DETAILED PROCEDURES

1. Add 100µl blocking buffer (PBS/20% Normal goat serum/NaN₃) buffer to wells.
2. Add 15µl whole blood to each well and mix.
3. Cover and incubate for 10 minutes.
4. Centrifuge at 3000 rpm for 5 minutes at 4°C.
5. Splash out supernatant.
6. Suspend cells in 200µl FACS buffer.
7. Centrifuge at 3000 rpm for 5 minutes at 4°C.
8. Splash out supernatant.
9. Add 50 µl of primary antibody dilution (1:100) to each sample well.
10. Add 50µl of FACS buffer to control cells.
11. Incubate for 20 minutes on ice.
12. Centrifuge at 3000 rpm for 5 minutes at 4°C.
13. Splash out supernatant.
14. Suspend cells in 200µl FACS buffer.
15. Centrifuge at 3000 rpm for 5 minutes at 4°C.
16. Splash out supernatant.
17. Add 50 ul of secondary antibody dilution (1:100) to each well.
18. Cover and incubate for 20 minutes on ice.
19. Centrifuge at 3000 rpm for 5 minutes at 4°C.
20. Splash out supernatant.
21. Wash with 200µl FACS buffer.
22. Centrifuge at 3000 rpm for 5 minutes at 4°C.
23. Splash out supernatant.
24. Add 200µl lysing solution dilution (1:25) to each well.
25. Incubate for 90-120 seconds.
26. Add 10ul of fixative solution to each well.
27. Centrifuge at 3000 rpm for 5 minutes at 4°C.
28. Splash out supernatant.
29. Add 200µl PBS to each well.
30. Cover plate with foil, label, and refrigerate at 4°C until FACS analysis.

APPENDIX E

WHITE BLOOD CELL ANALYSIS

DETAILED PROCEDURES

1. Sign in on daily use log and turn on Coulter Counter.
2. Place 40ml of fresh ionized solution into vial, insert into device, and press start. Wait until the monitor no longer says “standardizing” to continue.
3. If the count is greater than 10,000, press start again. Repeat process until count is less than 10,000.
4. Place 40µl of your sample into a tube with 20ml fresh ionizing solution. Add 4 drops of immunolyse solution.
5. Add 20ml fresh ionizing solution and place in coulter counter and press start to read.
6. Manually record white blood cell count displayed on monitor.
7. Repeat Steps 4-6 for each sample to be tested.
8. After last sample has been analyzed, place 40ml of fresh ionized solution into little tube, insert into device, and press start.
9. Place 40ml of fresh blue ionized solution into little tube, insert into device, and press start. Once the device no longer says “standardizing”, leave the blue solution in the reading position and turn the device off.

APPENDIX F

EMBLETTA PDS DEVICE SETUP

DETAILED PROCEDURES

Embletta PDS Set-up Instructions

Step 1. Attach the respiratory effort sensors

1. Place the respiratory effort sensor with the yellow plug on your stomach. Center it just above your waistline and wrap the strap around you.
2. With the Velcro facing away from your body, run the free end of the respiratory effort strap through the loop on the side of the respiratory effort sensor.
3. Fasten the Velcro so the strap is snug and secure.
4. Place the respiratory effort sensor with the blue plug on your chest at the mid-line and tighten in the same manner.

Note: You may need to readjust the straps when you lie down.

Step 2. Attach snore sensor

1. Attach the snore sensor to you neck slightly off center
2. Use adhesive tape (included) to secure the sensor.

Step 3. Attach the nasal cannula

1. Place the nasal prongs in your nostrils so that they curve toward you.
2. Loop the tubing over your ears and then position under your chin.
3. Use adhesive tape to secure tubing to cheekbones.
4. Tighten the cannula tubing, if necessary, by pushing the bolo slide (toggle) towards your chin.

Step 4. Attach oximeter sensor

Note: You may wish to have the oximeter sensor wire run under your sleepwear from the Embletta to your finger. Simply feed the wire under your sleepwear before attaching the sensor to your finger.

Note: The oximeter sensor will not function properly if nail polish has been applied to your nail.

Note: This step will be easier if you have someone available to help you tape the sensor.

1. Place a small piece of tape at the connection of the wire and sensor, with the sticky side placed on the same side with the two slightly elevated sensors.
2. Carefully position the tip of the index finger in the middle of the sensor, so that when folded over the finger, both sensors are in the same approximate place on the top and bottom of the finger.
3. Attach the tape to the bottom of the finger, securing the sensor in place.
4. Attach another piece of tape across the top of the sensor, completely securing both sensors to the finger in the appropriate position.
5. Attach another small piece of tape just below the middle of your index finger to secure the wire against the skin and minimize movement.

Step 5. Attaching the wires to the sensor block

1. Connect the individual sensors to the sensor plug using the color codes. Be aware of the shape of the sensor plugs and adaptors.
2. Once all sensor wires are attached, plug the sensor adaptor into the top of the Embletta PDS recorder. Be careful not to put pressure on the sensor wires or connectors. You will hear a beep and the device will power up. Recording of data begins when the sensor block is inserted. Make sure the block is completely inserted. Do not insert the block until you are ready to lie down in bed to go to sleep.
3. Connect the nasal cannula to the Luer lock on top of the Embletta PDS. Twist clockwise to tighten.

Step 6. Testing procedure

1. Before going to sleep and beginning your study, check that the sensors are attached correctly and that there is enough battery power to complete the study.
2. Press the test button (check) and hold until you hear a beep. The yellow lights on the front of the Embletta PDS will illuminate for 1 minute.

Status Lights

- **Yellow** means recording has not yet begun.
- **Green** means recording has started.
- **Red** means that an error has occurred and you should contact an investigator before continuing.

Battery light

- **Green** means that there is enough battery power to complete your study.
- **Red** means battery power is low and you should contact the investigator before progressing any further.

Sensor lights

The nasal cannula, snore, and respiratory effort sensors should flash as you inhale and exhale. Ignore the thermistor light. During the signal test, the oximeter sensor light will also illuminate if a signal is detected.

If the sensor lights are not lighting up properly, do the following:

1. Check the placement of the sensors.
2. Check that the sensors are firmly connected to the sensor adaptor.
3. Check that the sensor adaptor is properly connected to the Embletta PDS recorder.
4. Contact one of the following investigators if the Embletta PDS is still not detecting signals properly.

Step 7. Starting a sleep study

Note: Before starting your sleep study, turn off any mobile phones near your bed. This is very important. Signals from a mobile phone may interfere with the Embletta PDS recording.

1. Wrap the Embletta PDS strap around your abdomen or wherever it is most comfortable for you. Push the free end of the strap through the remaining loop on the Embletta PDS holster and adjust the strap so it is snug and secure.
2. Lie down on your back in bed. While the Embletta PDS is recording data, the green status light will flash continuously.

Step 8. Concluding the sleep study

1. Disconnect the sensor adaptor from the Embletta PDS. This will turn off the device.
2. Remove the sensors from your body. You do not have to disconnect the sensors from the sensor adaptor.
3. Take off the Embletta PDS strap.
4. Place all Embletta PDS components in the carry case.
5. Discard any used tape.

APPENDIX G

VASCULAR INFLOW AND REACTIVE HYPEREMIA

DETAILED PROCEDURES

1. Have subject lie supine on testing table for 10 minutes.
2. Obtain blood pressure from left arm manually via auscultation.
3. Tightly position blue arm cuff above the elbow on the left arm.
4. Tightly position blue wrist cuff around the left wrist.
5. Measure circumference of the widest portion of the left forearm, truncating the value to the nearest centimeter.
6. Choose the appropriately numbered strain gauge that corresponds to the forearm diameter.
7. To measure resting inflow, set the pressure on the E20 Rapid Cuff Inflator to 50mmHg.
8. Inflate blue wrist cuff to 50mmHg above systolic blood pressure.
9. Re-zero the plethysmograph by pressing the Balance switch.
10. Indicate start readings on NIVP3 software program.
11. The blue arm cuff will rapidly inflate to 50mmHg and a 5 second recording will be obtained.
12. Repeat steps 10 and 11 four times to get a total of 3 measurements.
13. Average the 5 values to get resting inflow value (units are %/minute, or cc's of blood flow/100 cc's of tissue/minute).
14. To measure reactive hyperemia, inflate blue arm cuff and blue wrist cuff to 50mmHg above systolic blood pressure reading.
15. Leave cuffs inflated to suprasystolic pressures for 5 minutes.
16. Set measurement timer to measure in 15 second intervals.
17. After 4 minutes and 45 seconds have passed, re-zero the plethysmograph by pressing the Balance switch.
18. After 5 minutes, release the upper arm cuff to 50mmHg and press start readings.
19. Automated readings will be made every 15 seconds for three minutes.
20. Manually analyze data to eliminate cuff artifact, using a straight line to best characterize the slope of the 5 second pulse wave display for each measurement.

APPENDIX H

INFORMED CONSENT

VIRGINIA POLYTECHNIC INSTITUTE AND STATE UNIVERSITY

Informed Consent for Participants in Investigative Project

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

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

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

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 use (only non-smokers can participate);
- Any problem affecting your breathing (cold, sinus infection, etc.) during the previous 6 weeks;
- Current pregnancy;
- Use of birth control pills;

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

II. Procedures

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

Introduction, Informed Consent, and Advanced Screening (up to 3 meetings)

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

You will then report to the Laboratory for Health and Exercise Science in 231 War Memorial Hall on the Virginia Tech campus. Once there, a researcher will read through this form with you and will answer any questions or concerns that you may have. The researcher will also go over a more detailed health history form with you and may ask you more questions about your health. This allows researchers to identify if any 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 small amount of blood (~75ml, about 4 tablespoons), which will be taken from a blood vessel in your arm.

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

Session 3 – Blood Vessel Health and Bicycle Exercise Tests

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

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

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

a) Forms

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

b) Blood Pressure

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

c) Other Physical Tests

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

d) At-Home Sleep Test

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

e) Blood Sample

You will have blood samples drawn in order to look at blood glucose, lipids (fats), and several markers of blood vessel function. The total amount of blood that you will give will be small, i.e. less than 75 ml (about 5 tablespoons). A qualified technician will draw the blood samples, and accepted medical procedures will be followed. A laboratory specialist will examine, process, and store your blood to be analyzed at the end of the study.

If a technician or other person who handles your blood sample is accidentally exposed to your blood, you will be required to have your blood tested for HIV/AIDS. This testing will be confidential and will be done at the Montgomery County Health Department. This test will cost \$50 and funds provided by the research sponsor will cover this cost. It is required that you provide the Montgomery County Health Department with your social security number and your name; if you have a positive test for HIV/AIDS, and only then, this result must be reported to the State Health Department (this is a legal requirement). The names of persons with HIV/AIDS positive tests that are reported to the state remain confidential; however, this information will be placed in your permanent medical records. The test facility requires 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. This dose is very small and poses minimal risk. The following table lists the radiation limits for an adult research participant according to the National Institutes of Health, Office for Protection from Research Risk (NIH-OPRR), compared to the exposure during this study.

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

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

g) *Blood Vessel Health Test*

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

h) *Bicycle Exercise Test*

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

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

III. Risks

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). 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: _____

Signature

Date

Witness (Research Coordinator)

Date

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

Carol Haskell, MD 951-8814
Research Coordinator

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

Stephen Guill, M.S. 231-6374
Investigator

David M. Moore, Ph.D. 231-4991
Chair, IRB, Research Division

Trent Hargens, M.S. 231-6374
Investigator

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

Nadine Guignel, B.S. 231-6375
Investigator

APPENDIX I

HEALTH HISTORY QUESTIONNAIRE

Name: _____ Age: ____ yr Date of Birth: _____

 Ethnicity: _____
 Height: _____ ft Weight: _____ pounds
 Gender: Female _____ Male _____
 Campus Address: _____
 Campus Telephone Number: _____ Campus Email Address: _____

 Address for Permanent Residence: _____

 Person to contact in case of emergency: _____

 Relationship: _____ Daytime Telephone: _____
 Home Telephone: _____
 Primary Care Physician: _____ Telephone: _____

Medical History

Please indicate any current or previous conditions or problems you have experienced or have been told by a physician you have had:

	Yes	No
Heart disease or any heart problems:	_____	_____
Rheumatic fever:	_____	_____
Respiratory disease or breathing problems:	_____	_____
Circulation problems:	_____	_____
Kidney disease or problems:	_____	_____
Urinary problems:	_____	_____
Reproductive problems:	_____	_____
Musculoskeletal problems:	_____	_____
Fainting or dizziness, especially with exertion:	_____	_____
Neurological problems/disorders:	_____	_____
High blood pressure:	_____	_____
Low blood pressure:	_____	_____
High blood cholesterol:	_____	_____
Diabetes:	_____	_____
Thyroid problems:	_____	_____
Eating disorders (bulimia, anorexia):	_____	_____

Allergies: _____

If "yes" to any of the above please indicate the date, explain, and describe:

Please list any hospitalizations/operations/recent illnesses (Type/Date): _____

Family Health History

Has anyone in your family (blood relatives only) been diagnosed or treated for any of the following?

	Yes	No	Relationship	Age
Heart attack	_____	_____	_____	_____
Heart disease	_____	_____	_____	_____
High blood pressure	_____	_____	_____	_____
Stroke	_____	_____	_____	_____
Kidney disease	_____	_____	_____	_____
Diabetes	_____	_____	_____	_____

Health Habits

Do you add salt to your food? Yes ___ No ___ Are you on any special type of diet?

Yes ___ No ___

If "yes" please describe _____

Do you drink caffeinated beverages? Yes _____ No _____ How many cups per day? _____

Do you drink alcoholic beverages? Yes _____ No _____ How many drinks per week? _____

What is the average number of drinks that you consume on the weekend? _____

Did you use tobacco products in the past (more than 12 months ago)? Yes _____ No _____

Sleep Habits Evaluation

Do you have episodes of parasomnias (disorders such as sleep walking, sleep talking, night terrors, body rocking, bedwetting that will cause partial or full awakening?) Yes___
No_____

Do you show signs of sleep disturbances (such as insomnia, daytime sleepiness) when you are anxious, stressed? Yes_____ No_____

Do you have difficulties to fall asleep if a certain object or a certain situation is absent such as listening to the radio, watching the television, having a teddy bear ...?) Yes_____
_____ No_____

Do you have difficulties to fall asleep earlier or later of your usual bedtime? Yes_____
No_____

Do you wake up at night to get a little snack? Yes___ No_____

If "yes", do you think that the snack is helping you to go back to sleep? Yes_____ No_____

Do you ever feel very tired, sleepy at school? Yes_____ No_____

Do you have hallucinations (vivid images that look like dreams occurring when you sleep) or find yourself physically weak or paralyzed for a few seconds? Yes_____
_____ No_____

Tonsils and Adenoids evaluation questionnaire

Do you have a history of recurrent tonsillitis which is an inflammation of the tonsils (clusters of tissue that lie in bands on both sides of the back of the throat) caused by an infection? In tonsillitis, the tonsils are enlarged, red, and often coated either partly or entirely? Yes_____ No_____

Did you ever have inflammation of the adenoids (single clump of tissue in the back of the nose) causing a blockage of the back of the nose, chronic and recurrent fluid or infections of your ears, or chronic or recurrent sinus infections? Yes_____
No_____

Did you have tonsillectomy (tonsils removed) or adenoidectomy (adenoids removed)?
Yes_____ No_____

Exercise Habits

Do you engage in regular exercise? Yes _____ No _____

If "yes" please list:

Activity	Frequency (times per week)	Duration (minutes)
-----------------	-----------------------------------	---------------------------

Do you ever feel faint, short of breath, or chest discomfort with exertion? Yes: _____
No: _____
If "yes", please explain : _____

Are there any orthopedic limitations you have that may restrict your ability to perform hard running exercise or intense strength-type exercises? (back, hips, knees, ankles)
Yes _____ No _____
If "yes" please explain: _____

Questions Related to Reproductive Function (skip to the next part if you are a male)

Do you use birth control? Yes _____ No _____
Date of last menses: _____
Have you had any abnormal menses or absence of menses in the last 12 months? Yes _____ No _____
If "yes", describe this menstrual problem:

Medications

Please list all medications (prescription and over-the-counter) you are currently taking or have taken in the past week: _____

Please sign to indicate the above information is correct:

Print Name

Signature

Date

Follow Up Review and Interview by:

Signature of Project Staff Member

Date

Results of Screening - Routine Findings: Make certain that all questions on this form are properly completed. Query candidate, immediately after they complete this questionnaire, about any items left blank or for which clear answers are not provided. If no unusual problems are disclosed that may affect the candidate's safety or eligibility for the study, note this finding below and submit file materials to the Research Coordinator. **THIS CANDIDATE QUALIFIES FOR PARTICIPATION IN THE STUDY, SUBJECT TO VERIFICATION BY THE RESEARCH COORDINATOR. Yes:** ____ **No:** ____.
If No, complete next section, below.

Results of Screening - Uncertain Findings: Note and discuss ANY potential health problem listed on this Health History form. Next, contact the candidate for clarification and report outcome to the Research Coordinator, Carol Haskell, M.D. Follow-up to appropriate health care professionals will be recommended, if deemed necessary, based on published guidelines (e.g., fasting blood glucose >109 mg/dL, fasting total cholesterol >200 mg/dL). Any and all costs related to such referral will be borne by the subject and not by Virginia Tech. **CANDIDATE HAS THE FOLLOWING UNDEFINED/UNCLARIFIED HEALTH PROBLEM(S) THAT WARRANT FURTHER REVIEW AND POSSIBLE EXCLUSION FROM THIS STUDY:**

APPENDIX J

EPWORTH SLEEPINESS SCALE

Subject ID _____ Name _____ Date Completed ____/____/____

This questionnaire asks you to indicate the chances of you becoming drowsy during hours of the day that you are not in bed sleeping. "How likely are you to doze off or fall asleep in the following situations?"

Use the following scale and indicate the most appropriate number for each situation.

- 0 = would never doze
- 1 = slight chance of dozing
- 2 = moderate chance of dozing
- 3 = high chance of dozing

<u>Situation</u>	<u>Chance of Dozing</u>
1. Sitting and reading	_____
2. Watching T.V.	_____
3. Sitting, inactive in a public place (ex. Theatre or meeting)	_____
4. As a passenger in a car for an hour without a break	_____
5. Lying down to rest in the afternoon when circumstances permit	_____
6. Sitting and talking with someone	_____
7. Sitting quietly after a lunch without alcohol	_____
8. In a car, while stopped for a few minutes in the traffic	_____

Sum of Scores, items 1-8 (staff use only) _____/24

APPENDIX K

**INSTITUTIONAL REVIEW BOARD
PROTECTION OF HUMAN SUBJECTS APPROVAL**

Institutional Review Board

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

DATE: August 23, 2005

MEMORANDUM

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

FROM: David Moore



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

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

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

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

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

cc: File



VIRGINIA POLYTECHNIC INSTITUTE
AND STATE UNIVERSITY


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

APPENDIX L

RAW DATA

Table 1. Subject Characteristics of Overweight Subjects with OSAS (owOSAS)

Subject	Age (years)	Height (cm)	Weight (kg)	BMI (kg/m²)	AHI (events/hr)	ESS (x/24)	Neck Size (cm)	Total Fat (kg)	Ab Fat (kg)	Body Fat (%)
1	19	182.9	96.2	28.8	8.5	8	41.6	28.1	8.2	26.7
2	19	174.0	96.6	31.6	8.2	12	39.5	32.7	9.1	33.7
3	25	182.0	103.6	31.3	21.5	6	43.0	27.9	9.1	26.6
4	22	162.0	85.9	32.7	37.8	6	37.7	20.9	6.5	27.2
5	23	172.0	84.1	28.4	13.3	12	39.7	23.3	6.2	27.2
6	25	174.0	115.5	38.0	44.1	2	44.0	40.4	11.9	34.2
7	20	178.0	121.8	38.4	54.0	6	41.5	35.6	9.8	29.2
8	26	168.0	80.9	28.7	11.5	4	37.0	22.9	6.4	27.1
9	23	172.5	106.4	35.6	19.9	6	42.0	39.1	11.5	35.9
10	27	111.5	98.9	31.6	60.5	14	41.5	30.7	10.3	29.5
11	24	175.0	89.1	29.1	16.9	6	39.0	25.7	7.5	28.4
12	21	187.0	122.7	35.1	8.5	11	42.0	40.2	13.0	32.2

BMI = Body Mass Index; AHI = Apnea/Hypopnea Index, ESS = Epworth Sleepiness Score

Table 2. Subject Characteristics of Overweight Subjects without OSAS (owNOSAS)

Subject	Age (years)	Height (cm)	Weight (kg)	BMI (kg/m²)	AHI (events/hr)	ESS (x/24)	Neck Size (cm)	Total Fat (kg)	Ab Fat (kg)	Body Fat (%)
1	22	183.0	88.1	26.6	2.9	5	38.5	24.2	7.1	27.0
2	28	175.3	78.9	25.8	3.2	7	39.0	14.1	3.3	17.8
3	23	166.4	73.5	27.0	3.7	12	37.0	13.8	4.0	18.7
4	27	175.0	134.4	43.9	.9	7	48.8	47.6	13.2	35.2
5	21	177.8	112.0	35.5	1.1	15	42.0	31.0	8.2	29.1
6	18	184.0	108.9	32.2	2.3	1	41.0	34.3	8.0	31.2
7	18	178.0	112.0	35.5	3.7	8	41.0	39.9	11.1	35.3
8	23	185.4	108.2	31.6	3.4	9	38.5	21.9	5.5	24.7
9	21	172.3	120.0	40.1	1.8	8	47.5	39.2	10.6	31.8
10	23	184.0	105.9	31.3	3.4	9	-	27.6	7.7	26.3
11	26	184.0	100.5	30.1	1.2	12	41.0	24.8	8.2	24.3
12	21	187.5	98.4	28.1	3.4	6	38.5	25.0	6.2	25.7
13	23	173.0	102.7	34.3	1.1	8	41.5	26.4	6.4	25.3
14	26	178.0	98.6	30.9	2.9	8	42.0	26.9	7.4	27.0
15	21	177.0	102.3	32.7	2.7	8	42.0	23.1	6.1	22.2
16	20	177.0	85.0	27.1	.6	10	37.0	17.2	5.1	19.8
17	18	174.0	88.0	29.1	.3	12	41.0	20.1	5.6	22.3
18	26	175.0	84.0	27.4	1.0	5	37.0	23.7	7.0	28.1

BMI = Body Mass Index; AHI = Apnea/Hypopnea Index, ESS = Epworth Sleepiness Score
 (-) denotes missing data

Table 3. Subject Characteristics of Normal Weight Subjects without OSAS (nwNOSAS)

Subject	Age (years)	Height (cm)	Weight (kg)	BMI (kg/m²)	AHI (events/hr)	ESS (x/24)	Neck Size (cm)	Total Fat (kg)	Ab Fat (kg)	Body Fat (%)
1	21	172.7	68.0	22.8	2.6	7	35.5	12.1	3.6	17.7
2	19	174.0	67.2	22.0	1.8	9	35.5	7.2	1.7	10.9
3	24	189.0	76.4	21.4	1.4	2	37.0	16.0	3.6	21.2
4	20	173.0	64.0	21.4	2.0	8	36.2	10.0	2.4	15.4
5	24	182.2	68.0	22.5	3.9	-	36.5	14.3	3.7	18.8
6	21	167.6	66.7	23.7	2.3	5	35.0	12.6	3.2	18.7
7	24	182.0	81.6	24.6	1.0	7	38.0	21.9	6.0	27.2
8	19	181.1	67.7	20.2	.7	5	35.5	11.4	2.8	16.6
9	23	179.0	66.0	20.6	3.0	5	35.0	12.4	3.6	18.5
10	19	179.0	74.0	23.1	1.0	7	38.0	10.9	2.6	14.5
11	21	184.0	77.0	22.7	2.8	6	37.5	11.2	2.7	14.3
12	23	166.0	57.3	20.8	.9	8	33.0	13.9	3.2	24.2
13	19	174.0	72.0	22.5	2.3	9	37.0	18.8	4.5	25.8
14	23	170.0	60.0	20.8	3.7	4	35.0	8.3	2.3	13.7
15	19	177.0	85.3	24.1	1.6	11	38.5	14.9	5.0	18.3
16	18	180.0	68.5	21.2	.4	3	38.0	11.5	3.2	16.9

BMI = Body Mass Index; AHI = Apnea/Hypopnea Index, ESS = Epworth Sleepiness Score
(-) denotes missing data

Table 4. owOSAS Markers of Insulin Resistance

Subject	Insulin (μU/L)	Glucose (mg/dl)	HOMA-IR	Adiponectin (μg/ml)	IL-6 (ng/ml)	TNF-α (ng/ml)	Leptin (ng/ml)
1	16.00	94	3.71	5.33	1.50	.74	11.50
2	17.20	94	3.99	8.30	12.00	.76	13.29
3	18.20	100	4.49	8.27	3.70	1.22	6.10
4	18.50	79	3.61	8.96	6.00	1.05	13.51
5	4.20	93	.96	13.56	1.60	.86	11.98
6	26.20	75	4.85	8.73	2.00	.70	16.20
7	11.30	76	2.12	8.69	-	1.04	11.30
8	8.70	74	1.59	9.43	14.30	.76	9.50
9	24.80	84	5.14	15.25	3.30	.86	17.80
10	17.90	90	3.98	8.54	.40	1.18	9.60
11	19.20	95	4.50	13.85	.80	.79	6.80
12	20.40	88	4.43	10.86	25.60	1.48	15.70

HOMA-IR = Homeostasis Model of Assessment of Insulin Resistance; IL-6 = Interleukin-6; TNF- α = Tumor Necrosis Factor Alpha.
(-) denotes missing data

Table 5. ownNOSAS Markers of Insulin Resistance

Subject	Insulin (μU/L)	Glucose (mg/dl)	HOMA-IR	Adiponectin (μg/ml)	IL-6 (ng/ml)	TNF-α (ng/ml)	Leptin (ng/ml)
1	25.80	87	5.54	10.79	16.70	.99	12.71
2	3.00	-	-	16.25	.60	.64	2.63
3	4.20	104	1.08	10.44	.40	1.22	2.53
4	38.60	109	10.39	5.47	.70	.63	21.82
5	15.30	104	3.93	6.19	.40	1.17	14.84
6	28.90	104	7.42	16.24	3.60	.86	20.34
7	16.80	91	3.77	12.16	2.20	.91	16.25
8	7.10	94	1.65	17.21	3.60	1.16	5.37
9	14.40	87	3.09	12.28	.50	.64	11.76
10	4.00	81	.80	27.42	15.80	.90	8.30
11	4.60	81	.92	11.37	1.80	.78	6.50
12	7.90	84	1.64	21.94	28.20	.86	12.40
13	4.00	85	.84	14.85	2.20	.93	6.50
14	14.90	98	3.61	10.39	.40	.64	9.00
15	14.90	107	3.94	18.68	.80	1.07	5.00
16	7.40	86	1.57	15.82	.40	.50	3.70
17	8.80	92	2.00	3.05	15.50	.72	7.10
18	4.90	88	1.06	19.67	26.30	.79	7.10

HOMA-IR = Homeostasis Model of Assessment of Insulin Resistance; IL-6 = Interleukin-6; TNF- α = Tumor Necrosis Factor Alpha.
 (-) denotes missing data

Table 6. nwNOSAS Markers of Insulin Resistance

Subject	Insulin (μU/L)	Glucose (mg/dl)	HOMA-IR	Adiponectin (μg/ml)	IL-6 (ng/ml)	TNF-α (ng/ml)	Leptin (ng/ml)
1	8.30	95	1.95	5.34	14.20	.78	3.47
2	6.20	94	1.44	6.51	6.20	.97	.90
3	5.40	72	.96	21.47	4.90	.78	4.80
4	3.40	99	.83	11.49	8.60	1.49	-
5	3.00	82	.61	14.23	.40	.73	4.80
6	5.20	81	1.04	-	-	-	6.83
7	5.50	96	1.30	11.60	15.30	.77	4.14
8	11.00	69	1.87	17.02	2.30	.78	5.90
9	7.20	94	1.67	16.87	3.90	.80	2.40
10	14.80	103	3.76	13.27	2.00	.88	4.00
11	4.00	65	.64	22.48	4.10	.56	3.20
12	5.50	84	1.14	12.64	23.80	.99	7.80
13	5.30	85	1.11	17.91	7.10	.84	1.80
14	6.00	77	1.14	12.36	.40	.79	3.50
15	5.60	84	1.16	18.93	-	.98	3.10

HOMA-IR = Homeostasis Model of Assessment of Insulin Resistance; IL-6 = Interleukin-6; TNF- α = Tumor Necrosis Factor Alpha.
(-) denotes missing data

Table 7. owOSAS Markers of Metabolic Syndrome

Subject	Waist Size (cm)	Triglycerides (mg/dl)	Glucose (mg/dl)	HDL cholesterol (mg/dl)	Systolic BP (mmHg)	Diastolic BP (mmHg)	Number of MetS Components
1	119.0	133	94	48	122	88	2
2	110.0	222	94	30	116	88	4
3	115.0	128	100	29	132	78	4
4	107.0	290	79	41	120	80	1
5	112.0	45	93	34	112	82	1
6	129.0	213	75	33	126	90	4
7	221.0	144	76	28	122	86	2
8	107.0	66	74	31	110	86	2
9	224.5	61	84	37	124	74	2
10	111.5	131	90	19	140	86	3
11	109.0	73	95	54	134	88	1
12	129.0	134	88	25	132	90	2

Table 8. owNOSAS Markers of Metabolic Syndrome

Subject	Waist Size (cm)	Triglycerides (mg/dl)	Glucose (mg/dl)	HDL cholesterol (mg/dl)	Systolic BP (mmHg)	Diastolic BP (mmHg)	Number of MetS Components
1	92.8	92	87	26	122	98	2
2	82.0	56	104	34	124	82	2
3	123.0	154	109	25	102	72	4
4	98.5	135	104	32	120	88	3
5	98.5	95	104	33	122	84	2
6	115.0	66	91	27	124	82	2
7	92.8	150	94	49	132	100	2
8	115.0	60	87	38	118	88	3
9	95.5	69	81	29	110	78	1
10	91.0	45	85	70	158	102	1
11	96.0	114	98	43	128	100	1
12	103.0	100	107	33	124	86	4
13	86.0	63	86	40	118	82	0
14	86.0	109	92	35	132	82	2
15	93.0	85	88	52	108	78	0

Table 9. nwNOSAS Markers of Metabolic Syndrome

Subject	Waist Size (cm)	Triglycerides (mg/dl)	Glucose (mg/dl)	HDL cholesterol (mg/dl)	Systolic BP (mmHg)	Diastolic BP (mmHg)	Number of MetS Components
1	80.0	175	95	36	130	90	3
2	75.0	50	94	31	108	78	1
3	75.0	44	72	34	118	86	2
4	90.0	93	99	26	112	80	1
5	77.5	172	82	33	124	78	2
6	86.0	65	81	38	122	86	2
7	75.5	45	96	22	132	84	2
8	76.0	120	69	38	116	84	1
9	77.0	106	94	40	128	82	0
10	78.0	79	103	60	124	82	1
11	68.0	45	65	66	108	70	0
12	85.0	52	84	25	106	84	1
13	69.0	45	85	63	120	70	0
14	85.5	45	77	42	128	80	0
15	76.5	54	84	54	138	72	1

Table 10. owOSAS Biomarkers of Endothelial Dysfunction

Subject	CRP (mg/L)	VEGF (pg/ml)	VEGFR2r (%)	ADMA (μmol/L)
1	.63	19.10	-	.54
2	.63	84.40	-	.75
3	1.31	297.90	-	.47
4	.46	121.50	.70	.43
5	1.76	156.20	1.00	.46
6	2.10	249.90	1.10	.36
7	1.04	322.80	1.10	.44
8	.76	203.60	2.00	.52
9	1.49	92.70	1.60	.41
10	1.00	37.50	10.40	.37
11	8.75	542.40	8.40	.47
12	3.35	69.40	8.70	.81

CRP = C – Reactive Protein; VEGF = Vascular Endothelial Growth Factor; VEGFR2 = Expression of Vascular Endothelial Growth Factor Receptor 2 in Monocytes; ADMA = Asymmetric Dimethylarginine.
(-) denotes missing data

Table 11. owNOSAS Biomarkers of Endothelial Dysfunction

Subject	CRP (mg/L)	VEGF (pg/ml)	VEGFR2r (%)	ADMA (μmol/L)
1	1.02	512.40	1.20	.62
2	.29	8.30	-	.67
3	.11	54.50	-	.53
4	5.01	199.50	-	.53
5	1.26	94.80	1.80	.47
6	4.05	51.60	-	.61
7	10.22	124.70	-	.41
8	5.93	84.10	-	.53
9	.49	144.40	4.00	.44
10	.98	51.10	10.70	.78
11	.95	306.00	1.40	.40
12	.58	86.30	1.70	.41
13	.44	134.70	11.70	.44
14	3.22	198.70	.80	.48
15	.16	403.30	4.80	.52
16	.08	207.00	4.90	.55
17	.74	397.50	10.20	.43
18	.20	149.60	5.70	.68

CRP = C – Reactive Protein; VEGF = Vascular Endothelial Growth Factor; VEGFR2 = Expression of Vascular Endothelial Growth Factor Receptor 2 in Monocytes; ADMA = Asymmetric Dimethylarginine.
 (-) denotes missing data

Table 12. nwnOSAS Biomarkers of Endothelial Dysfunction

Subject	CRP (mg/L)	VEGF (pg/ml)	VEGFR2r (%)	ADMA (μ mol/L)
1	.31	12.70	-	.37
2	.16	51.50	-	.49
3	.23	41.30	8.40	.33
4	.33	45.20	-	.38
5	.95	206.20	3.01	.55
6	-	38.40	-	.79
7	.25	44.80	-	.57
8	.33	11.10	3.50	.48
9	.06	184.60	.90	.49
10	.34	80.10	13.10	.54
11	.37	144.10	5.80	.61
12	2.94	31.50	5.00	.51
13	.54	36.10	16.20	.57
14	.56	120.10	9.20	.66
15	.14	146.50	2.60	.55

CRP = C – Reactive Protein; VEGF = Vascular Endothelial Growth Factor; VEGFR2r= Expression of Vascular Endothelial Growth Factor Receptor 2 in Monocytes; ADMA = Asymmetric Dimethylarginine.
 (-) denotes missing data

Table 13. owOSAS Postocclusive Reactive Hyperemia

Subject	Inflow	RH0	RH15	RH30	RH45	RH60	RH75	RH90	RH105	RH120
1	3.03	20.06	11.58	7.52	6.27	5.49	3.56	2.83	3.88	2.66
2	2.54	18.38	9.86	7.43	4.08	2.82	2.63	2.75	1.98	1.31
3	9.51	23.75	16.02	15.06	17.79	13.32	9.10	11.20	9.79	10.50
4	1.93	22.44	13.21	14.09	9.56	6.39	4.71	3.82	2.82	3.10
5	2.29	23.98	18.82	15.09	10.68	5.51	3.80	3.39	2.63	2.51
6	3.51	30.04	13.24	11.37	9.87	8.24	7.24	5.13	5.75	3.49
7	2.48	37.06	14.48	11.13	10.46	9.54	8.05	6.07	5.69	3.99
8	3.91	20.26	15.99	16.55	12.83	13.20	7.71	5.13	8.16	4.41
9	2.47	21.72	14.26	9.41	9.13	6.84	4.68	3.12	3.62	2.08
10	4.29	33.92	22.53	22.01	18.94	13.45	10.77	7.65	6.02	4.92
11	3.23	28.45	15.00	9.23	8.45	6.58	4.71	3.76	3.16	2.70
12	2.49	28.67	16.23	13.36	9.27	7.24	5.13	5.48	5.42	5.95

* Units are ml/min/100ml

RH0 = Reactive Hyperemia at 0 seconds; RH15 = Reactive Hyperemia at 15 seconds; RH30 = Reactive Hyperemia at 30 seconds; RH45 = Reactive Hyperemia at 45 seconds; RH60 = Reactive Hyperemia at 60 seconds; RH75 = Reactive Hyperemia at 75 seconds; RH90 = Reactive Hyperemia at 90 seconds; RH105 = Reactive Hyperemia at 105 seconds; RH120 = Reactive Hyperemia at 120 seconds.

Table 14. owNOSAS Postocclusive Reactive Hyperemia

Subject	Inflow	RH0	RH15	RH30	RH45	RH60	RH75	RH90	RH105	RH120
1	2.32	19.33	12.70	12.34	8.17	4.97	6.00	3.57	3.98	2.08
2	2.49	16.70	11.97	7.39	5.50	5.03	4.22	3.33	2.52	2.61
3	3.49	29.98	22.90	22.38	14.77	8.96	4.39	4.76	5.12	3.31
4	2.64	16.94	14.86	12.64	9.75	7.54	5.91	5.08	4.28	3.73
5	2.14	19.67	7.42	4.21	3.19	2.75	2.67	1.61	1.92	1.55
6	2.22	26.14	10.63	5.25	3.75	2.85	2.44	2.17	2.11	1.98
7	1.82	11.15	6.50	4.65	3.34	2.54	2.02	1.40	1.66	1.48
8	1.95	10.30	9.72	6.02	4.72	4.56	3.29	2.60	2.57	1.39
9	3.18	35.38	17.90	14.69	11.00	7.92	6.33	4.92	4.30	4.63
10	2.03	17.16	10.08	8.96	5.94	5.13	3.92	2.13	3.14	2.68
11	4.46	30.17	15.23	11.55	8.79	7.71	6.54	5.15	4.83	4.39
12	2.75	19.75	10.21	6.03	4.44	3.64	3.44	2.87	2.45	2.19
13	2.76	24.93	15.39	14.90	11.33	9.77	7.79	5.26	3.98	3.60
14	2.33	15.95	16.76	9.01	6.72	5.29	3.15	2.63	2.39	1.98
15	3.53	28.15	14.63	11.45	9.93	7.49	6.31	4.35	4.89	3.76
16	4.77	36.91	16.95	18.15	14.31	10.46	9.17	5.29	4.31	3.12
17	5.71	21.10	16.19	10.64	8.51	6.49	5.17	4.47	3.81	3.05
18	4.08	26.51	12.63	9.14	7.57	7.65	5.75	5.88	4.62	4.14

* Units are ml/min/100ml

RH0 = Reactive Hyperemia at 0 seconds; RH15 = Reactive Hyperemia at 15 seconds; RH30 = Reactive Hyperemia at 30 seconds; RH45 = Reactive Hyperemia at 45 seconds; RH60 = Reactive Hyperemia at 60 seconds; RH75 = Reactive Hyperemia at 75 seconds; RH90 = Reactive Hyperemia at 90 seconds; RH105 = Reactive Hyperemia at 105 seconds; RH120 = Reactive Hyperemia at 120 seconds.

Table 15. nwNOSAS Postocclusive Reactive Hyperemia

Subject	Inflow	RH0	RH15	RH30	RH45	RH60	RH75	RH90	RH105	RH120
1	2.70	22.94	21.76	11.33	8.58	6.30	5.03	4.08	3.80	3.48
2	4.13	32.47	12.67	7.24	5.51	5.25	6.32	4.78	4.00	4.28
3	1.78	23.53	10.79	10.98	6.80	6.37	3.72	3.01	2.31	1.60
4	3.92	29.10	20.84	14.07	10.56	6.75	5.05	3.80	3.20	2.95
5	3.27	21.66	10.56	4.97	3.06	2.80	3.49	3.36	2.88	3.07
6	3.50	23.37	13.37	10.10	8.46	7.07	6.23	5.67	4.15	3.99
7	3.30	29.00	15.25	7.37	5.20	3.78	3.40	3.12	3.18	2.15
8	2.17	28.84	15.44	12.46	9.89	7.05	6.05	4.05	3.20	3.49
9	2.66	23.83	10.08	5.43	4.04	3.29	3.58	2.83	3.07	1.71
10	4.35	25.19	16.27	9.91	6.76	5.20	3.85	3.48	4.33	5.18
11	3.39	18.24	15.77	13.72	11.16	10.67	6.27	4.62	4.52	4.26
12	1.36	14.41	8.67	2.92	2.69	2.40	1.80	1.96	1.74	1.15
13	2.07	22.70	13.08	8.79	6.68	5.15	4.39	4.79	4.26	3.41
14	4.16	27.22	19.29	17.05	15.99	14.71	8.23	5.49	4.55	4.15
15	3.23	14.23	10.08	6.03	5.31	3.96	3.84	4.01	3.60	3.70
16	2.79	26.30	14.01	10.52	8.96	6.91	5.78	5.17	5.35	3.64

* Units are ml/min/100ml

RH0 = Reactive Hyperemia at 0 seconds; RH15 = Reactive Hyperemia at 15 seconds; RH30 = Reactive Hyperemia at 30 seconds; RH45 = Reactive Hyperemia at 45 seconds; RH60 = Reactive Hyperemia at 60 seconds; RH75 = Reactive Hyperemia at 75 seconds; RH90 = Reactive Hyperemia at 90 seconds; RH105 = Reactive Hyperemia at 105 seconds; RH120 = Reactive Hyperemia at 120 seconds.

VITA

Stephen Gregory Guill was born March 12, 1979 in South Boston, Virginia. He graduated from Halifax County High School in 1996, and is also an alumnus to the Southside Virginia Governor's School for Global Economics and Technology in Keysville, Virginia. Steve began working in the exercise science field in his junior year at Virginia Tech, and volunteered to help train subjects in a study funded by the US Army. After graduating from Virginia Tech with honors in 2001, Steve enrolled in a master's program in Health and Exercise Science at Wake Forest University. While there, he became coordinator for the stress testing laboratory for their cardiac rehabilitation center. Stephen graduated from Wake Forest in 2003, and knew he wanted to continue a career that allowed him to conduct research and assist in clinical exercise programs. Virginia Tech presented the ideal opportunity for this, and Stephen began working on his doctorate in clinical exercise physiology in the summer of 2003. During this time, Stephen developed an interest in both gerontology and molecular cell biology and biotechnology, and decided to pursue doctoral certificates in both of these areas. Stephen also happily served as the laboratory coordinator for Virginia Tech's cardiac rehabilitation program, and played a vital role in the expansion of the program into an exercise program that welcomed those individuals wishing to prevent chronic disease as well. Stephen successfully defended his dissertation on April 20, 2007. As graduation approached, Stephen accepted an offer from the Virginia College of Osteopathic Medicine to become the Program Director for their clinical exercise program. Currently, Stephen also teaches clinical nutrition at VCOM and continues to pursue research in clinical exercise physiology and obstructive sleep apnea syndrome.