Adverse Effects of Obstructive Sleep Apnea:
Interindividual Differences in Symptoms and Biomarkers

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Thesis for the degree of Philosophiae Doctor
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Doctoral committee: Karl Andersen, Gudmundur H. Gudmundsson and Greg Maislin

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Neikvæðar afleiðingar kæfisvefns:
Breytileg einkenni og lífmerki eftir einstaklingum

Erna Sif Arnardóttir

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To my Mom and Dad

Doing a PhD is a journey, not a destination. Enjoy it!

Thorarinn Gislason

Ágrip

Inngangur: Kæfisvefn er algengur svefnsjúkdómur sem einkennist af tíðum öndunarstoppum í svefni og óeðlilegri dagsyfju. Faraldsfræði-rannsóknir sýna að a.m.k. 2 - 4% almennings þjáist af kæfisvefni en tíðnin er mun hærri hjá einstaklingum með offitu. Kæfisvefnssjúklingar hrjóta flestir hátt en eru að auki með ýmis önnur einkenni í breytilegum mæli t.d. nætursvita. Mikilvægt er að auka þekkingu á einkennum kæfisvefns því slík einkenni veita m.a. vísbendingu um hvenær ástæða er til svefnmælingar hjá einstaklingum með ógreindan kæfisvefn. Kæfisvefn er þekktur áhættuþáttur fyrir hjarta- og æðasjúkdóma, en hluti sjúklinga virðist þó verndaður fyrir þeim neikvæðu afleiðingum kæfisvefns. Af þessum sökum er áríðandi að skilja betur breytileika milli einstaklinga m.a. með mælingum á bólguboðefnum sem gefa til kynna áhættu á hjarta- og æðasjúkdómum. Þær upplýsingar nýtast til þróunar á einstaklingsbundinni meðferð kæfisvefns og markvissari forvarna á tengdum sjúkdómum.

Tilgangur: Að meta hvort tíður nætursviti sé einkenni kæfisvefns. Að meta hver séu sjálfstæð áhrif kæfisvefns á styrk bólguboðefnanna interleukin-6 (IL-6), C-reactive protein (CRP) og leptíns í blóði, óháð offitu. Að meta þátt einstaklingsbreytileika í nætursvita og boðefnastyrk í blóði.

Aðferðir: Samband kæfisvefns og einkenna nætursvita var metið með samanburði á 822 ómeðhöndluðum kæfisvefnssjúklingum (Íslenska kæfisvefnsrannsóknin, 666 karlar og 156 konur) og 703 þátttakendum úr almennu þýði (374 karlar og 329 konur). Kæfisvefnssjúklingarnir voru endurmetnir tveimur árum eftir að meðferð með svefnöndunartæki hófst (n = 741, 90,1%). Kæfisvefnssjúklingarnir fóru í svefnmælingu þar sem alvarleiki kæfisvefns var mældur með fjórum mismunandi stöðlum: fjöldi öndunarstoppa/klst. (AHI), fjöldi súrefnismettunarfalla/klst. (ODI), tími í súrefnisskorti (< 90% súrefnismettun) og lágmarksgildi súrefnismettunar yfir nóttina. Segulómun af kvið m.t.t. iðrafitu og fitu undir húð var einnig gerð. Í úrtaki 15 annars heilbrigðra karla með kæfisvefn, var nætursviti metinn sem húðleiðnistuðull (EDA) í svefni (fjöldi atburða/klst.) bæði fyrir og eftir 3 mánaða meðferð. Mæling á IL-6, CRP og leptíni í blóðvökva var framkvæmd hjá fyrstu 454 ómeðhöndluðu kæfisvefnssjúklingunum í Íslensku kæfisvefnsrannsókninni.

Niðurstöður: Tíðum nætursvita (≥ 3x viku) var lýst hjá 31,1% kæfisvefnssjúklinga en aðeins 11,1% af almennu þýði ($p < 0,001$). Þessi munur hélst marktækur eftir að tekið hafði verið tillit til lýðfræðilegra þátta. Tíður nætursviti tengdist lægri aldri, greindum hjarta- og æðasjúkdómum ásamt háþrýstingi, dagsyfju og erfiðleikum við að sofna og viðhalda svefni en enginn kynjamunur fannst. Algengi tíðs nætursvita minnkaði í 11,5%

við meðferð með svefnöndunartæki ($p < 0,003$). Hjá þeim 15 kæfisvefnssjúklingum þar sem sviti var mældur, lækkaði húðleiðnistuðull einnig marktækt við meðferð, að meðaltali (± staðalfrávik) úr 132 (± 22) í 79 (± 18) atburði/klst ($p = 0,04$). Ómeðhöndlaðir kæfisvefnssjúklingar með háan húðleiðnistuðul voru einnig líklegri til að vera með háan slagþrýsting, bæði kvölds og morgna ($p < 0,01$). Fylgni var á milli lækkunar á húðleiðnistuðli við meðferð og lækkunar á blóðþrýstingi, bæði slag- og þanþrýstingi ($p < 0,05$).

Hjá ómeðhöndluðum kæfisvefnssjúklingum ($n = 454$) var marktæk fylgni á milli aukins alvarleika súrefnismettunarfalla og hærri styrks IL-6, CRP og leptíns en engin fylgni við fjölda öndunarstoppa. Þegar hópnum var skipt í þrennt eftir líkamsþyngdarstuðli (body mass index, BMI) (BMI < 30, BMI 30 - < 35 and BMI ≥ 35 kg/m^2), var alvarleiki kæfisvefns einungis tengdur blóðstyrk IL-6 og CRP hjá þátttakendum með offitu. Hins vegar fundust engin tengsl á milli alvarleika kæfisvefns og leptínstyrks, innan neins BMI hóps. Fjölþátta línuleg aðhvarfsgreining staðfesti sjálfstæð tengsl milli alvarleika kæfisvefns og IL-6 styrks og víxlverkun milli alvarleika kæfisvefns og BMI. Offita breytti tengslum milli alvarleika kæfisvefns og IL-6 þannig að lítil sem engin tengsl voru hjá einstaklingum með BMI < 30 en alvarleiki kæfisvefns var einungis tengdur IL-6 styrk hjá einstaklingum með offitu. Svipuð en veikari tengsl fundust milli alvarleika kæfisvefns og BMI á CRP blóðstyrk hjá karlmönnum og en eingöngu hjá konum eftir tíðahvörf. Hins vegar fundust engin marktæk tengsl milli alvarleika kæfisvefns og leptínstyrks í blóði, en kyn útskýrði 21,2% af breytileika og BMI 38,7% í leptínstyrk.

Ályktanir: Tíður nætursviti er einkenni kæfisvefns sem a.m.k. þriðjungur kæfisvefnssjúklinga upplifir. Einkenni tíðs nætursvita voru þrisvar sinnum algengari hjá ómeðhöndluðum kæfisvefnssjúklingum en í almennu þýði og lækkaði niður í algengi almenns þýðis við reglubundna meðferð með svefnöndunartæki. Mælingar sýndu einnig minnkaðan nætursvita við meðferð kæfisvefns. Tíður nætursviti hjá kæfisvefnssjúklingum er mögulega merki um aukna áhættu á sjúkdómum í hjarta- og æðakerfi. Alvarleiki kæfisvefns hefur sjálfstætt forspárgildi fyrir styrk IL-6 og CRP bólguboðefna í blóði og aukna áhættu á hjarta- og æðasjúkdómum en þessi tengsl finnast eingöngu hjá kæfisvefnssjúklingum með offitu. Hins vegar eru offita og kyn helstu spáþættir fyrir leptínstyrk. Við mat á neikvæðum afleiðingum kæfisvefns þarf því að hafa í huga einstaklingsbundinn breytileika í viðbrögðum við kæfisvefni.

Lykilorð: Kæfisvefn, offita, nætursviti, boðefni, bólguviðbrögð

Abstract

Introduction: Obstructive sleep apnea (OSA) is a common sleep disorder that is characterised by frequent cessation of breathing during sleep and excessive daytime sleepiness. Epidemiological studies show that at least 2 - 4% of the general population suffers from OSA and that the prevalence is much higher in obese subjects. OSA patients usually snore loudly but also have other more variable symptoms such as nocturnal sweating. It is important to recognise better the clinical symptoms of OSA as such knowledge is e.g. informative for the need of a sleep study in undiagnosed OSA patients. OSA is a known risk factor for cardiovascular disease (CVD) but some patients appear to be protected against the adverse consequences of OSA. It is important to understand this inter-individual difference better, e.g. by measuring inflammatory biomarkers as indicators of increased CVD risk. This information can be used for the development of personalized treatment of OSA and better prevention of its comorbidities.

Objectives: To assess whether frequent nocturnal sweating is a symptom of OSA. To assess the relationship of OSA severity with levels of inflammatory biomarkers interleukin-6 (IL-6), C-reactive protein (CRP) and leptin in blood, independent of obesity. To evaluate the role of interindividual differences in both nocturnal sweating and levels of inflammatory biomarkers in blood.

Methods: The relationship between OSA and reported nocturnal sweating was assessed by comparing 822 untreated OSA subjects in the Icelandic Sleep Apnea Cohort (ISAC, 666 males and 156 females) to 703 subjects in a general population cohort (374 males and 329 females). The ISAC cohort was re-assessed two years after starting positive airway pressure (PAP) treatment (n = 741, 90.1%). The OSA patients underwent a sleep study assessing OSA severity by four different indices; the apnea-hypopnea index (AHI), the oxygen desaturation index (ODI), hypoxia time (minutes with oxygen saturation < 90%) and the minimum oxygen saturation ($minSaO_2$) during the night. Magnetic resonance imaging of abdominal visceral and subcutaneous fat volume was performed. Objective nocturnal sweating was assessed by the electrodermal activity (EDA) index during sleep in a subset of 15 otherwise healthy OSA males while untreated and after 3 months of PAP treatment. Measurement of serum IL-6, CRP and leptin levels was performed cross-sectionally for the first 454 untreated OSA subjects in the ISAC cohort.

Results: Frequent nocturnal sweating (≥ 3x a week) was reported by 31.1% of the OSA cohort *vs*. only 11.1% of the general population cohort

($p < 0.0001$). This difference remained significant after adjustment for demographic factors. Nocturnal sweating was related to younger age, presence of CVD and hypertension, daytime sleepiness, and difficulties initiating and maintaining sleep but no gender differences were found. The prevalence of frequent nocturnal sweating decreased to 11.5% with PAP treatment ($p < 0.003$). Also, in the 15 OSA patients with objective measures of sweating, the mean (± standard deviation) EDA index during sleep decreased from 132 (± 22) events/hr. in untreated patients to 79 (± 18) events/hr. on PAP treatment ($p = 0.04$). Untreated patients with high EDA indices also had high systolic blood pressure in the evening and morning ($p < 0.01$). A decrease in EDA index with treatment correlated with a decrease in systolic and diastolic blood pressure ($p < 0.05$).

In untreated ISAC participants ($n = 454$), oxygen desaturation severity indices were significantly correlated with higher levels of IL-6, CRP and leptin, but AHI was not. When stratified by BMI category (BMI < 30, BMI 30 - 35 and BMI ≥ 35 kg/m^2), OSA severity was associated with IL-6 and CRP levels in obese participants only. However, no relationship was found between OSA severity and leptin levels, in any of the BMI groups. A multiple linear regression model confirmed an independent association between OSA severity and IL-6 levels as well as an interaction between OSA severity and BMI. The degree of obesity altered the relationship between OSA severity and IL-6 levels, so that no relationship was found in the nonobese but only in the obese subjects. A similar but weaker relationship was found between OSA severity and BMI on CRP levels for males and postmenopausal women. However, no relationship of OSA severity was found with leptin levels, but gender explained 21.2% of the variance and BMI 38.7% in leptin levels.

Conclusions: Frequent nocturnal sweating is reported by a third of OSA patients. The prevalence of reported frequent nocturnal sweating was threefold higher in untreated OSA patients than in the general population and decreased to general population levels with full PAP treatment. Objective measurements also showed a decrease of nocturnal sweating with treatment. Frequent nocturnal sweating in OSA patients may be an indicator of increased CVD risk. OSA severity is an independent predictor of increased IL-6 and CRP levels and increased CVD risk but this association is only found in obese patients. However, obesity and gender are the dominant determinants of leptin levels. When assessing the adverse effects of OSA, interindividual differences in the response to OSA need to be considered.

Keywords: obstructive sleep apnea, obesity, nocturnal sweating, biomarkers, inflammatory response

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I think the African proverb “It takes a village to raise a child” fully applies to PhD students as well, as I truly have a village to thank aside from the co-authors of the papers. I am grateful to all my friends and co-workers at the Landspitali – The National University Hospital of Iceland, who have supported me throughout all this work. I would especially like to thank my favorite sleep technologists: Magdalena Ósk Sigurgunnarsdóttir, Sigríður Sigurðardóttir, Kristín Anna Ólafsdóttir, Sigrún Sigmundsdóttir, Atli Jósefsson, Ólafía Ása Jóhannesdóttir and Halldóra Brynjólfsdóttir. These papers would also never have been published without the amazing and tireless work of Lovísa Guðmundsdóttir, Sigrún Guðmundsdóttir and Kristín Bára Jörundsdóttir, who recruited and met with the majority of the ISAC and BOLD participants. The nurses administering sleep apnea treatment at the hospital are also wonderful, always hard at work and willing to help, especially Oddný Fjóla Lárusdóttir, Björg Eysteinsdóttir and Bryndís Halldórsdóttir whom I am especially grateful. The other statistical people in ISAC: Kristján Andri Kristjánsson, Brendan Keenan and Robert Hachadoorian for database management. Guðmundur Sævarsson, software engineer at Nox Medical, for his help in creating an automatic analysis of the EDA signal, which saved me many hours of manual work, and the whole team at Nox Medical and former Flaga for their help with the devices and software used in this thesis. Elizabeth Cook for her work on biomarker measurement (CRP and sympathetic markers) as well as Heather Collins and the other staff at the Radioimmunoassay and Biomarker Core, Diabetes and Endocrinology Research Center, University of Pennsylvania (NIH DK 19525), who performed the leptin measurements. Frances Pack for all her hard work and very friendly manner. Matthew Thorne-Fitzgerald and the other staff at the University of Pennsylvania who helped assemble and analyze the data, as well as Daniel Barrett, Karen McLaughlin, Jennifer Montoya, Anna Sveinbjörg Hansdóttir and Terry G. Lacy for their help in the preparation of the manuscripts and thesis. I would also like to thank the graphical designers who assisted with the preparation of some of the figures in the papers and thesis; Þórhildur Ögn Jónsdóttir, Ólöf Jóna Guðmundsdóttir, Guðný Þórarinsdóttir and Árni Collett as well as Björg Þorleifsdóttir (again), Una Björk Ómarsdóttir, Auður Eiríksdóttir and Þröstur Freyr Gylfason for their corrections of the Icelandic abstract. Also my utmost thanks go to Björgvin Ragnarsson at Háskólaprent for all his assistance with the printing of this thesis.

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Contents

List of abbreviations

AHI	Apnea-hypopnea index
ANOVA	analysis of variance
biPAP	Bilevel positive airway pressure
BMI	Body mass index
BOLD	Burden of Lung Disease
CI	Confidence interval
CIH	Chronic intermittent hypoxia
COPD	Chronic obstructive pulmonary disease
CPAP	Continuous positive airway pressure
CRP	C-reactive protein
CVD	Cardiovascular disease
EDA	Electrodermal activity index
EEG	Electroencephalography
EMG	Electromyography
ESS	Epworth Sleepiness Scale
FFA	Free fatty acids
GER	Gastroesophageal reflux
HIF-1α	Hypoxia induction factor-1 alpha
HRT	Hormone replacement therapy
ICAM-1	Intercellular adhesion molecule-1
ICC	Intraclass correlation coefficient
IL-6	Interleukin-6
ISAC	The Icelandic Sleep Apnea Cohort
MAP	Multivariable Apnea Prediction
MI	myocardial infarction
MRI	Magnetic resonance imaging
NF-κB	Nuclear factor kappa B
ODI	Oxygen desaturation index
OR	Odds ratio
OSA	Obstructive sleep apnea
OSAS	Obstructive sleep apnea syndrome
PAP	Positive airway pressure
PSG	Polysomnography

REM	Rapid eye movement
RLS	Restless legs syndrome
RMI	Respiratory mechanics instability
ROS	Reactive oxygen species
SaO_2	Oxygen saturation
SAT	Subcutaneous adipose tissue
SD	Standard deviation
TNF-α	Tumor necrosis factor-alpha
VAT	Visceral adipose tissue
VCAM-1	Vascular adhesion molecule-1

List of figures

List of tables

This thesis is based on the following original publications, which are referred to in the text by their Roman numerals (I-V):

I. ES Arnardottir, B Thorleifsdottir, E Svanborg, I Olafsson and T Gislason. “Sleep-related sweating in obstructive sleep apnoea: association with sleep stages and blood pressure”. *Journal of Sleep Research* 2009; 19 (1p2): 122-130.

II. ES Arnardottir, C Janson, E Bjornsdottir, B Benediktsdottir, S Juliusson, S Kuna, AI Pack, T Gislason. “Nocturnal Sweating – A Common Symptom of Obstructive Sleep Apnea: The Icelandic Sleep Apnea Cohort”. *BMJ Open* 2013; 3 (5) e002795.

III. ES Arnardottir, G Maislin, RJ Schwab, B Staley, B Benediktsdottir, I Olafsson, S Juliusson, M Romer, T Gislason*, AI Pack*. *Co-senior authors. “The Interaction of obstructive sleep apnea and obesity on the inflammatory markers C-reactive protein and interleukin-6: The Icelandic Sleep Apnea Cohort”. *Sleep* 2012, 35 (7): 921-932.

IV. ES Arnardottir, G Maislin, N Jackson, RJ Schwab, B Benediktsdottir, K Teff, S Juliusson, AI Pack*, T Gislason*. *Co-senior authors. “The Role of Obesity, Different Fat Compartments and Sleep Apnea Severity in Circulating Leptin Levels: The Icelandic Sleep Apnea Cohort Study”. *International Journal of Obesity*, Advance online publication Sept 11, 2012.

Additional review paper in thesis:

V. ES Arnardottir, M Mackiewicz, T Gislason, KL Teff, A.I. Pack. “Molecular signatures of obstructive sleep apnea in adults: A review and perspective”. *Sleep* 2009; 32 (4): 447-470.

In addition, some unpublished data are presented regarding CRP measurements in blood related to papers I and II, sleepiness in paper I and blood pressure measurements in paper II.

Declaration of contribution

Below is a declaration of my contribution to each paper, on which the thesis is based. The contribution of co-authors is acknowledged in a special section above.

Paper I: I designed the study with the mentors of my MS thesis, Thorarinn Gislason, Bjorg Thorleifsdottir and Eva Svanborg. I applied for ethical permission, study funding, performed all the subject recruitment, measurements and data analysis, except Isleifur Olafsson and his staff performed the measurements of sympathetic metabolites. I performed all statistical analyses, drafted the paper and participated in all revisions of the paper with the co-authors.

Paper II: I designed the study with my PhD mentors, Thorarinn Gislason and Allan I. Pack. I participated in the data collection (mainly database quality assurance) but this large data collection involved the staff of both Sleep Centers of Landspitali – The National University Hospital of Iceland and the University of Pennsylvania. I performed all statistical analyses with the help of Christer Janson, drafted the paper and participated in all revisions of the paper with the co-authors.

Paper III: I designed the study with my PhD mentors, Thorarinn Gislason and Allan I. Pack and Greg Maislin, a member of the PhD committee. I participated in the data collection (mainly database quality assurance) but this large data collection involved the staff of both Sleep Centers of Landspitali – The National University Hospital of Iceland and the University of Pennsylvania. Specifically, the biomarker measurements were performed by Isleifur Olafsson (CRP) and his team as well as Micah Romer, under my supervision (IL-6), the MRI assessments were supervised by Richard Schwab and sleep scoring by Bethany Staley. Together with Greg Maislin, I performed all statistical analyses. I drafted the paper and participated in all revisions of the paper with the co-authors.

Paper IV: I designed the study with my PhD mentors, Thorarinn Gislason and Allan I. Pack and Greg Maislin, a member of the PhD committee. I participated in the data collection (mainly database quality assurance) but this large data collection involved the staff of both Sleep Centers of Landspitali – The National University Hospital of Iceland and the University of Pennsylvania. Specifically, the biomarker measurements

were performed by Heather Collins at the Radioimmunoassay and Biomarker Core, Diabetes and Endocrinology Research Center, University of Pennsylvania (NIH DK 19525), the MRI assessments were supervised by Richard Schwab and sleep scoring by Bethany Staley. Together with Nicholas Jackson, I performed all statistical analyses. I drafted the paper and participated in all revisions of the paper with the co-authors.

Paper V: I designed the format of the review and perspective with Allan I. Pack. I collected and read all relevant references and drafted the paper and participated in all revisions of the paper from co-authors.

1 Introduction

1.1 What is obstructive sleep apnea?

Obstructive sleep apnea (OSA) is characterized by repeated cessation of breathing (apneas) or declines in breathing (hypopneas) during sleep due to closure of the upper airway (Figure 1). The severity of OSA is measured by the apnea-hypopnea index (AHI), i.e., the number of apneas and hypopneas per hour of sleep. An AHI less than 5 is considered normal, 5 - ≤15 mild disease, 15-≤ 30 moderate disease, and ≥ 30, a breathing stop at least every other minute, a severe disease (for a review see[1]).

These breathing disturbances result in the following: repetitive decreases in oxygen saturation causing cyclical deoxygenation/reoxygenation; interruption of sleep with frequent arousals (sleep fragmentation) causing loss of rapid eye movement (REM) sleep and slow wave sleep (stages 3-4); episodic hypercapnia and repeated changes in intrathoracic pressure (for reviews, see[1, 2]).

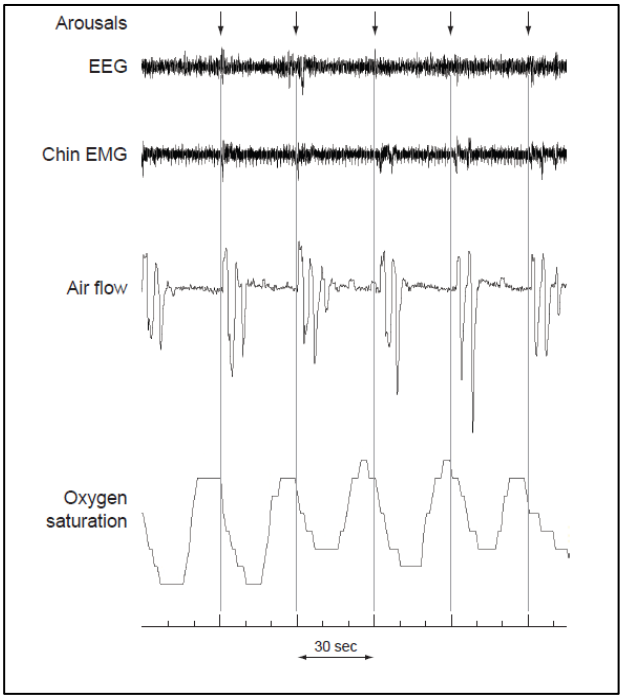

Figure 1. Typical sleep recording of a subject with obstructive sleep apnea (OSA). Recording of airflow shows apneas followed by oxygen desaturations and arousals (arrows) in the electroencephalography (EEG) during sleep. Chin electromyography (EMG) is also shown.

Obstructive sleep apnea is common. It affects at least 4% of middle-aged males and 2% of middle-aged females[3]. Among obese subjects, these percentages are much higher[3, 4]. Moreover, these prevalence estimates are based on a conservative definition of OSA: the presence AHI ≥ 5 and the presence of excessive daytime sleepiness, named *obstructive sleep apnea syndrome* (OSAS). Considering only the presence of disordered breathing during sleep, i.e., with or without complaints of excessive sleepiness, the prevalence is even higher[3].

Interestingly, one of the first OSA patient described in history was likely a character in the Icelandic Snorra-Edda by Snorri Sturluson (1179-1241). There he presents a giant sleeping on his back, snoring massively with symptoms of extreme sleepiness (Figure 2). However, recognition of OSA as an important clinical syndrome began much later and, while clinically described, was considered a rare disorder until 20 – 30 years ago (reviewed by[1]).

The main symptoms of OSA during sleep are loud and disturbing snoring together with witnessed respiration cessations. Other night symptoms may include disturbed sleep and less well described symptoms such as nocturnal sweating, nocturia and gastroesophageal reflux (GER). Daytime symptoms include excessive sleepiness, fatigue and poor concentration (reviewed by[5]).

Figure 2. The giant snoring on his back causing the ground to shake. Þór is trying to smash the giant's head with his hammer. In *Snorra-Edda* by Snorri Sturluson (1179-1241). Reproduced from www.hurstwic.org/history/articles/mythology/myths/text/thor_utgard.htm

Obesity, in particular central obesity, is the most important risk factor for OSA[3, 6, 7]. Among obese subjects the prevalence of OSA is much higher than in general population cohorts, up to ~90% in the morbidly obese[4, 8, 9]. Increased adipose tissue (e.g., parapharyngeal fat pads), as well as enlargement of soft tissues around the airway, such as the tongue, cause a reduction in airway size (Figure 3) and decreased lung volume (reviewed by[10]). Visceral fat accumulation is more associated with adverse health effects than other fat deposits (reviewed by[11]) and may also be more associated with the presence of OSA than other measures of obesity[6, 12, 13]. Other factors that narrow the size of the upper airways such as retroposed mandible and adenotonsillar hypertrophy (reviewed by[14]) are also a risk factor for OSA. A family aggregation is found, even though little is known about specific genes conferring risk (reviewed by[1]).

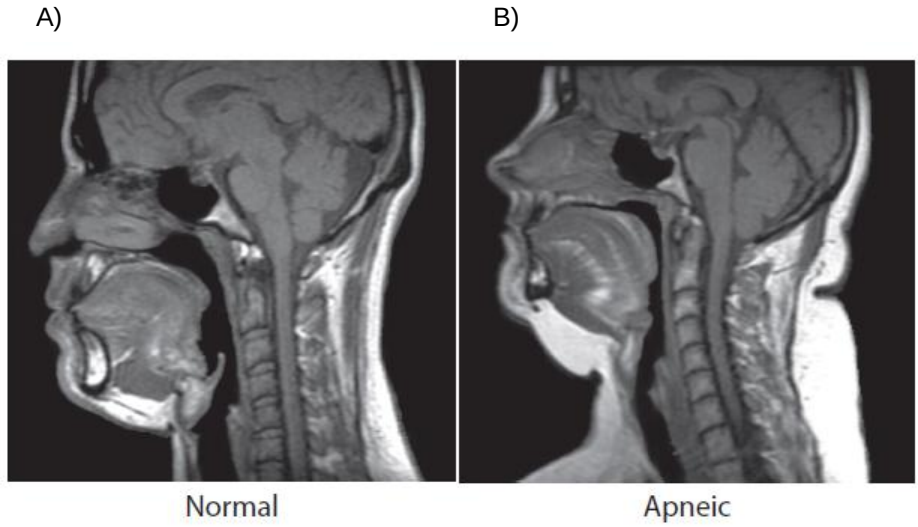

Figure 3. Magnetic resonance imaging (MRI) of the upper airway of a) a normal individual and b) a typical sleep apnea patient. The decreased size of the upper airway due to obesity and narrower upper airway anatomy is clear (e.g. larger tongue and smaller mandible). Fat is shown in white. Reproduced from a) Schwab *et al*. 2005[14] and b) from Schwab, personal files.

A particular advantage in studying this common condition is that there is a safe, effective therapy—nasal positive airway pressure (PAP, Figure 4), which prevents the closure of the upper airway and its consequences (see meta-analysis[15]). The meta-analysis showed a significant decrease in sleepiness (both objective and subjective), quality of life improved and blood pressure decreased with PAP treatment[15]. The majority of patients show reasonable compliance (device use >4 hours per night)[16-18] and improvements in morbidity and mortality with PAP treatment have been found in longitudinal observational studies[19-21].

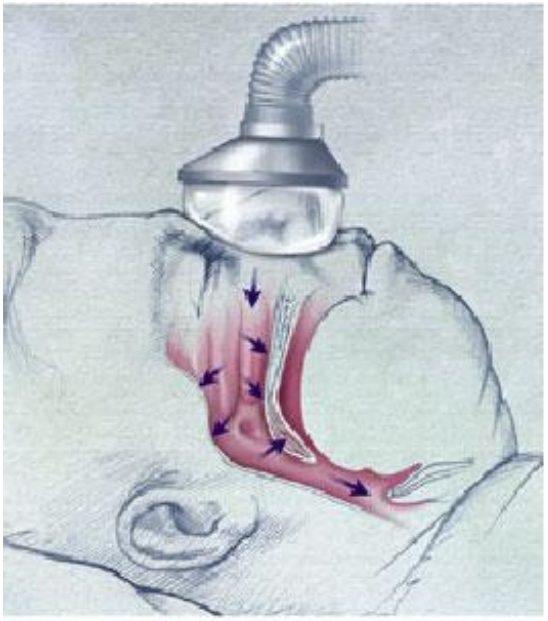

Figure 4. A schematic of how positive airway pressure (PAP) treatment prevents the closing of the airway and allows for normal breathing. Reproduced from www.aafp.org/afp/20040201/561.html.

1.2 Pathogenic mechanisms of OSA

An important question is how OSA causes its adverse physiological effects and how to show the independent effects of OSA in the presence of comorbid conditions like obesity. A schematic figure from Paper V focusing on the molecular signature of OSA and the role of obesity is shown below (Figure 5). In the following sections, the evidence supporting the role of the main molecular pathways causing OSA-related comorbidities are discussed and how they may lead to the increased prevalence of hypertension, cardiovascular disease and diabetes. The confounding effects of obesity and other comorbidities are discussed, as well as the role of inter-individual differences in the response to OSA.

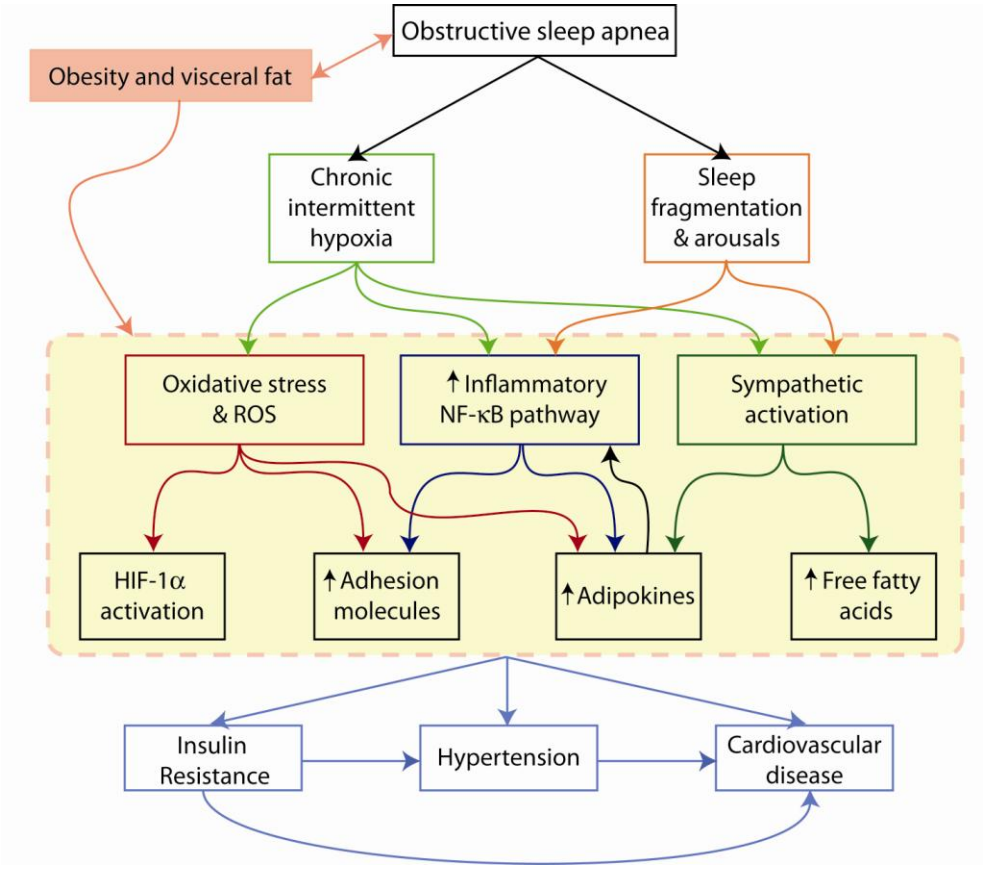

Figure 5. The possible pathogenic mechanisms of obstructive sleep apnea, leading to changes in molecular biomarkers and comorbidities. The confounding effect of obesity that affects the same molecular pathways is highlighted. Abbreviations: NF-κB, Nuclear factor kappa B; ROS, reactive oxygen species; HIF-1α, hypoxia inducible factor-1 alpha. Reproduced from paper V.

1.2.1 The physiological challenges of OSA

1.2.1.1 Chronic intermittent hypoxia

Chronic intermittent hypoxia (CIH) is thought to play a key role in the OSA pathogenic mechanisms (Figure 5 and for reviews, see[22-24]). Cyclical hypoxia with reoxygenation is thought to be like repeated ischemia-reperfusion damage with increased reactive oxygen species production during the restoration of oxygen, as occurs in an ischemic region when blood flow is restored (ischemia reperfusion). The increased production of reactive oxygen species is thought to cause oxidative stress, activation of proinflammatory cytokines, and adhesion molecules through activation of the proinflammatory transcription factor nuclear factor kappa B (NF-κB). The activation of inflammatory and adhesion molecules

is then believed to activate circulating cells (e.g., monocytes, endothelial cells and lymphocytes) causing endothelial dysfunction followed by cardiovascular and cerebrovascular disease.

Animal and cell models generally support the idea of CIH as an important player in the OSA pathophysiology. CIH in rodent models has been shown to cause vascular remodeling[25, 26] and atherosclerosis, but only when combined with a high fat diet[27]. Additionally CIH in rodent models has been found to cause increased sympathetic activation and hypertension[25, 28], persistent sleepiness[29], neuronal injury[29-31] and insulin resistance[32, 33].

There is also activation of the transcriptional factor hypoxia induction factor-1 alpha (HIF-1α), a key factor in oxygen homeostasis, which causes direct activation of >50 downstream molecules such as erythropoietin (reviewed by[22]) in response to CIH in animal and cell models[34-36]. Activation of HIF-1α plays an important role in the sympathetic response, increased blood pressure and increased triglyceride levels in animal models[35, 36]. It has also been shown that NF-κB is a key transcriptional activator of HIF-1α, linking the proinflammatory and hypoxic response pathways together[37].

It is important to note, however, that the CIH referred to in animal and cell models is usually more acute and with much more severe hypoxia than is found in OSA, limiting generalizability to the studying of real-life OSA patients (reviewed in Paper V). Additionally, the CIH may occur during waking periods in animals and the methodology used in different laboratories to produce CIH is very diverse[38], further complicating the comparison with OSA. In addition, not all organs may experience systemic CIH in the same manner. A recent study showed that during CIH, oxygen fluctuations were found in the liver, attenuated fluctuations in muscle and sustained hypoxia in fat[39]. Also, adipose tissue hypoxia appears to occur in obesity *per se*, likely due to the lower perfusion of adipose tissue and capillary rarefaction of arteries, causing inflammation in the absence of OSA[40-43].

The effect of intermittent hypoxia has also been assessed in humans. Studies in awake[44, 45] and more recently in sleeping[46-48] healthy human subjects for up to 28 nights have shown that intermittent hypoxia causes increased sympathetic activation and an increase in blood pressure. Moreover, administration of 100% oxygen to OSA patients during obstructive events diminishes the sympathetic response to apneas.[49] These studies therefore support the findings from animal models that chronic intermittent hypoxia leads to increased sympathetic activation. However, no changes were found in inflammatory biomarkers in blood during 14 nights of intermittent hypoxia[47].

1.2.1.2 Arousals and sleep fragmentation

CIH is not the only physiological challenge that occurs during apneic events; there is also the challenge of sleep fragmentation with repeated arousals. Each of these arousals is associated with a burst of sympathetic activity[50-52] and cardiac changes, including surges in blood pressure[53, 54]. Repetitive arousals lead to elevated cortisol and lipid levels[55], increased metabolism[56] and neurobehavioral deficits as a consequence of sleep fragmentation[56, 57]. The role of sleep fragmentation has not received as much attention as intermittent hypoxia. However, short sleep duration is increasingly common in our society[58]. It not only causes behavioral and neurobehavioral consequences (reviewed by[59]) but is also associated with increased risk of hypertension, weight gain, type 2 diabetes and mortality (meta-analysis by[60-63]). Studies of partial sleep deprivation for 1-10 nights have also shown increases in systemic inflammation[64-66].

1.2.2 Molecular mechanisms affected by OSA

1.2.2.1 Oxidative stress

Studies of oxidative stress in OSA have shown conflicting results, and many have used measures that are controversial regarding their ability to assess oxidative stress (reviewed in Paper V). However, a growing number of human studies support the presence of systemic oxidative stress in OSA[67-86]. Studies showing no oxidative stress in OSA are also reported but are much fewer in number[87-90]. It is important to note that some of the studies performed have used poorly matched control groups, usually with large BMI differences, that limit the significance of their findings[71, 78, 79, 81, 84].

1.2.2.2 Inflammation

Based on the overall oxidative stress model described above, increased reactive oxygen species production should cause increased expression of inflammatory cytokines through activation of NF-κB in OSA patients.

Interleukin-6 (IL-6) and C-reactive protein (CRP) are key inflammatory biomarkers associated with an increased risk of atherosclerosis and cardiovascular disease (CVD)[91]. IL-6 stimulates production of the proinflammatory CRP in the liver (the main production site for CRP)[92]. A total of 15-30% of circulating IL-6 levels come from fat tissue[92]. The levels of both IL-6 and CRP are increased in obesity[92] and IL-6 release has been found to be two- to threefold higher in visceral than subcutaneous fat[93, 94]. However, the increase in IL-6 comes mostly from non-fat cells within the adipose tissue, not the adipocytes themselves. Therefore IL-6 can be considered an adipokine but it is also released by other cell types such as endothelial cells and monocytes[92].

Some studies have found significant associations between OSA and IL-6 and/or CRP levels[13, 95-107] and/or decrease with continuous positive airway pressure (CPAP) treatment[97, 106, 108-113]. There are, however, conflicting studies that do not show independent associations of OSA and IL-6 and/or CRP levels[103, 114-129] or no change with CPAP treatment[105, 124, 130-136]. Two recent meta-analyses did show an overall significant decrease in CRP levels with >4 weeks of PAP treatment[137, 138] but did suggest potential racial differences, as well as a publication bias, with a lack of negative studies being published. The role of the level of obesity, visceral fat and other potential confounding effects on inflammation in these earlier studies has not been adequately addressed. Due to the common co-existence of obesity and OSA, it is important to understand how the two disorders may interact in determining inflammatory levels. In the most obese patients the effects of OSA on inflammation may be small due to the already high stimulation by obesity. Alternatively, it may be that the effect of OSA is amplified by the increased number of inflammatory cells in fat in more obese patients[139].

1.2.2.3 Sympathetic Activity

As shown above, both CIH and sleep fragmentation can lead to increased sympathetic activity. Indeed, increased sympathetic activity has been shown in untreated OSA compared to controls and PAP treatment by microneurography, a direct measure of muscle nerve sympathetic activity[140, 141]. Another way is to measure the levels of catecholamines epinephrine and norepinephrine in plasma or urine, which reflect different functions of the catecholamine systems; adrenomedullary hormonal activation and sympathetic neuronal activation, respectively[142]. OSA patients have been found to have higher levels of plasma and urinary norepinephrine levels[143-146], albeit not in all studies[147]. More severe OSA is also correlated with higher daytime sympathetic activity and a decrease with PAP treatment[140, 148]. The results for epinephrine levels are less convincing, as levels were similar in OSA subjects compared to well-matched controls in two studies[144, 145]. Some studies have found a small decrease with PAP[149, 150]. but not all[151.] Factors influencing the decrease in sympathetic activity with PAP treatment include PAP compliance[152], length of treatment[153] and whether subjects are normotensive or hypertensive to begin with[154]. The increase in blood pressure found in many untreated OSA patients is considered, at least partly, a result of their high sympathetic activity[155, 156.]

1.2.2.4 Adipokines

Sympathetic activity, oxidative stress and inflammation may be the most important domains affected by the pathogenic mechanisms of OSA, surely the most studied so far. However other domains such as adipokines produced by white adipose tissue (reviewed by[157, 158]), are potentially affected as they are

influenced by the primary mechanisms occurring in OSA: sympathetic activity, oxidative stress, and inflammation (reviewed in V).

One of the most researched adipokines in OSA subjects is leptin. Leptin is an adipokine released peripherally from fat cells that contributes to regulating body adiposity through a feedback loop whereby elevations in leptin are recognized by the central nervous system, acting as a satiety signal to suppress appetite (reviewed by[159]). Leptin also has an immunomodulatory role[160-163] and is both activated by proinflammatory mediators[164-166] and works as a stimulant of proinflammatory cytokine production such as IL-6 and tumor necrosis factor-alpha (TNF-α)[167]. Leptin resistance in the brain is thought to be responsible for a failure of the high levels of circulating leptin to suppress appetite, ultimately resulting in increased food intake and adiposity. The central leptin resistance is site-specific since other functions of leptin in regulating inflammation and sympathetic activation remain intact. Therefore, high leptin levels may be a part of the cascade causing the low-grade systemic inflammation and sympathetic nervous system-mediated hypertension commonly seen in obesity[159] and high leptin levels are considered an independent risk factor for cardiovascular disease[168, 169].

Some studies have found that OSA subjects have higher leptin levels than controls[12, 13, 145, 170-180] even when matched for BMI[12, 145, 171, 172, 177, 179] and a decrease in leptin levels with PAP treatment[172, 180-187]. There are, however, conflicting data showing no differences in leptin levels between OSA subjects and controls[129, 188-194] and no change with PAP treatment[134, 188, 194-198]. Finally, three small studies suggest differential effects of OSA on leptin levels depending on obesity; two studies showing effects of OSA in lean subjects only but the third showing effects in obese subjects only[190, 199, 200]. None of these studies have adequately addressed the relative role of subcutaneous and visceral fat, which may be an important factor since subcutaneous fat has been found to produce more leptin than visceral fat[201-203].

1.3 Adverse consequences of OSA

1.3.1 Hypertension

OSA is an independent risk factor for hypertension[204-207], in a dose-dependent manner[204, 205, 208]. Randomized controlled PAP trials have repeatedly shown a modest decrease in 24-hour ambulatory mean blood pressure as confirmed by meta-analysis[209], especially in more severe OSA and with higher PAP usage[210]. Hypertensive patients are also more likely to show a decrease in blood pressure with PAP than normotensive ones[211, 212]. However, no beneficial effect of PAP on blood pressure has been found in nonsleepy OSA patients[213, 214]. Data from a canine OSA model support a causal OSA effect on hypertension as dogs with simulated OSA for 1-3 months developed increased day- and nighttime blood

pressure, while dogs with recurrent arousals developed increased nighttime blood pressure only[215].

Diastolic blood pressure may rise before systolic in the development of hypertension in OSA[216] and both diastolic, nighttime and masked hypertension are underdiagnosed in untreated OSA patients[217, 218]. OSA is also an independent risk factor for pulmonary hypertension, which then decreases with PAP treatment[219]. OSA is highly prevalent in hypertensive patients[220-223], especially in younger subjects with drug-resistant hypertension[220, 224], and PAP treatment has been shown to effectively lower blood pressure in these subjects[225].

Interindividual differences exist in the development of hypertension among OSA patients as about half of OSA patients develop hypertension[205, 226, 227] (Figure 6). One potential component to the differences is genetic variability. There is now a large literature on genes conferring risk for hypertension, insulin resistance and cardiovascular disease[228-232] but a limited number of studies has addressed the question of causes for interindividual differences in the development of hypertension in OSA patients.

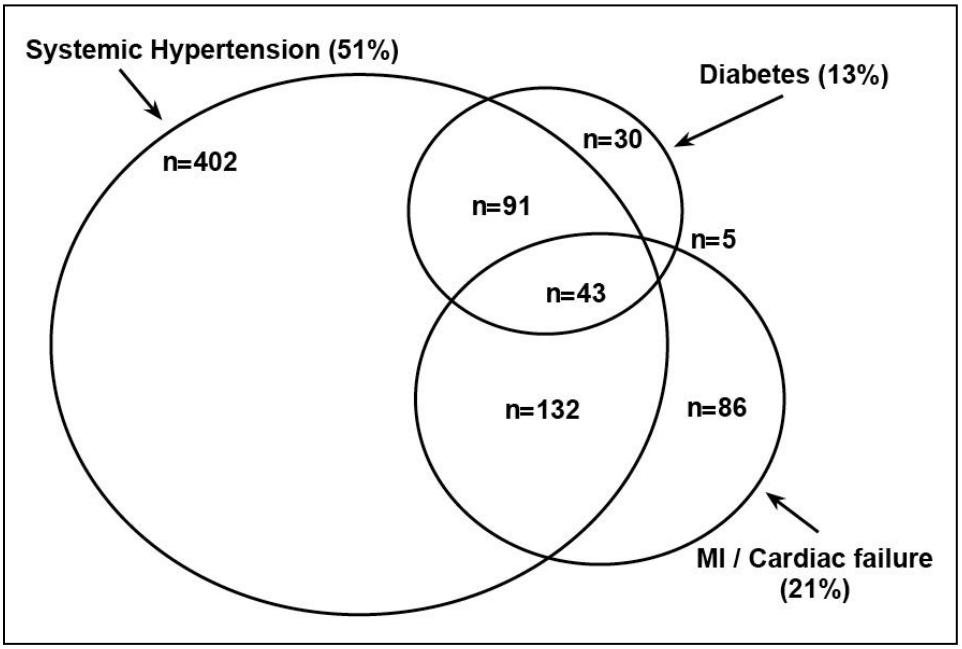

Figure 6. The prevalence of systemic hypertension, myocardial infarction (MI) and diabetes in Icelandic sleep apnea patients (n = 1303). Note the clustering of comorbidities within a subset of the cohort and that 41% of subjects have no comorbidities. Reproduced from Pack & Gislason 2009[227].

1.3.2 Cardiovascular disease

Recent epidemiology research has clearly shown an increased risk of cardiovascular events and mortality rate among untreated OSA subjects which is reduced with PAP treatment[19-21, 233-240]. Males under the age of fifty with OSA appear to be especially vulnerable as they have a higher mortality rate compared to the general population and older males with OSA[235, 236]. Risk factors for mortality in sleep apnea patients include diabetes, obesity and chronic heart failure[241]. Also, OSA subjects have an increased risk of sudden death when sleeping compared to subjects with no OSA and the general population[242].

Cardiovascular risk factors such as endothelial dysfunction, increased carotid intima-media thickness and arterial stiffness have been shown to be increased in OSA patients compared to age and BMI-matched controls[95, 243-247]. This increased risk is correlated to OSA severity[248-250] and has been shown to decrease with PAP treatment[113, 243, 251, 252].

Endothelial function has been proposed to be the link between OSA and cardiovascular disease as endothelial dysfunction promotes atherosclerosis and increases the risk of cardiovascular disease (reviewed by[23, 253]). The intermittent hypoxia is thought to cause an increase in reactive oxygen species which evoke inflammatory responses. The inflammation in turn activates circulating blood cells, platelets and endothelial cells, causing increased adhesiveness, injuries to endothelial cells and endothelial dysfunction (reviewed by [24]).

Interindividual differences exist again as the results for individual subjects are very diverse[243, 244] and some OSA subjects have similar endothelial function to that of the controls, indicating that some OSA patients may not be at as high risk for cardiovascular comorbidity[243].

1.3.3 Insulin Resistance and Diabetes

Studies in animal models support the notion that chronic intermittent hypoxia is a key factor leading to insulin resistance[32, 33]. Sleep fragmentation may also play a role [254]. Many studies have reported an association between impaired glucose metabolism and OSA. However, two recent metaanalyses did not support the hypothesis that OSA independently influences glucose metabolism[255, 256]. A longer follow-up time than in traditional studies may, however, be needed to see changes[257].

1.3.4 Sleepiness

Excessive daytime sleepiness is an important symptom of OSA and an integral part of the definition of OSAS[258], as explained above. Studies generally show more objectively and subjectively measured sleepiness in OSA patients than

controls [259-262] and a significant reduction in sleepiness with PAP[263-266]. The effects of PAP in mild to moderate OSA are small but significant (metaanalysis by[267]). Untreated OSA patients are at an increased risk of crashing while driving[268, 269], making excessive daytime sleepiness a serious consequence of OSA.

The number of subjects with OSA (even severe disease) but no hypersomnolence is, however, considerable[3, 214]. This raises the question why some people with OSA are sleepy[270] and some are not despite a similar severity of disease. Studies looking at interindividual differences in sleepiness in healthy individuals after sleep deprivation may help to answer this question as very diverse responses are seen in objective and subjective sleepiness and performance (reviewed by[271]), possibly due to differential activation of various brain regions[270, 272]. The same mechanisms that cause such interindividual differences in response to total sleep deprivation may be at work in OSA. Sleepy OSA patients are more likely to initiate PAP treatment than non-sleepy patients[273] and studies focusing on non-sleepy OSA patients have found no beneficial effect of PAP treatment on blood pressure, hypertension and cardiovascular events nor on quality of life, objective sleepiness, and memory[213, 214, 274, 275]. Additional research in this area is required to understand whether non-sleepy OSA patients are in need of PAP treatment or not.

1.3.5 Night symptoms - Nocturnal sweating

The adverse effects of OSA include the night symptoms experienced by the untreated OSA patients. Many patients experience troubled sleep with many nighttime awakenings. Also some patients describe severe nocturnal sweating. It has been reported clinically that half of OSA patients report nocturnal sweating, usually around the neck and upper body area[276]. and two small studies have shown a reduction in symptoms with PAP treatment[277, 278].

Sweating is solely controlled by the skin sympathetic nervous system and mainly functions to increase heat loss when necessary and maintain thermoregulation. Body temperature and sleep are highly interrelated as the lowering of body temperature and increased distal vasodilation shorten sleep onset latency (reviewed by[279]). Also sleep quality is affected by slight changes in core body temperature, a decrease of 0.2°C due to external cooling causing an increase in deep sleep[280] and an increase of 0.3°C causes more disturbed sleep[281]. Sweating can be assessed objectively by measuring electrodermal activity (EDA), a measurement of the sympathetic activation of the eccrine sweat glands[282] (Figure 7). EDA correlates highly with a measurement of actual sweat quantity on the skin as assessed by the ventilated capsule method ($r = 0.88$)[283].

Nocturnal sweating is a symptom commonly encountered in clinical medicine (reviewed by[284]), but has only been evaluated to a limited degree. Nocturnal sweating can be very bothersome to the patient and the bed partner, but only a minority of patients report this symptom to their physician[285, 286]. The possible causes of nocturnal sweating include, but are not limited to, malignancy, infections, endocrine and neurologic disorders, menopause, GER, medications (mainly antidepressants and antipyretics), substance abuse and panic attacks, as well as sleep disorders such as OSA and insomnia[286-288] (for reviews, see[284, 289-291]). Other causes can be as simple as an overheated room or too thick bed clothes (reviewed by[284]). Nocturnal sweating has been associated with increased daytime tiredness and sleep problems in a general population cohort [285, 286] as well as in subjects referred to a sleep laboratory[292]. A recent review article by Mold *et al*. stated that much is still unknown about the causation, evaluation and management of nocturnal sweating[291].

Larger studies comparing the prevalence of nocturnal sweating in untreated OSA patients with subjects from the general population as well as changes with PAP treatment are needed in order to confirm nocturnal sweating as a true symptom of OSA. In addition, studies assessing the reliability of subjective assessment of sweating to objective measurements are needed[291].

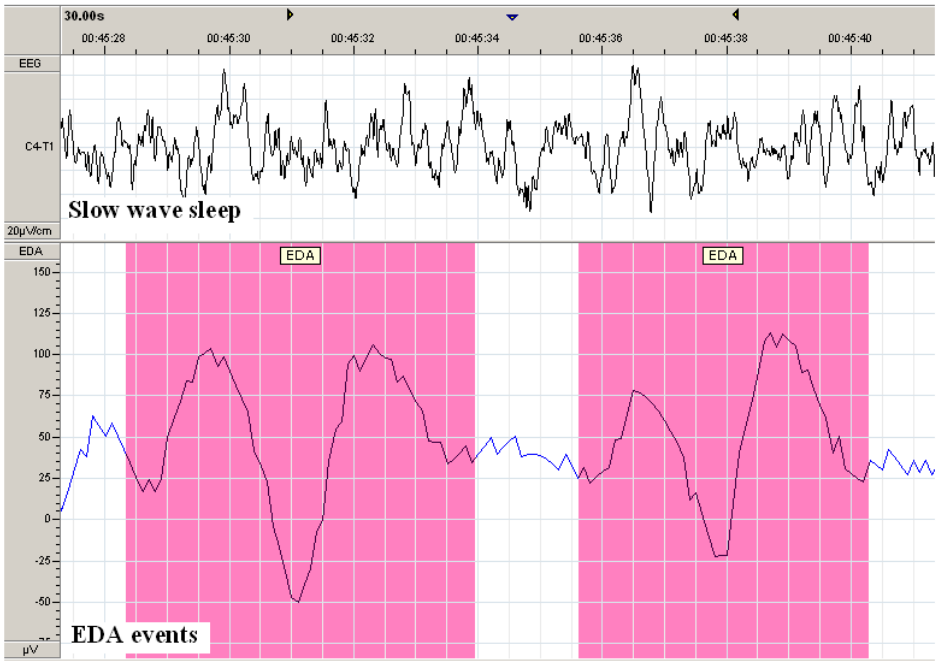

Figure 7. Objective measurement of sweating by measuring electrodermal activity (EDA) events during sleep (total screen shot is 14 seconds).

1.4 Confounding effects of obesity

Both OSA and obesity have been shown to be independent risk factors for hypertension, cardiovascular disease and insulin resistance (for reviews on OSA, see[1, 293]; for reviews on obesity, see[294, 295]). Due to the common co-existence of OSA and obesity, it is important to consider their relative roles in adverse clinical consequences. To complicate matters further, the key molecular mechanisms causing adverse consequences in OSA, i.e., oxidative stress, inflammation and sympathetic activation, also occur in obesity[296-300]. Unfortunately, until now the large literature on obesity has simply ignored this issue and not considered OSA as a factor in their studies, as shown by a metaanalysis of obesity and cardiovascular disease published in *Lancet in 2006*[301], where OSA was not assessed in a single study.

How to determine the relative role of OSA is the vital question. A commonly used strategy is to assess differences between OSA patients and controls, usually BMI-matched. This strategy may, however, not be sufficient as abdominal visceral fat is a risk factor for OSA and has been shown to be increased in OSA patients compared to BMI-matched controls[6, 13, 302]. This is problematic as abdominal visceral fat is associated more strongly with adverse health consequences such as hypertension, insulin resistance, diabetes and the metabolic syndrome than other fat deposits[303-307]. A direct measure of visceral and subcutaneous abdominal fat is therefore important to further understanding of the relative roles of OSA and obesity in adverse clinical consequences.

Another common strategy to assess the role of OSA is to assess differences before and after effective PAP therapy, a within-subject strategy. This strategy has been used in multiple studies (for examples see[77, 97, 209, 308]). There are, however, problems with this strategy that need to be considered. First of all, OSA appears to have a direct effect on visceral fat gene expression[309] and successful PAP therapy has been shown to reduce the amount of visceral fat,[181, 187] albeit by a small amount (8% to 16% over a period of 3-6 months). Therefore, changes with PAP treatment could be due, at least in part, to reductions in visceral fat mass. Moreover, in patients with OSA who are effectively treated with PAP, there could be irreversible effects of OSA, e.g. in residual sleepiness [310]. OSA is a chronic, slowly progressive disorder,[311] and it can be present for years before it is diagnosed[8, 312]. Currently the magnitude of irreversible effects of untreated OSA is largely unknown. Studies are needed to estimate the reversible effects of OSA (differences from pre- to post-PAP in effectively treated individuals) and irreversible effects of OSA, i.e., to estimate the difference between patients with OSA after effective treatment when compared to controls with similar levels of obesity but not with OSA.

1.4.1 Confounding effects of other comorbid conditions

The main molecular mechanisms of OSA - systemic inflammation, oxidative stress and sympathetic activation - are all considered key pathological mechanisms for the adverse consequences of OSA, as shown above. However, these mechanisms are not unique to OSA but also occur in many other diseases and conditions which are commonly found in patients with OSA, e.g., cardiovascular disease, type 2 diabetes, metabolic syndrome and smoking (Figure 8 and reviewed in Paper V). Study designs that assess the confounding effect of obesity and other co-morbid conditions that affect the same mechanisms as OSA does are required to determine the relative role of OSA *per se* in adverse consequences.

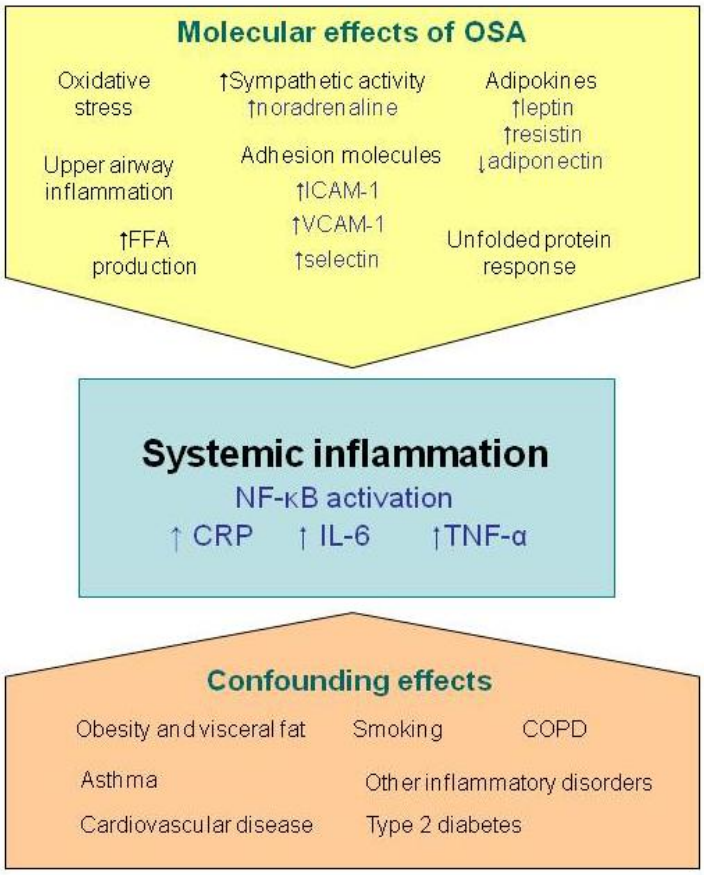

Figure 8. The different molecular effects of obstructive sleep apnea (OSA) that can lead to systemic inflammation as well as potential confounding effects by other comorbid disorders. Abbreviations: FFA, free fatty acids; ICAM-1, intercellular adhesion molecule 1; VCAM, vascular adhesion molecule 1; NF-κB, nuclear factor kappa B; CRP, C-reactive protein; IL-6, interleukin-6; TNF-α, tumor necrosis factor-alpha; COPD, chronic obstructive pulmonary disease. Reproduced from Paper V.

1.5 Interindividual differences in the response to OSA

Differences between individuals with OSA may be directly related to the nature of their sleep disordered breathing events. Some individuals with OSA have marked sleep fragmentation with little, if any, intermittent hypoxia, while others may show marked intermittent hypoxia with lesser degrees of fragmentation despite similar AHI, the index now used to determine OSA severity (reviewed in Paper V). Also the length of apneas and hypopneas can vary considerably between subjects, from the minimum of 10 seconds to over a minute. Therefore simply using AHI as a measure of OSA severity may not be adequate in determining adverse outcomes of the disease.

Also as mentioned above, we know that there is heterogeneity in the biological response to OSA. Many individuals with OSA do not have excessive sleepiness[3] and only about 50% of OSA subjects develop hypertension[205, 226, 227]. One potential component of the differences is genetic variability. One possibility is that individuals who develop hypertension and cardiovascular disease have increased pathogenic responses or decreased adaptive response for an equivalent degree of OSA severity. Such heterogeneity in response might also be related to differences in protective mechanisms such as antioxidant ability, anti-inflammatory response and in adaptive responses affecting sympathetic activation (reviewed in V).

2 Primary aims of this study

The overall aim of this thesis was to investigate interindividual differences in the response to sleep apnea, for physiological symptoms (nocturnal sweating) as well as molecular biomarkers (IL-6, CRP and leptin levels). The research questions and hypotheses were the following:

Research question 1: Is frequent nocturnal sweating a symptom of OSA?

a) What is the relationship between electrodermal activity, an objective measure of sweating during sleep to subjective complaints of nocturnal sweating?

b) Do OSA patients report frequent nocturnal sweating more often than subjects in the general population (Paper II)?

c) Is nocturnal sweating associated with OSA severity?

d) Are subjects with this symptom different from other subjects (both in the general population and OSA patients) with regards to demographic factors, cardiovascular risk profile and other symptoms (Papers I and II)?

e) Does nocturnal sweating change with PAP treatment (Papers I and II)?

f) Is frequent nocturnal sweating related to temperature changes and/or sympathetic activity (Paper I)?

Hypothesis 1: Frequent nocturnal sweating is a clinical symptom of OSA that is related to higher OSA severity. These patients have an increased cardiovascular risk compared to other OSA patients due to increased sympathetic activity. They also show decreased sweating with successful PAP treatment, both measured objectively and subjectively.

Research question 2: Is OSA severity associated with increased levels of inflammatory biomarkers; CRP and IL-6 (Paper III) and leptin (Paper IV)?

a) If a relationship exists, is it independent of obesity level?

b) If a relationship exists, is it affected by other covariates such as gender and/or co morbid diseases?

Hypothesis 2: OSA severity is independently associated with increased CRP, IL-6 and leptin levels after adjusting for obesity. The effects of OSA may vary depending on obesity level and the characteristics of excess fat distribution. Other covariates influence the biomarker levels but do not change the effects of obesity and OSA on biomarker levels.

3 Materials and methods

3.1 Study cohorts

The four research papers described in this thesis are based on results from three study cohorts (Table 1). Paper I is based on the clinical OSA cohort, Paper II on the baseline and follow-up data in the Icelandic Sleep Apnea Cohort (ISAC) as well as the general population cohort, and Papers III and IV on the baseline data from the first 532 subjects in the Icelandic Sleep Apnea Cohort (ISAC). The consent of the National Bioethics Committee and the Data Protection Authority of Iceland was granted for all three study cohorts and written consent obtained from all research subjects. Additionally for the ISAC study, the consent of the Institutional Review Board of the University of Pennsylvania was granted for the study.

3.1.1 Icelandic Sleep Apnea Cohort (ISAC)

All patients with moderate to severe OSA (AHI ≥15 events/hr) who were referred to the Department of Respiratory Medicine and Sleep, Landspitali – The National University Hospital of Iceland for treatment with PAP from September 2005 to December 2009 were invited to participate in the Icelandic Sleep Apnea Cohort (ISAC) study. No other inclusion or exclusion criteria were used. All ISAC subjects were invited to a two year follow-up study after starting PAP treatment. Paper II included all ISAC subjects with the relevant baseline and follow-up data. Paper III included 532 ISAC subjects, from September 2005 to September 2008, baseline data only. Paper IV included 530 ISAC subjects, enrolled from September 2005 to September 2008, baseline data only.

The data collection at baseline that relates to this thesis was the following: Untreated OSA patients underwent a type 3 sleep study, fasting morning blood draw, questionnaires and body measurements, as well as an MRI of the whole abdomen. At follow-up the assessment was the following: objective and subjective PAP adherence, fasting morning blood draw, questionnaires and body measurements.

3.1.2 General population cohort

The general population cohort was primarily invited to participate in the Burden of Obstructive Lung Diseases (BOLD) initiative in Iceland; a multicenter international study aiming to estimate the burden of chronic obstructive pulmonary disorder (COPD) worldwide[313, 314].

The general population sample was a random sample of Icelandic citizens, ≥40 years living in the capital area of Reykjavik. At the end of November 2004, of the 73,391 subjects ≥40 years living in the area, 939 subjects were randomly selected to participate. Altogether 762 of the 939 eligible subjects (81.2%) responded. No significant differences were found with regards to age or smoking status, between responders and non-responders in this cohort. However, there was a significant difference in gender with proportionally more men participating ($p < 0.01$).

The data collection that relates to this thesis was the following: questionnaires, body measurements and a nonfasting blood draw during the day.

3.1.3 Clinical cohort

Males with moderate to severe OSA (AHI ≥ 15 events/hr) who were referred to the Pulmonary Department, Landspitali – The National University Hospital of Iceland for treatment with PAP from August 2005 to September 2006 were invited to participate in this study. The exclusion criteria for the participants included female gender, current use of medication for a heart condition or hypertension, known diabetes or other chronic disorders, prior PAP treatment and current smoking. The majority of subjects in this study also took part in the ISAC study.

The data collection that relates to this thesis was the following: in-laboratory polysomnography with additional measurements of objective sweating and temperature. Evening and fasting morning blood draws were collected as well as overnigh urine collection, questionnaires and body measurements.

Table 1. The cohorts described in this thesis, by research papers. Abbreviations: ISAC, Icelandic Sleep Apnea Cohort; OSA, obstructive sleep apnea.

	ISAC baseline (n=822)	**ISAC 2 year follow-up (n=700)**	**General population cohort (n=703)**	**Clinical OSA cohort (n=15)**
Paper I				X
Paper II	X	X	X	
Paper III	X (first 532)			
Paper IV	X (first 530)			

3.2 Measurements

3.2.1 Questionnaires and body measurements

Subjects in all three study cohorts were evaluated in the same manner at the Department of Respiratory Medicine and Sleep, Landspitali – The National University Hospital of Iceland. All subjects answered the same core questionnaire on general health status, current smoking (smoking or tobacco use during the past month) and whether they had hypertension and/or diabetes (doctor diagnosis and medication), cardiovascular disease which was defined as a doctor diagnosis of coronary artery occlusion (myocardial infarction or heart attack), heart failure and/or stroke. The Basic Nordic Sleep Questionnaire[315] was administered to all subjects (includes questions on sleep quality, insomnia symptoms, snoring, nocturnal sweating, and GER). The response alternatives for those questions were on a frequency scale of 1 - 5: (1) never or very seldom, (2) less than once a week, (3) once to twice a week, (4) three to five times a week, and (5) every day or almost every day of the week. Frequent nocturnal sweating was defined as a score of 4 or 5, i.e., reporting nocturnal sweating ≥3x week. Insomnia symptoms were defined as difficulties initiating sleep or maintaining sleep ≥3x week[316]. Daytime and nocturnal GER symptoms were defined, respectively as reporting heartburn during the daytime and after going to bed ≥1x week[317, 318]. Other questionnaires included the Epworth Sleepiness Scale (ESS) [264], a 12-item Short-Form Health Survey for physical and mental quality of life[319] and questions on restless legs syndrome (RLS) symptoms based on recommendations from the International Restless Legs Syndrome Study Group[320]. Those who answered the questionnaire as follows were regarded as having symptoms of RLS: a strong urge to move the legs often or very often; the discomfort in the legs was relieved by moving the legs or walking; the symptoms had to be most prominent in the evening, at bedtime or not different between times of day (same definition as our previous paper[314]). Subjects in all studies listed their medication use for hypertension and diabetes (pharmacological treatment was coded according to the Anatomical Therapeutic Chemical drug classification system http://www.whocc.no/atcddd) but subjects in ISAC and the clinical OSA cohort also gave a detailed list of all other medications they used.

Subjects in the general population (Paper II) were defined as high or low risk for OSA based on the Multivariable Apnea Prediction (MAP) index[321]. The MAP score is based on self-reported frequency of occurrence of apnea symptoms (snoring or gasping, breathing stops, choking or struggling for breath during the night) as well as BMI, age and gender. The MAP index ranges from 0 - 1 where subjects with a score of 0 are least likely to have OSA. A MAP index cut-off of 0.75 was used to define high risk of OSA similar to our previous

publication[316] All questionnaires were translated from English into Icelandic and back-translated to assure accuracy.

Height and weight were measured in the same manner for all participants. Subjects were asked to remove their shoes and heavy outer garments for the measurements. Additionally, for ISAC and the clinical OSA cohort participants; neck, waist and hip circumferences were measured using standardized procedures.

OSA subjects in the ISAC and clinical OSA study cohorts answered the same questionnaires and had repeated measurements at the follow-up visit (Papers I and II).

3.2.2 Sleep studies

3.2.2.1 Polysomnography

In the clinical cohort (Paper I), all subjects underwent in-laboratory polysomnography (PSG) with an Embla A10 device (Natus Medical Inc., Ontario Canada). The PSG included two channels for electroencephalography, electrooculography, submental electromyography, electrocardiography and bilateral anterior tibialis electromyography. Nasal airflow was recorded through a cannula (nasal pressure transducer). Chest and abdominal movements were measured by respiratory inductive plethysmography belts. Pulse and oxygen saturation were measured by a finger probe oximeter based on a 4 beat exponential average (Flex Sensor 8000J and XPOD oximeter, Nonin Medical Inc., Plymouth, Minnesota) and body position and activity by a sensor situated on the chest.

The scoring of sleep stages (scored visually in 30-s periods) was performed in accordance with Rechtschaffen and Kales scoring rules[322], cortical arousals and leg movement scoring in accordance with the Atlas Task Force recommendations[323, 324]. An apnea was defined as a cessation of airflow (<10% of normal airflow) for ≥10 seconds. Apneas were further divided into obstructive, mixed and central apneas according to respiratory movements of the chest and abdomen. A hypopnea was scored when the airflow was < 50% of baseline for ≥ 10 seconds or when the airflow was diminished ≥ 30% from baseline for 10 seconds or longer with ≥ 4% drop of blood oxygen concentration or an arousal. These definitions are in agreement with recommendations from the American Academy of Sleep Medicine[258]. The AHI was defined as the number of apneas and hypopneas/hr sleep. An oxygen desaturation index (ODI) was calculated as the number of oxygen desaturations ≥ 4%/hr sleep. Hypoxia time was defined as the number of minutes with oxygen saturation (SaO_2) < 90% during sleep.

Respiratory movements were analyzed automatically to measure respiratory mechanics instability. A respiratory mechanics instability (RMI) state

was scored when there was a thoraco-abdominal asynchrony, as indicated by more than a 10 degree phase angle deviation of the thorax and abdomen (Somnologica Science 3.3.1; Natus Medical Inc.). A RMI index was calculated as the percentage of the night spent in the state of respiratory mechanics instability.

Scoring of all recordings was performed by the same person (Arnardottir) and used in all analyses. A qualified PSG scorer at another sleep center in the United States made a second blind scoring of the recordings. Concordance was assessed by a two-way mixed intraclass correlation coefficient (ICC) for absolute agreement between the two scorers and calculated with a 95% CI for selected variables. The concordance for sleep scoring was very high (ICC >0.90 for AHI, ODI, total sleep time, sleep stages 1, 2, REM and arousals). The only major difference in scoring was for slow wave sleep (ICC 0.71) due to differences in amplitude criteria in the two sleep centers.

3.2.2.2 Type 3 sleep studies

Prior to referral for PAP treatment, all ISAC subjects had a type 3 sleep study with an Embletta type 3 portable monitor, an Embla 12 channel system (Natus Medical Inc, Ontario, Canada) or a T3 device (Nox Medical, Reykjavik, Iceland). The same signals were recorded on all studies. The type 3 monitoring is what is used clinically in Iceland to determine sleep apnea. However, all patients are also screened for other sleep disorders using questionnaires and an interview with a physician. Patients believed to suffer from sleep disorders other than sleep apnea then have a PSG study or additional periodic leg movement measurement instead of a simple type 3 monitoring for sleep apnea. The differences between a type 3 study and a full in-laboratory PSG are shown in Figure 9. Patients with obesity-hypoventilation syndrome would be detected by evaluation of their serum bicarbonate level and arterial blood gases. Such patients were not included in our study.

All sleep studies were re-scored by trained sleep technologists based on the following criteria: a minimum of 4 hours of a scorable oxygen saturation signal (n = 15 subjects excluded in total cohort). These subjects were not excluded from other analyses in Paper II but the subset of these patients (n=9) in papers III and IV were excluded from all analyses as OSA severity was one of the main variables tested. Patients were instructed to switch the recorder off when they woke up in the morning. Scoring was started 30 minutes into the recording and ended 5 minutes before the study recording was completed. Events were scored according to the following definitions: a classification of hypopnea required a ≥30% decrease in flow with ≥4% oxygen desaturation or a ≥50% decrease in flow for ≥10 sec with a sudden increase in flow at the end of the event. A classification of an obstructive apnea required a ≥80% decrease in

flow for ≥10 sec. The AHI was calculated as the number of apneas plus hypopneas/hr of recording (excluding upright time). ODI was calculated as the number of falls in oxygen of ≥4% per hour of recording (excluding upright time). Hypoxia time was defined as the number of minutes with SaO_2 < 90%. The minimum SaO_2 was defined as the lowest oxygen saturation reached during the study.

Measurement reliability of the scoring of the AHI and ODI was assessed based on intraclass correlation coefficient (ICC) analysis. The ICC for n = 51 randomly selected recordings scored on two different occasions was 0.99 for AHI and almost 1.00 for ODI. These data indicate essentially ignorable variability due to scoring. To test for systematic differences in the measurement of OSA severity by Embletta *vs*. T3 devices, 13 subjects slept with both devices simultaneously during their laboratory diagnostic OSA study. Their AHI ranged from 0 - 58 events/hr. The intraclass correlation coefficient (ICC) for AHI was 0.99 ($p < 0.001$) and for ODI 0.97 ($p < 0.001$), showing no significant differences in measured OSA severity between devices.

3.2.2.3 General population cohort

Sleep studies were not performed in this cohort. Therefore some subjects may have undiagnosed sleep apnea. The likelihood of subjects in this cohort having sleep apnea was assessed by calculating the MAP index for OSA risk[316].

A) B)

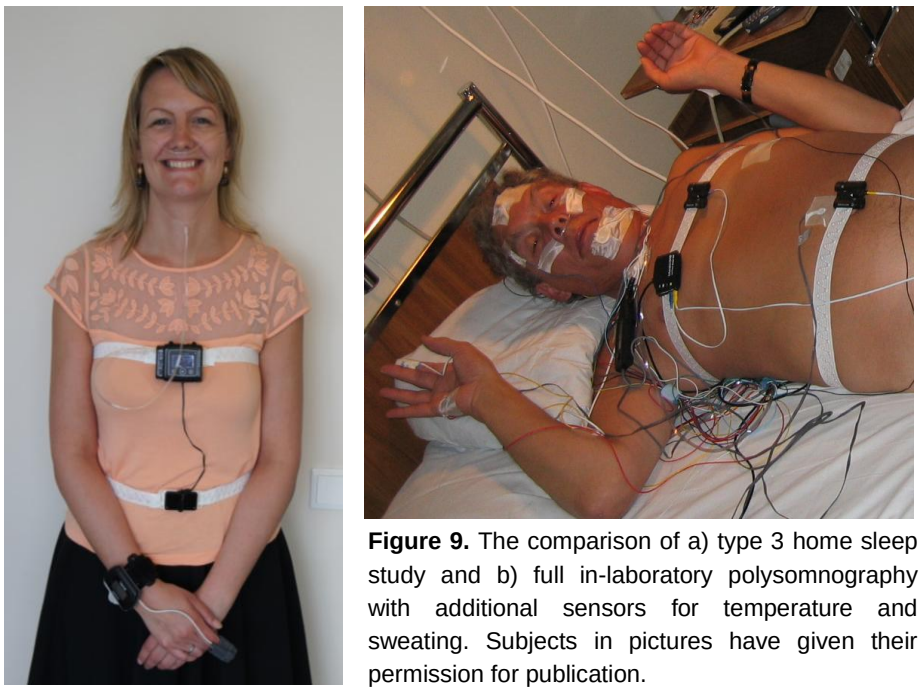

Figure 9. The comparison of a) type 3 home sleep study and b) full in-laboratory polysomnography with additional sensors for temperature and sweating. Subjects in pictures have given their permission for publication.

3.2.3 Measurement of electrodermal activity

In paper I electrodermal activity (EDA) was measured with NoiseFree™ single bio-potential silver-silver/chloride electrodes (Rochester Electro-Medical, Tampa, FL, USA) connected to the Embla device. EDA was recorded using the skin potential method by placing one electrode on the hypothenar eminence as an active site and another on a lightly abraded site on the volar surface of the forearm as an inactive site (two-thirds of the distance from wrist to elbow) on the right arm[325, 326]. An EDA index (events per hour sleep) was calculated, considering an EDA event a change in skin potential of >50 µV amplitude and >1.5 s duration (low-cut filter 0.3 Hz)[327]. EDA percentage was calculated as the proportion of total sleep time spent in EDA events.

3.2.4 Measurement of temperature

In paper I core body temperature was measured 10 cm into the rectum (YSI4491E; YSI Inc., Yellow Springs, Ohio, USA) and skin temperature (YSI409B; YSI Inc.) at eight different locations on the body for the overnight recording. The temperature probes were connected to the Embla™ digital recording device. Both core and skin temperature were measured continuously at 1-s intervals with ± 0.1°C accuracy. The Krauchi *et al*.[328] method was used for location of skin temperature probes and calculation of the distal-to-proximal skin temperature gradient, the difference between the average temperature of distal and proximal areas. The room temperature was kept constant around 22°C and the patients had light bed covers. Core body temperature measurements were missing for two subjects, one of whom declined use of the core body temperature probe and another whose probe was disconnected during one study. Other data for these subjects were used in the analyses.

The sleep period from sleep onset until waking was divided into 30-minute periods and the average temperature of the first two hours calculated to examine the lowering in core body temperature after sleep onset. The minimum 30-minute core body temperature period of all the 30-minute periods for each recording was also calculated. The distal–proximal skin temperature gradient, EDA index, EDA percentage, breathing parameters and arousals for each of these 30-minute periods were also calculated. Only periods with >20 minutes of sleep were used in the calculations. The Signal Workshop software was used for these analysis (Nox Medical, Reykjavik, Iceland).

3.2.5 Biomarker assessment

3.2.5.1 Urinary biomarkers

In paper I, urine was collected from 22:00 h until the first post-awakening morning void (c. 10 h). As the urine collections were not 24-h samples, all

values were corrected for creatinine clearance[329]. Urinary adrenaline and noradrenaline were measured by high-performance liquid chromatography and adrenaline–creatinine and noradrenaline–creatinine ratios calculated. After addition of an internal standard (3,4-dihydroxybenzylamine hydrobromide), catecholamines were extracted by alumina adsorption using reagents from Chromsystems (Munich, Germany). Isocratic separations were performed on a C18 reverse phase column using electrochemical detection[330]. Measurements of urinary metabolites were missing for one subject who declined to participate in this part of the study but other data for this subject were used in the analyses.

3.2.5.2 Blood biomarkers

In the ISAC study cohort, blood was drawn in the morning after sleep from the antecubital vein of fasting untreated participants. Specimens were collected in SST vacutainers (Greiner, Kremsmünster, Austria) and allowed to clot at room temperature for 20 minutes before centrifugation for 10 minutes at 3,000 rpm. After separation, the serum samples were stored at -20°C.

Paper III: Enzyme-linked immunosorbent assay was used to determine serum interleukin-6 (IL-6) in Paper III (R&D Systems, Minneapolis, MN). The IL-6 assay was performed according to the recommendations of the manufacturer. The lower detection limit of the assay is 0.70 pg/mL. In total, 48 patients had levels below 0.70 pg/mL and their values were treated in the analysis as 0.70 pg/mL. Despite the lower bound restriction of 0.70 pg/mL on the distribution of IL-6 values, the multiple linear regression model residuals continued to satisfy parametric assumptions after log transformation. IL-6 levels were measured in duplicates and samples, where results varied more than 15%, were reanalyzed. The intra-assay coefficient of variation for all duplicate samples was 5.0%. Using control samples, the inter-assay coefficient of variation of the IL-6 assay was found to be 11.2% at a concentration of 2.18 pg/mL and 9.2% at a concentration of 0.68 pg/mL.

Paper III: Serum C-reactive protein (CRP) concentrations were measured (Paper III) on a Kone 30 analyzer using a commercially available latex-enhanced immunoturbidimetric high-sensitivity assay from Roche Diagnostic Systems (Mannheim, Germany). The lower detection limit of the assay is 0.1 mg/L. Patient levels ranged from 0.38-53.87 mg/L. Thus, all patients had a CRP level greater than the lower limit of detection. The inter-assay coefficient of variation for the CRP measurements was 1.1% at a concentration of 3.73 mg/L and 1.9% at a concentration of 0.68 mg/L (control samples were analyzed each day). CRP measurements were missing for five patients but other data for these subjects were used in the analyses. Blood was sampled and CRP assessed in the clinical OSA cohort in the same manner before and after CPAP treatment.

Paper IV: A radioimmunoassay was used to determine serum leptin levels in ng/ml, using the double antibody/PEG technique with iodine-125 labeled human leptin and human leptin antiserum (Millipore, HL-81K). Leptin levels were measured in duplicate. The average intra-assay coefficient of variation (CV) of leptin levels for all the duplicate serum samples in the radioimmunoassay was 3.0% and the inter-assay CV for a control sample with an average leptin level of 14.2 ng/ml was 11.5%. The limit of sensitivity for the assay is 0.5 ng/ml - 100ng/ml. The variance of the assay is increased at the high end (inter-assay CV at 41.5 ng/ml was 22.0%) and therefore all samples > 30 ng/ml were repeated in a 4x dilution (n = 34).

All biomarker data required natural log transformation for analyses (See Figure S1 in Paper III).

3.2.6 Magnetic resonance imaging

Subjects in the ISAC cohort underwent MRI of the abdomen using a 1.5T scanner (Siemens Avanto, Germany) while untreated. Briefly, the abdominal compartment was defined from the superior aspect of the xiphoid process to the anterior portion of the L5-S1 interspace. MR images were obtained in 1 cm contiguous intervals through the abdominal compartment and summed to assess the total abdominal, subcutaneous and visceral fat volume (Figure 10). Intraclass correlation coefficient (ICC) analyses for visceral and subcutaneous fat volumes, for two trained raters, were both essentially 1.0, showing ignorable technical variability. For further details, see Papers III and IV.

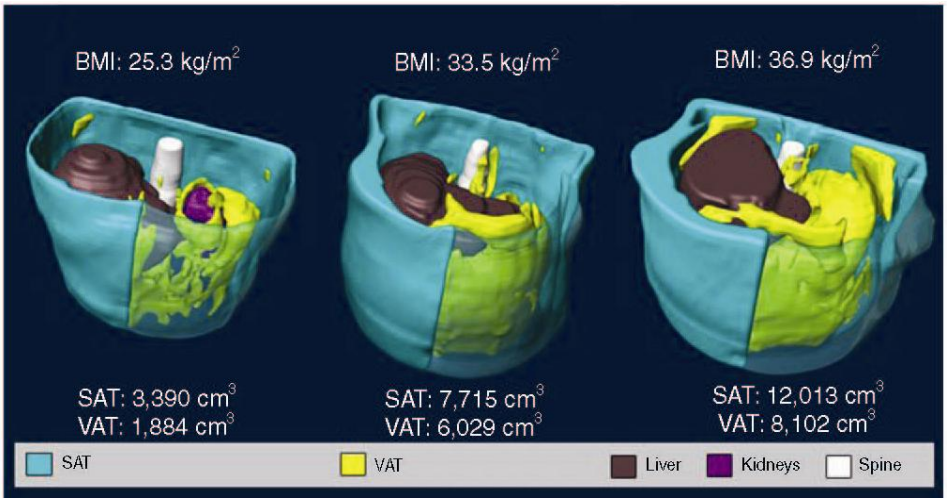

Figure 10. Three-dimensional reconstructions of abdominal fat in three males in the Icelandic Sleep Apnea Cohort and different body mass index (BMI) levels. Visceral abdominal fat (VAT), the fat the surrounds the organs, is shown in yellow and subcutaneous abdominal fat (SAT), the fat below the skin, in blue. Reproduced from our publication, Maislin *et al*., 2012[331].

3.2.7 PAP use

All PAP treatment in Iceland is administered by the Department of Respiratory Medicine and Sleep at Landspitali – The National University Hospital of Iceland, the sole provider of ventilator treatment in Iceland. All subjects with OSA were treated with autoPAP or continuous PAP (CPAP) units (ResMed Corp. San Diego, CA, USA) and treatment was only changed to bilevel PAP (BiPAP) or adaptive servoventilation if treatment efficacy on autoPAP or CPAP was inadequate (defined by AHI ≥ 15 using PAP and/or persistent patient complaints).

Subjects in Paper 1 had ResMed S7 machines allowing for PAP compliance assessment at the 3 month follow-up from the back of the device (total hours of usage/days since PAP installation).

In the ISAC 2 year follow-up (Paper II), PAP adherence was objectively measured by downloading the mask on-time stored by the PAP unit in the previous 4 weeks if available (in those patients on ResMed S8 machines). Subjects recruited at the beginning of the study period had older models of PAP devices which did not provide this type of information. Self-report data from all patients were also collected at the follow-up visit, based on three multiple choice questions about average PAP use: 1) Do you use PAP for your sleep apnea? (Yes, no or don't know), 2) How many nights per week do you use PAP? (Response alternatives: 1, 2, 3, 4, 5, 6 or 7 nights/week), 3) How much of the sleeping time each night do you use PAP? (Response alternatives: all the sleeping time [100%]; almost all the sleeping time [80 - 99%]; most of the sleeping time [60 - 79%]; about half of the sleeping time [40 - 59%]; about one third of the sleeping time [20 - 39%]; almost none of the sleeping time [1 - 19%]; none of the sleeping time [0%]; don´t know).

3.3 Statistical analysis

For baseline analysis in all papers, the chi-square test and t-test/one-way analysis of variance (ANOVA) were used for categorical and continuous variables respectively. For the comparison of data from the untreated versus the PAP-treated conditions, a paired sample t-test was used for parametric data while the Wilcoxon-signed rank test was used for non-parametric data. Initial evaluations of the strengths of linear associations were based on the Pearson correlation (parametric data) and the Spearman rank (non-parametric data and clinical OSA cohort due to low n) correlations. A p value of ≤0.05 was considered significant for all analyses. STATA 11.0 was used for all statistical analysis except SPSS 11.0 was used in Paper I and SAS 9.2 for model and bootstrap results in Paper III.

Paper I: A one-way ANOVA for repeated measures was employed for within-subject comparison of more than two conditions.

Paper II: Logistic regression was used for multivariable analyses and assessment of adjusted odds ratios (OR) with 95% CI. The significance of change in the prevalence of nocturnal sweating symptoms with PAP treatment was assessed using population averaged generalized estimating equations for a binomial outcome. The Wald test was used to examine differences in change of prevalence in nocturnal sweating symptoms by level of PAP use.

Papers III and IV: Biomarker levels were (natural) log transformed for normality in all analyses. Statistical tests that compared the strengths of linear associations between leptin levels and different obesity metrics were produced using a non-parametric bootstrap re-sampling procedure (n=1000 replications) that accounted for within-subject correlations[332].

Subjects were stratified by obesity level based on BMI (BMI < 30 kg/m^2, BMI ≥ 30 and < 35 and BMI ≥ 35) to provide a comparison of biomarker response to increasing OSA severity across different BMI levels. The range of OSA severity was different among the three BMI groups as the highest BMI group had individuals with very high OSA severity (AHI > 80 and ODI > 65) not found in the lowest BMI group (Figure 11A and 11C). Also, there were a few participants in the lowest BMI group with low OSA severity (AHI < 14 and ODI < 10, n = 24) not found in the highest BMI group. To avoid extrapolation beyond the experience of the data, those participants who had OSA severity not found in the other BMI groups were excluded (n = 69, Figure 11B and D). In this way the range of AHI and ODI was kept comparable across obesity strata (selected *a priori*). This permitted valid application of analysis of covariance in which remaining differences in AHI and ODI could be subsequently adjusted for. Therefore the total number of subjects used in the analyses was n = 454 for Paper III and n= 452 for paper IV (see further in Paper III and IV).

The goal of the primary analysis was to estimate the simultaneous statistical effects of OSA and obesity severity and their interaction on biomarker levels (IL-6, CRP and leptin). The parameters of the multiple linear regression model included higher order and interaction terms (response surface modeling[333]) to permit the association of OSA severity to depend on obesity in both linear and non-linear ways. Different obesity and OSA severity markers were compared for their ability to explain variance in log(biomarker) levels. The prediction equation included linear and quadratic terms for selected OSA and obesity severity measures as well as interactions between obesity and OSA linear and higher orders terms as follows: E(log(biomarker)) = $\beta_0 + \beta_1$*OSA + β_2*(OSA)^2 + β_3*Obesity + β_4*(Obesity)^2 + β_5*(OSA*Obesity) + β_6*OSA*(Obesity)^2 + β_7*BMI*(Obesity)^2. Squared partial correlations were used to evaluate the relative importance of various model factors. The squared partial correlation is the proportion of unexplained variance that is eliminated when the

factor in question is added to the model. For further details on analytical strategies, see Papers III and IV.

The influences of a selected set of covariates were evaluated for confounding factors and effect modification. Confounding was assessed by adding the covariate to the model as a main effect and seeing if the significance of factors involving sleep apnea and obesity changed substantially. Effect modification arises when factors interacted with OSA and/or obesity to amplify or attenuate the inflammatory response. The independent role of these covariates was not the subject of this study, but the nature of significant independent associations was noted. A significant covariate that was not a confounder merely altered the variability explained by the model but did not change its shape or interpretations involving the obesity OSA interaction. Covariates assessed included gender, age (10-yr intervals), excessive alcohol consumption (≥ 7 drinks/wk for females and >14 drinks/wk for males), current smoking (smoking or tobacco use during the past month), pack-yr of smoking (quartiles; 0 pack-yr, > 0-17 pack-yr, 17-35 pack-yr, and > 35 pack-yr), cardiovascular disease (doctor diagnosis of coronary artery occlusion [myocardial infarction or heart attack], heart failure and/or stroke), hypertension (doctor diagnosis of hypertension and current hypertensive medication), diabetes (doctor diagnosis), regular exercise (yes/no) as well as the use of statins, both any use of statins (n = 103) and specifically the 2 most widely used in the study, atorvastatin (n = 49) and simvastatin (n = 39). A total of 15 patients were on other statins. Chronic obstructive pulmonary disease was not assessed as a confounder because it and/or emphysema were diagnosed in only 19 patients. Women were considered postmenopausal if they reported having stopped menstruation for ≥ 6 months.

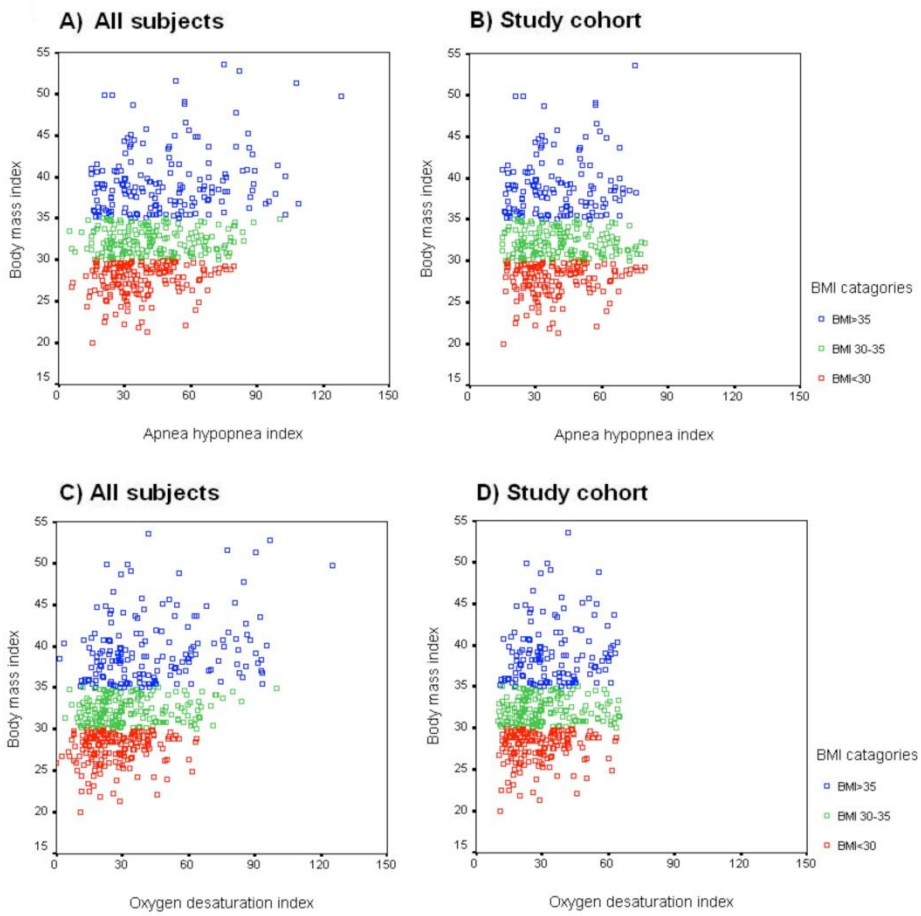

Figure 11. The range of the apnea-hypopnea index (AHI, upper panel) and oxygen desaturation index (ODI, lower panel) within the three body mass index (BMI) categories in all patients considered (a and c) and the study cohort with AHI 14-80 and ODI 10-65 only (b and d) in papers III and IV. Reproduced from Paper IV.

4 Results

4.1 The study cohorts

4.1.1 Baseline characteristics

In the ISAC cohort (Papers II – IV), over 90% of eligible and approached subjects agreed to participate in the study, a total of 826 subjects. Four subjects withdrew from the study, leaving 822 subjects in the baseline cohort, n = 666 males and 156 females with an age range of 21 - 83 years (Figure 12).

The general population cohort (Paper II) included a total of 762 subjects. Of these, n = 30 did not answer the questions on nocturnal sweating. Another n = 13 were > 83 years of age and n = 20 had a BMI < 20 kg/m^2, not found in the ISAC cohort. They were therefore excluded from further analysis. The final general population cohort for analysis was n = 703, n = 374 males and n = 329 females (Figure 12).

A total of 24 OSA patients agreed to take part in the clinical OSA cohort (Paper I) and all of the eligible and approached subjects agreed to participation. Nine participants were excluded from the data analyses because of CPAP noncompliance and referral to other treatment options (n = 7), predominant central apneas (n = 1) and current use of heart medication (n = 1). A total of 15 participants were therefore included in the study (Figure 12).

The characteristics of the three study cohorts are shown in Table 2. The clinical OSA cohort included only the subset of "otherwise healthy", non-smoking OSA patients as shown outside the rings in Figure 6, while the ISAC cohort was a clinical cohort reflecting the obesity levels and comorbidities found in the Icelandic sleep apnea population offered treatment for their disease. The ISAC sub-cohorts used for analysis in papers III and IV had very similar characteristics to the total ISAC cohort.

The subjects in the clinical OSA cohort were five years younger on average than the ISAC subjects but were otherwise similar in BMI and OSA severity. OSA subjects had a higher BMI on average than the general population subjects (Table 2) but the BMI range was similar (ISAC: 20.1 - 58.6 kg/m^2, controls: 20.0 - 55.0 kg/m^2). Participants in the ISAC cohort had a higher prevalence of treated hypertension and diabetes than subjects from the general population and reported more sleepiness on the Epworth Sleepiness Scale. Additionally, males in the ISAC cohort were two years younger on average, more likely to be current smokers, and have a diagnosis of cardiovascular disease than the males from the general population. These differences were not found in females but proportionally more females were found in the general population cohort than in the ISAC cohort (46.8% *vs*. 19%, p<0.001).

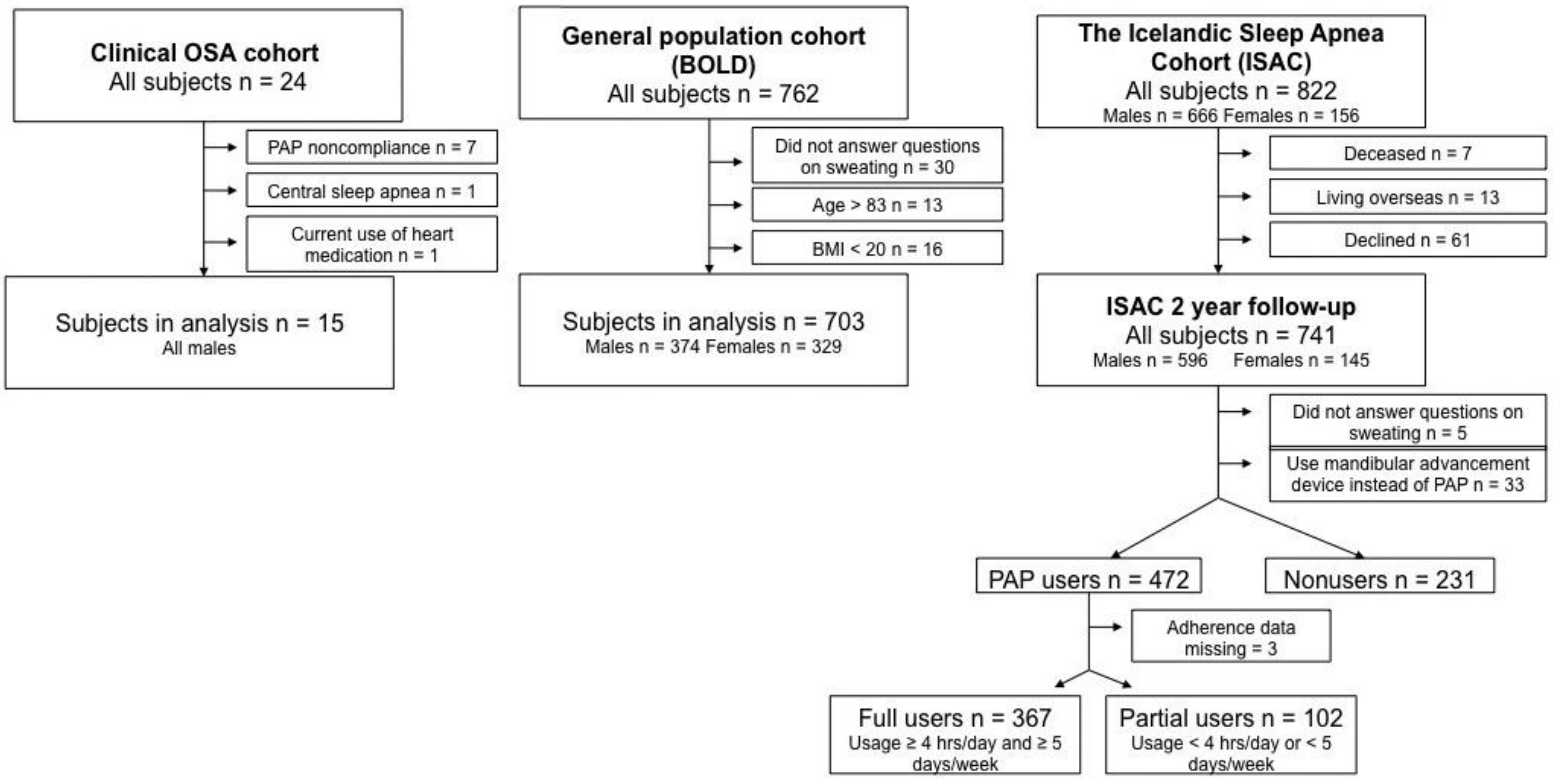

Figure 12. The three complete study cohorts. Abbreviations: OSA, obstructive sleep apnea; PAP, positive airway pressure; BOLD, burden of lung disease; BMI, body mass index; ISAC, Icelandic Sleep Apnea Cohort. Adapted from papers I-II.

Table 2. Demographic factors for all three study cohorts; The Icelandic Sleep Apnea Cohort (ISAC), the complete cohort in paper II and subset of cohort in papers III and IV, the clinical OSA cohort in paper I, and the general population cohort in paper II.

	Clinical OSA cohort (paper I)	**ISAC total cohort** (paper II)	**ISAC subset** (paper III)	**ISAC subset** (paper IV)	**General population cohort** (paper II)
	n=15	*n = 822*	*n=454*	*n=452*	*n=703*
Age (years)	49.4 ± 10.1	54.5 ± 10.6	54.4 ± 10.6	54.4 ± 10.6	56.4 ±11.1
Males (%)	100	81.0%	83.7	83.4	53.2%
BMI	34.6 ± 8.0	33.5 ± 5.7	32.6 ± 5.3	32.7 ± 5.3	28.1 ± 4.8
Current smokers (%)	0.0	21.1	23.8	24.0	18.0
AHI	45.3 ± 15.1	44.9 ± 20.7	40.1 ± 16.1	40.2 ± 16.1	N/A
ODI	34.8 ± 19.4	35.5 ± 20.2	31.5 ± 14.0	31.5 ± 14.1	N/A
ESS score	11.3 ± 4.4	11.7 ± 5.1	11.7 ± 4.9	11.6 ± 4.9	6.1 ± 3.9
Hypertension (%)	0.0	45.7	44.4	44.4	24.6
CVD (%)	0.0	18.4	18.2	17.8	14.4
Diabetes (%)	0.0	8.7	11.1	11.1	2.9

Abbreviations: OSA, obstructive sleep apnea; BMI, body mass index; AHI, apnea-hypopnea index; ODI, Oxygen desaturation index; ESS, Epworth Sleepiness Scale, CVD, cardiovascular disease, defined as a doctor diagnosis of coronary artery occlusion (myocardial infarction or heart attack), heart failure and/or stroke. Hypertension and diabetes were defined as a doctor diagnosis and medication.

4.1.1.1 ISAC subsets in Papers III and IV

All untreated ISAC participants (n = 532) at the time of analysis (Sept 2005 - Sept 2008) were assessed for the simultaneous assessment of the role of OSA severity and obesity level on biomarker levels (Figure 13). Nine subjects had <4 hours of a scorable O_2 saturation signal and were excluded from further analysis. Another 69 subjects were excluded from the primary analysis due to non-overlapping OSA severity (AHI or ODI) in the different BMI groups (see further in Methods). The study cohort, therefore, included 454 patients across three BMI strata, all having AHI 14-80 and ODI 10-65 in Paper III. The study cohort in paper IV included almost all the same subjects (n=452 out of n=454 in final analysis, Paper IV - Figure 1). Among these study participants, complete MRI was obtained in 373 subjects (82.2%). Failure to obtain MRI assessment occurred more commonly in the most obese group, usually due to claustrophobia or image quality issues. Subjects who did not have MRI date but had complete data from other assessments were included in other analyses. Out of the total cohort of 454 patients in analysis, 380 were males and 74 were females; 82.4% (61 of 74) of the women were postmenopausal and four were on hormone replacement therapy (HRT). Additional complementary analyses were done, including all 523 participants. The results of these additional analyses lead to the same conclusions and can be found in the supplements of papers III and IV.

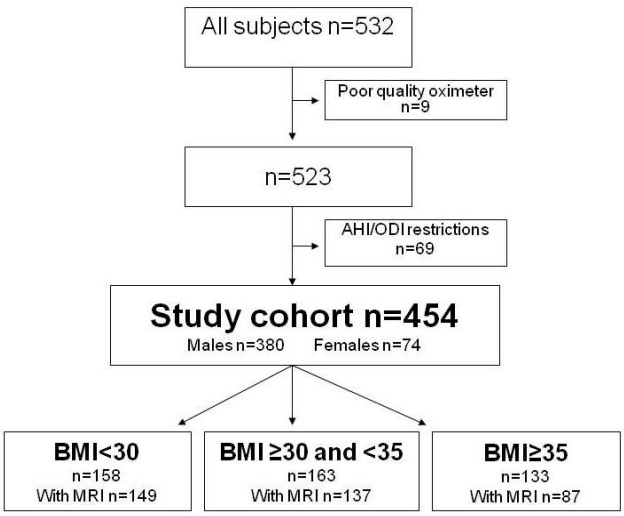

Figure 13. The Icelandic Sleep Apnea cohort used for biomarker analysis. Reproduced from Paper III but the study cohort in Paper IV was almost the same (n=452 instead of n=454). Abbreviations: BMI, body mass index; AHI, apnea hypopnea index; ODI, oxygen desaturation index; MRI, magnetic resonance imaging.

4.1.2 PAP follow-up

In the clinical OSA cohort, on average the PAP compliance for the three months was 5.5 ± 1.3 hrs and subjects with little or no use were excluded from the study (Paper I).

The subjects in ISAC were invited to a 2 year follow-up visit, done in the same manner as the initial visit (Paper II). This follow-up was completed in 741 (90.1%) subjects from October 2007 to January 2012. No significant differences were found in age, BMI, OSA severity (as assessed by AHI or ODI) or gender between non-responders (n = 81) and responders (n = 741). At follow-up, five subjects did not complete the questions on nocturnal sweating, 33 subjects were using a mandibular advancement device instead of PAP, and three had missing adherence data on PAP, leaving n = 700 in follow-up analysis in Paper II; 469 PAP users and 231 nonusers (Figure 12).

At the two year follow-up, objective PAP data were available for 357 out of 469 PAP users (76.1%). Out of the PAP users, 53% were on autoPAP, 43% on CPAP, 3% on BiPAP and 1% on adaptive servoventilation. Participants with objective data who used PAP for ≥ 20 days and ≥ 4 hrs/day on average for the past month were considered full users (n = 285 of 357 with objective data).

Full users used their device on average 26.7 ± 2.0 days in the preceding four weeks and 6.8 ± 1.2 hrs/day. Partial users were defined as those using PAP≤19 days or <4 hrs/day on average (n = 72). The partial users used their device on average for 14.5 ± 7.1 days and 3.6 ± 2.1 hrs/day in the preceding four weeks.

In those participants for whom objective PAP data were not available (n = 112), the self-report data were used to define subjects as full or partial PAP users. To validate this approach, the data among the 355 subjects with both objective (memory cards) and self-reported data on frequency of PAP use were compared. Self-report of PAP use ≥ 5 nights per week and ≥ 60% of the sleeping time had 98.6% sensitivity and 45.1% specificity in distinguishing full versus partial users.

Among the non-PAP users (n = 231) in the ISAC cohort, 56.7% (n = 129) had a repeat sleep study when followed after two years. On average, the AHI increased from 37.8 ± 15.0 events/hr at baseline to 48.4 ± 18.6 events/hr at follow-up, $p < 0.0001$, despite a similar weight on average at baseline and follow-up. Altogether 98% of the 129 subjects still had AHI ≥ 15 events/hr at the two year follow-up. A total of n = 17 nonusers underwent upper airway surgery on the uvula and/or soft palate between baseline and follow-up and n = 13 had >10% weight loss. Of these, n = 18 had a repeat sleep study at follow-up and had no significant change on average in their AHI between baseline and follow-up despite the alternative treatment.

4.2 Frequent nocturnal sweating

4.2.1 Objective *vs*. subjective measurements

In the otherwise healthy OSA patients in the clinical OSA cohort (Paper I) the distribution of reported nocturnal sweating was very broad. When untreated, one patient of fifteen reported sweating every night or almost every night, two patients 3-5 times a week, six patients 1-2 a week, two less than once a week, and four never or very seldom. The reported subjective nocturnal sweating of OSA patients significantly lowered with PAP treatment ($p = 0.008$), with all subjects now reporting nocturnal sweating <3 times a week.

The distribution of measured objective sweating as assessed by the EDA index was also very broad (27.3 – 337.6 events/hr sleep). A high correlation was found between subjective measurement of sweating in untreated patients and the objective measurement of sweating as measured by the EDA index (Spearman $r = 0.61$, $p = 0.01$, Figure 14). This correlation was not seen for the patients on PAP treatment (Spearman $r = -0.03$, $p=0.91$) but these findings are inconclusive due to the limited range of reported nocturnal sweating on PAP (all <3 times a week).

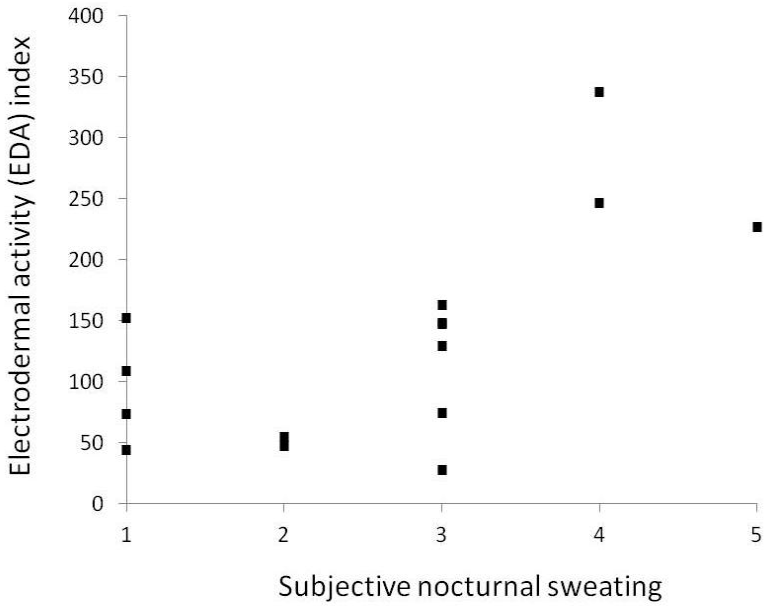

Figure 14. The correlation of subjectively reported nocturnal sweating to the measured electrodermal activity (EDA) index per hour of sleep (Spearman $r=0.61$, $p=0.01$). Subjective nocturnal sweating was assessed on a frequency scale of 1-5: (1) never or very seldom, (2) less than once a week, (3) once to twice a week, (4) three to five times a week, and (5) every day or almost every day of the week. Adapted from Paper I.

4.2.2 Is frequent nocturnal sweating a symptom of OSA?

Participants in the ISAC cohort had a three-fold higher prevalence of frequent nocturnal sweating (≥ 3 times a week) than subjects in the general population cohort (31.1% *vs*. 11.1%, $p < 0.0001$). Subjects in the ISAC cohort had a higher prevalence of frequent nocturnal sweating within all comparable gender and age groups (40 - 49, 50 - 59, 60 - 69, and ≥ 70 years) except that the difference was not statistically significant in women ≥70 years, likely due to low number of subjects in that group (Figure 15). No significant gender differences in prevalence of nocturnal sweating were found. The distribution of sweating on the original 5-point scale was also much more skewed to the left in the general population than in the untreated OSA cohort, where more subjects report some degree of nocturnal sweating (Figure 16).

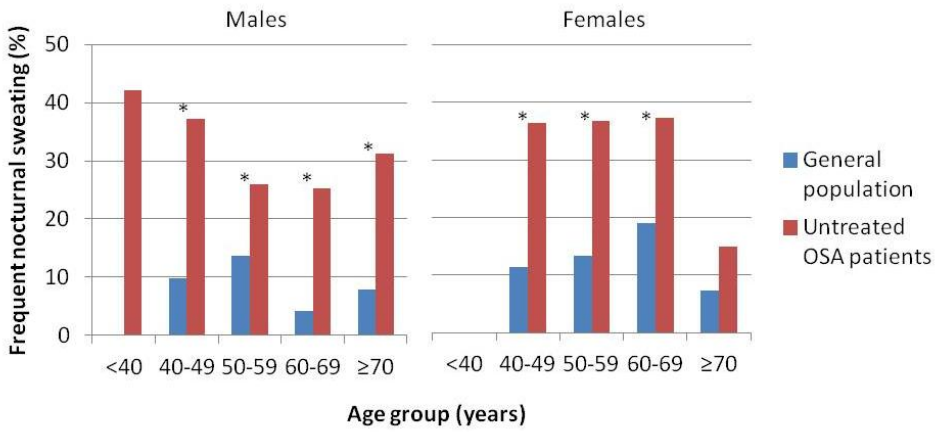

Figure 15. The prevalence of frequent nocturnal sweating (≥ 3x week) in subjects in the general population and the untreated obstructive sleep apnea (OSA) patient cohort, divided by gender and age groups. Significant differences ($p < 0.05$) are shown by an asterisk (*). Reproduced from Paper II.

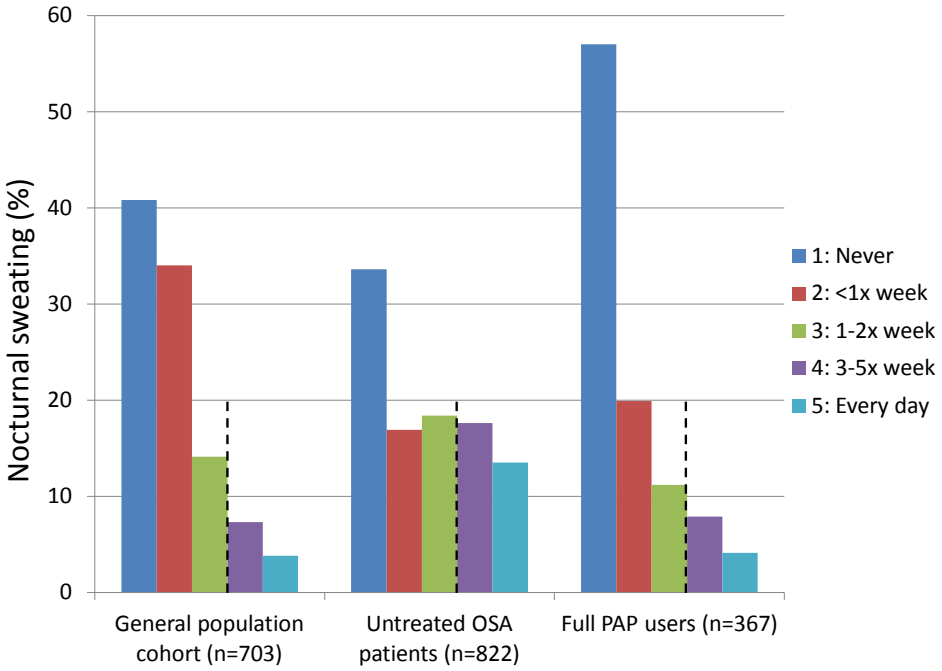

Figure 16. The distribution of reported nocturnal sweating (%) on a 1 - 5 frequency scale for the general population and untreated OSA cohort. Additionally the full positive airway pressure (PAP) users at the 2 year follow-up in the Icelandic Sleep Apnea Cohort(n = 367) are shown. Subjects with frequent nocturnal sweating were defined by a prevalence ≥ 3 times a week, as shown by the broken line. Reproduced from Paper II.

4.2.3 Demographic factors related to nocturnal sweating

The demographic characteristics of subjects with and without frequent nocturnal sweating in the ISAC and general population cohort were compared. Younger age was significantly related to nocturnal sweating among OSA patients ($p = 0.003$) but not in the general population ($p = 0.44$). In the general population cohort only, subjects with medically treated diabetes (n = 20) were more likely to report frequent nocturnal sweating ($p < 0.05$). No relationship was found with gender, current smoking, diagnosed hypertension or cardiovascular disease in either cohort in unadjusted analysis (data not shown). However, those reporting frequent nocturnal sweating reported lower mental and physical quality of life, both in the general population and the OSA cohort. For further details, see Table 2 in Paper II.

To understand better which factors were associated with frequent nocturnal sweating after adjustment for other covariates, a multivariable logistic regression model was created. OSA patients and general population subjects were assessed separately. Variables tested in the model were gender, age, BMI, current smoking, diagnosed hypertension, cardiovascular disease, and

diabetes. For both cohorts, lower age was a significant predictor of nocturnal sweating (Table 3). For the general population subjects only, diagnosed cardiovascular disease was an additional significant factor and for the OSA cohort only, hypertension was an additional significant factor. The effect of beta-blockers and calcium-channel blockers was tested specifically in multivariate analysis, and was not significantly related to frequent nocturnal sweating (data not shown). Similarly in the otherwise healthy OSA subjects in the clinical OSA cohort (Paper I), higher objective sweating as measured by the EDA index correlated with higher systolic blood pressure in the evening and morning in untreated patients ($r= 0.63$, $p=0.001$ and $r=0.67$ $p=0.006$, respectively). In the ISAC study, only morning blood pressure was measured. Significantly higher diastolic blood pressure was found for non-hypertensive subjects reporting frequent nocturnal sweating (n=131) compared to those reporting sweating only seldom (n=314), but the differences were small (83.8 ± 8.8 *vs*. 81.5 ± 9.7 in those that reported sweating seldom, $p=0.02$, unpublished data).

When we combined both the ISAC and general population cohorts into one analysis (Paper II), being a part of the OSA cohort remained significantly related to increased nocturnal sweating after adjustment for demographic factors, with an odds ratio of 3.11 (95% CI 2.25 to 4.30). No interaction with gender was found, neither within the OSA cohort, general population cohort nor for all subjects combined. Decreased mental and physical quality of life remained significantly lower in those reporting frequent nocturnal sweating after adjustment for demographic factors, both in the OSA and general population cohorts ($p<0.01$).

Table 3. Demographic factors associated with frequent nocturnal sweating (≥ 3 x a week) in the general population cohort and Icelandic Sleep Apnea Cohort (ISAC). The associations are expressed as adjusted odds ratio with a 95% confidence interval (OR (95% CI)). Significant findings ($p < 0.05$) are shown in bold. Reproduced from Paper II.

	General population only		**ISAC cohort only**	
	Unadjusted OR (95% CI)	Adjusted OR (95% CI)*	Unadjusted OR (95% CI)	Adjusted OR (95% CI)*
Female gender	1.37 (0.86 - 2.2)	1.38 (0.85 - 2.24)	1.13 (0.78 – 1.64)	1.24 (0.84 - 1.82)
Age per 10 years	0.92 (0.74 - 1.14)	**0.75 (0.57 – 0.99)**	**0.81 (0.70 – 0.93)**	**0.79 (0.67 – 0.93)**
BMI per 5 units	1.22 (0.97 - 1.52)	1.21 (0.96 - 1.52)	1.12 (0.99 - 1.28)	1.05 (0.92 - 1.21)
Current smoker	1.56 (0.89 - 2.72)	1.77 (0.98 - 3.19)	1.27 (0.89 – 1.81)	1.22 (0.85 – 1.76)
Hypertension	1.32 (0.79 – 2.23)	1.37 (0.75 – 2.51)	1.20 (0.89 – 1.62)	**1.48 (1.06 – 2.08)**
CVD	1.64 (0.90 -2.97)	**2.38 (1.14 – 4.95)**	0.69 (0.46 – 1.04)	0.68 (0.38 – 1.23)
Diabetes	2.77 (0.98 – 7.8)	2.66 (0.87 – 8.16)	0.73 (0.42 – 1.27)	0.83 (0.54 – 1.28)

Abbreviations: BMI, body mass index; CVD, cardiovascular disease, defined as a doctor diagnosis of coronary artery occlusion (myocardial infarction or heart attack), heart failure and/or stroke. Hypertension and diabetes were defined as a doctor diagnosis and treatment with medication. *Adjusted for all other variables in table.

4.2.4 Measurements, biomarkers and nocturnal sweating

No association was found between reported frequent nocturnal sweating and sleep apnea severity in the ISAC cohort (Paper II) on continuous or categorical comparison with AHI and ODI (categories: < 20, 20 - 40, 40 - 60 and > 60 events/hr). Similarly in the clinical OSA cohort (Paper I), no correlation was found between the EDA index and AHI or ODI in untreated OSA patients. No significant correlation was found between temperature measurements and sweating in the clinical OSA cohort. Also, no temporal relationship was found between temperature measurements or the EDA index to breathing disturbances and arousals in untreated OSA patients. No significant relationship was found between the EDA index and the nocturnal urinary sympathetic markers measured (epinephrine-creatinine and norepinephrine-creatinine ratio) in untreated OSA patients in the clinical OSA cohort.

In the ISAC study a borderline significant relationship was found between reported frequent nocturnal sweating and higher CRP levels. The geometric mean of the subjects reporting frequent sweating was 2.94 (95% CI 2.57-3.35) but for those reporting seldom sweating 2.52 (95% CI 2.31-2.75), p= 0.055 (unpublished data). No such relationship was found in the general population cohort. These differences in CRP in the ISAC cohort were, however, small and no correlation was found between CRP levels and objective sweating in untreated OSA subjects in the clinical OSA cohort (unpublished data).

4.2.5 Symptoms related to nocturnal sweating

Many sleep and respiratory symptoms were significantly related to nocturnal sweating in the univariate analysis (see Paper II - Table 2). After adjustment for demographic factors, in both the OSA cohort and the general population cohort, nocturnal sweating was associated with insomnia symptoms, excessive daytime sleepiness and RLS symptoms (Table 4). However, only participants in the ISAC cohort showed a relationship between daytime and nocturnal GER and nocturnal sweating. Subjects in the general population reporting frequent nocturnal sweating were more likely to report snoring. In addition, those with increased risk of OSA as calculated by the MAP score (n=46, 6.5%) were twice as likely to report frequent nocturnal sweating, albeit this was borderline statisticially significant (Adjusted odds ratio 2.08, 95% CI 0.94 to 4.59).

Fully adjusted models taking into account the effect of other sleep and respiratory complaints were also evaluated. Difficulties initiating sleep was most strongly related to nocturnal sweating in the general population (OR 4.05, 95% CI 1.72 to 9.54). For the participants in the OSA cohort, however, difficulties maintaining sleep, daytime sleepiness and GER symptoms were most strongly associated with frequent nocturnal sweating (Table 4).

Table 4: Independent associations between reported symptoms with nocturnal sweating in the general population cohort and Icelandic Sleep Apnea Cohort. Odds ratios (OR) are shown with 95% confidence intervals (CI) and significant findings ($p < 0.05$) are shown in bold. Reproduced from Paper II.

	General population only		**OSA patients only**	
	OR (95% CI) Partially adjusted[a]	OR (95% CI) Fully adjusted[b]	OR (95% CI) Partially adjusted[a]	OR (95% CI) Fully adjusted[b]
Reported snoring ≥ 3d/w	**1.92 (1.04 – 3.54)**	1.00 (0.47 – 2.11)	2.47 (0.94 – 6.53)	1.77 (0.62 – 5.02)
Difficulties initiating sleep ≥ 3d/w	**4.70 (2.66 – 8.32)**	**4.31 (1.84 – 10.10)**	**1.64 (1.09 – 2.46)**	1.49 (0.88 – 2.52)
Difficulties maintaining sleep ≥ 3d/w	**2.68 (1.63 - 4.41)**	1.12 (0.53 - 2.37)	**2.01 (1.46 – 2.78)**	**1.71 (1.15- 2.55)**
Feeling sleepy/drowsy during the day (6 - 7d/w)	**2.31 (1.16 - 4.58)**	2.28 (0.81 – 6.48)	**2.34 (1.57 – 3.49)**	**1.96 (1.28 – 3.01)**
Epworth Sleepiness Scale per 5 unit[c]	1.24 (0.91 - 1.68)	1.26 (0.83 – 1.91)	**1.24 (1.07 - 1.45)**	**1.19 (1.01 - 1.40)**
Restless legs syndrome symptoms	**2.00 (1.15 - 3.46)**	1.27 (0.57 – 2.87)	**1.47 (1.07 – 2.00)**	1.24 (0.84 – 1.82)
Nocturnal GER symptoms ≥ 1d/w	1.75 (0.74 – 4.17)	0.97 (0.12 - 7.78)	**2.21 (1.46 – 3.33)**	**1.84 (1.06 – 3.20)**
Daytime GER symptoms ≥ 1d/w	1.19 (0.39 - 3.66)	0.66 (0.08 – 5.37)	**1.99 (1.41 – 2.80)**	1.76 (0.95 – 2.42)
High risk OSA (MAP index ≥ 0.75)[d]	2.08 (0.94 – 4.59)	1.43 (0.54 – 3.79)	N/A	N/A

Abbreviations: d/w, days per week; GER, gastroesophageal reflux; MAP, multivariate apnea prediction. [a]Partially adjusted OR are adjusted for the demographics shown in Table 3. [b]Fully adjusted OR are adjusted for demographics as well as snoring, difficulties initiating and maintaining sleep, feeling sleepy/drowsy, restless legs syndrome, daytime and nocturnal GER. [c]The fully adjusted Epworth score is not adjusted for feeling sleepy/drowsy during the day.[d]The partial MAP index is not adjusted for gender, age and BMI, as it forms a part of the MAP score itself. Additionally the fully adjusted MAP index is not adjusted for snoring (see further in Methods).

4.2.6 The effect of PAP treatment on nocturnal sweating

The average EDA index decreased significantly from 131.9 ± 22.4 events/hr in untreated patients to 78.5 ± 17.7 events/hr with PAP treatment (p = 0.04, Paper I). The percentage of time during the sleep time spent in sweating lowered from 16.2 ± 3.3 % to 8.3 ± 2.0 % with PAP (p = 0.04). The variability in the EDA index both before and after treatment was considerable, and the EDA index did increase in 4 out of 15 patients with PAP treatment (Figure 17).

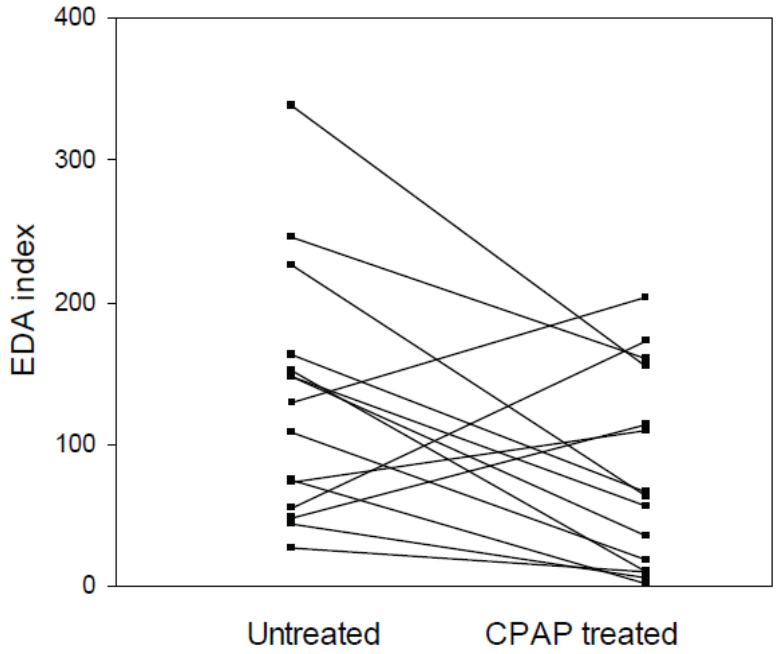

Figure 17. The individual change in electrodermal activity (EDA) index per hour sleep from untreated OSA patients to continuous positive airway pressure (CPAP) treated patients. Reproduced from Paper I.

The change in the prevalence of reported frequent nocturnal sweating with PAP treatment in the ISAC cohort was tested by comparing full PAP users, partial PAP users, and nonusers (Paper II). A significant decrease in the prevalence of frequent nocturnal sweating was found in the full PAP users as well as nonusers at the 2 year follow-up (p < 0.001), but no significant change was found in partial PAP users. The full PAP users had a significantly greater decrease in frequent nocturnal sweating than nonusers (33.5% to 12% *vs*. 31.6% to 20.4%, p = 0.01). See Table 5. Nonusers and partial PAP users were also more likely than full PAP users to report the continuation or development of frequent sweating (≥3 times a week) at follow-up (p < 0.01, Paper II - Figure 4).

Additional analysis was performed using only subjects with objective data of PAP use (n = 357) to determine if the number of hours of PAP use were correlated with changes in nocturnal sweating by frequency scale. A borderline significant but small correlation was found between decreased sweating at follow-up and higher average daily use of PAP (r = -0.09, p = 0.08).

Frequent nocturnal sweating at baseline did not predict whether an ISAC subject became a full PAP user, partial PAP user or nonuser. The distribution of nocturnal sweating on the 1 - 5 frequency scale for full PAP users was similar to the distribution of the general population (Figure 16).

Table 5. Frequent nocturnal sweating at 2-year follow-up for the different PAP adherence groups in the Icelandic Sleep Apnea Cohort. Significant findings ($p < 0.05$) are shown in bold. Reproduced from Paper II.

Frequent nocturnal sweating (≥ 3d/w)	**Baseline (%)**	**Follow-up (%)**	**p_{change}**	**p compared to nonusers**
Nonusers (n = 231)	**31.6**	**20.4**	**<0.001**	-
Partial PAP users (n = 102)	31.4	26.5	0.33	0.28
Full PAP users (n = 367)	**33.5**	**12.0**	**<0.001**	**0.01**

Abbreviations: d/w, days per week; PAP, positive airway pressure

4.2.7 Changes in measurements and biomarkers

In the clinical OSA cohort epinephrine levels in the overnight urine sample decreased significantly in the OSA patients when they were treated with PAP for 3 months (p = 0.02, Paper I). No significant change was found in norepinephrine levels. A significant decrease was also found in diastolic morning blood pressure with PAP treatment (p = 0.008) but no change in morning systolic blood pressure or evening blood pressure or CRP levels in blood (CRP, unpublished data).

No relationship was found between traditional markers of OSA severity with objective or subjective measures of sweating (Papers I and II). However, a decrease in respiratory mechanics instability index with PAP treatment was shown to correlate with a decrease in EDA index (r = 0.60, p= 0.02, Paper I). On PAP treatment, a higher respiratory mechanics instability index also correlated with a higher EDA index (r = 0.56, p = 0.03). In addition, a significant relationship was found between the EDA index in untreated patients and the change in REM% with CPAP treatment. The higher the EDA index was in untreated patients, the more the REM% increased with PAP treatment (Paper I

- Figure 3). This relationship was maintained when controlled for AHI. No correlation was found between the EDA index and other sleep stages or the number of arousals and awakenings in the patients when untreated or PAP treated. No significant difference in temperature measurements (both core and distal-proximal skin temperature gradient) was observed between subjects when untreated and PAP treated. For more details, see Paper I.

Additionally a decrease in the EDA index with PAP treatment correlated with a decrease in evening systolic and diastolic blood pressure (r=0.52, p=0.05 and r=0.67, p=0.006, respectively, Figure 18). In the ISAC cohort, no significant relationship was, however, found between changes in subjective sweating and systolic or diastolic blood pressure measured in the morning (unpublished data).

No changes were found in sympathetic urinary biomarkers or CRP levels in relation to changes in sweating, neither in the clinical OSA nor the ISAC study cohorts (CRP, unpublished data).

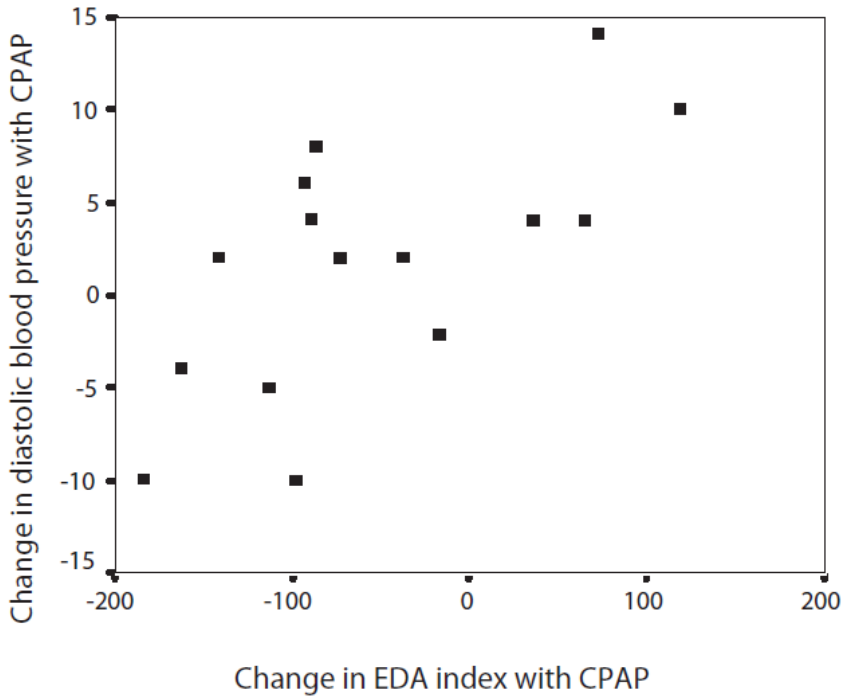

Figure 18. The change in objective sweating as measured by the electrodermal activity (EDA) index during sleep correlated highly with the change in diastolic blood pressure with continuous positive airway pressure (CPAP) treatment (r=0.67, p=0.006). Reproduced from Paper I.

4.2.8 Symptom changes

Subjects with decreased sweating had less difficulties maintaining sleep ≥ 3x week at follow-up compared to those with persistent sweating (Paper II). This was found both for full PAP users and nonusers ($p < 0.01$ for both groups), but no difference was found for partial PAP users. Similarly full PAP users that developed frequent nocturnal sweating at follow-up (n = 33) had more difficulties maintaining sleep ($p = 0.003$) than subjects with no frequent sweating at both baseline and follow-up (n = 439).

In nonusers only (Paper II), subjects with decreased sweating also had decreased daytime sleepiness as assessed by the ESS compared to those with persistent frequent sweating (decrease by -3.5 ± 4.4 *vs*. -0.6 ± 4.1 points, $p = 0.006$). No significant changes were found in reported difficulties initiating sleep, GER symptoms or RLS symptoms. Also, full PAP users were more likely to remain feeling sleepy/drowsy ($p = 0.003$) at follow-up if they developed frequent sweating compared to subjects with no frequent sweating. A borderline significant relationship between higher objective sweating (Paper I) and more sleepiness as assessed by the ESS on PAP treatment was also found in the clinical OSA cohort ($r=0.45$, $p=0.09$, unpublished data).

No significant changes were found in mental or physical quality of life for any of the PAP groups related to changes in reported frequent nocturnal sweating (Paper II).

4.3 Is OSA severity independently associated with biomarker levels after adjustment for obesity?

The 3 BMI groups (BMI<30, BMI 30 - <35 and BMI ≥35) in the subset of the ISAC study cohort (n=454 in Paper III and n = 452 in Paper IV) used for analysis of inflammatory biomarkers were similar with regard to demographics, although the prevalence of hypertension and diabetes increased with increasing BMI (Table 6). In addition, with increasing BMI the average degree of hypoxia increased even though the ranges for AHI and ODI were restricted to be equal (AHI range 14-80 and ODI range 10-65, see further in Methods). No difference was found in statin use or prevalence of chronic obstructive pulmonary disease (COPD) between the three BMI groups (see Paper III – Table 1).

Table 6. Demographic and sleep-disordered breathing data in the Icelandic Sleep Apnea Cohort (n = 454) and by body mass index (BMI) in the three BMI categories. Significant differences are shown in bold (p < 0.05). Adapted from Papers III and IV.

	BMI < 30 kg/m^2	**BMI 30- < 35 kg/m^2**	**BMI ≥ 35 kg/m^2**	**P value[a]**
	n = 158	n = 163	n = 133	
Age (years)	55.4 ± 9.5	54.8 ± 10.9	52.7 ± 11.2	0.08
% of males	86.7	83.4	80.5	0.35
BMI (kg/m^2)	27.6 ± 2.0	32.2 ± 1.4	39.2 ± 3.7	N/A
Current smokers (%)	27.2	22.7	21.2	0.45
AHI	38.8 ± 15.2	41.3 ± 16.8	40.4 ± 16.3	0.39
ODI	**28.5 ± 12.4**	**31.6 ± 14.8**	**34.8 ± 14.3**	**0.001**
Minimum SaO_2	**78.7 ± 5.9**	**77.7 ± 7.3**	**74.9 ± 8.0**	**< 0.001**
Hypoxia time (min)	**30.3 ± 42.1**	**38.9 ± 60.7**	**60.8 ± 73.9**	**< 0.001**
ESS score	12.1 ± 4.9	11.3 ± 4.8	11.7 ± 5.0	0.34
Hypertension (%)	**35.0**	**44.1**	**56.1**	**0.002**
CVD (%)	17.3	17.3	20.5	0.75
Diabetes (%)	**5.8**	**8.6**	**20.3**	**< 0.001**
CRP(mg/L)[b]	**1.8 (1.6 - 2.0)**	**2.6 (2.3 - 2.9)**	**4.1 (3.6 - 4.7)**	**< 0.001**
IL-6 (pg/ml)[b]	**1.3 (1.2 - 1.4)**	**1.6 (1.5 - 1.8)**	**2.2 (2.0 - 2.4)**	**< 0.001**
Leptin (ng/ml)[c]	**6.7 (6.0 - 7.3)**	**10.8 (9.8 - 11.8)**	**17.9 (16.1 - 19.9)**	**<0.001**

Note: Data are presented as mean ± standard deviation or % where indicated. [a]One-way analysis of variance for continuous variables and Pearson chi-square test for percentages. [b]Exponentiated means and 95% confidence intervals determined from log-transformed values (i.e., geometric mean). [c]Total n=452 instead of n=454. Abbreviations: AHI, apnea-hypopnea index; ODI, oxygen desaturation index; SaO_2, oxygen saturation; min, minutes; ESS, Epworth Sleepiness Scale; CVD, cardiovascular disease defined as a doctor diagnosis of coronary artery occlusion (myocardial infarction or heart attack), heart failure and/or stroke..; CRP, C-reactive protein; IL-6, interleukin-6. Hypertension and diabetes were defined as a doctor‘s diagnosis and treatment with medication.

4.3.1 The relationship between biomarkers and obesity

Both IL-6, CRP and leptin levels increased significantly across the three BMI groups (Table 6, $p < 0.001$). For leptin levels, a large gender difference was found, but was not found for IL-6 or CRP. In males, the geometric mean (95% CI) leptin level was 9.0 (8.5 - 9.6) ng/ml and for females 24.0 (20.7 - 27.8) ng/ml, $p<0.0001$). Menopausal status was, however, not significantly associated with leptin levels.

Of all the different fat measures, the correlation between inflammatory IL-6 and CRP levels and obesity level was highest for BMI (Table 7). The magnitude of correlation between BMI and CRP was significantly higher than for visceral fat volume alone, waist circumference, neck circumference, and waist-to-hip ratio in bootstrap analysis ($n = 373$, $P < 0.05$). However, BMI, total and subcutaneous fat volume had comparable magnitudes of correlation with CRP levels. Similar results were found for BMI and IL-6, i.e., the association with BMI was higher than for other fat measures. Total fat volume was significantly more highly correlated with both CRP and IL-6 levels than visceral fat volume (bootstrap analysis).

The correlations between leptin levels and different measures of obesity were assessed with and without adjustment for gender (Table 7). The magnitude of correlation between total fat volume and leptin levels, adjusting for gender, was significantly highest of all the obesity measures ($r = 0.73$, $p < 0.001$). The correlations for BMI, waist circumference and subcutaneous fat volume were significantly lower based on bootstrap-based comparisons ($r = 0.64 - 0.69$, $p < 0.001$). Visceral fat volume, neck circumference and waist-to-hip ratio were significantly less associated with leptin levels than all of the above.

For correlation analysis between the different measures of obesity and between different measures of obesity and OSA severity indices, see supplemental Table S2 in Paper III.

4.3.2 The relationship between biomarkers and sleep apnea

The correlation between IL-6, CRP and leptin levels and OSA severity was assessed by four different markers of OSA severity: a) the number of sleep disordered breathing events (AHI) and three desaturation indices; ODI, hypoxia time, and minimum SaO_2. When assessing all OSA subjects, the correlation was significant for the desaturation indices only with all three biomarkers (Table 8). However, AHI was not significantly correlated with IL-6, CRP or leptin levels.

When assessed within the 3 BMI categories, only minimum SaO_2 and hypoxia time remained significantly associated with IL-6 and CRP levels and only in the 2 groups of obese patients, not in those with BMI < 30 kg/m^2 (Table 8 and Figure 19). However, no association of leptin levels and OSA severity was found when assessed within the 3 BMI categories. No relationship was found between any biomarker level and subjective sleepiness as assessed by the Epworth Sleepiness Scale (data not shown).

Table 7. Pearson correlation coefficients between interleukin-6 (IL-6), C-reactive protein (CRP) and leptin levels in serum and various obesity measurements in all subjects with magnetic resonance imaging (MRI) data (n = 371). The analysis is shown both unadjusted for all three biomarkers and leptin additionally adjusted for gender. The highest correlation in each column is shown in bold. Adapted from Papers III and IV.

	Log(IL-6) levels (pg/ml)		**Log(CRP) levels (mg/L)**		**Leptin levels (ng/ml)**		**Leptin levels (ng/ml) Adjusted for gender**	
	r	p	r	p	r	p	r	p
Body mass index (kg/m^2)	**0.29**	**< 0.001**	**0.31**	**< 0.001**	0.61	<0.001	0.64	<0.001
Total abdominal fat volume (cm^3)	0.26	< 0.001	0.30	< 0.001	0.58	<0.001	**0.73**	**<0.001**
Subcutaneous fat volume (cm^3)	0.23	< 0.001	0.30	< 0.001	**0.67**	**<0.001**	0.69	<0.001
Waist circumference (cm)	0.24	< 0.001	0.24	< 0.001	0.44	<0.001	0.67	<0.001
Visceral fat volume (cm^3)	0.23	< 0.001	0.21	< 0.001	0.24	<0.001	0.59	<0.001
Neck circumference (cm)	0.21	< 0.001	0.15	0.002	0.04	0.50	0.44	<0.001
Waist-to-hip ratio (cm/cm)	0.06	0.22	0.09	0.10	0.03	0.61	0.42	<0.001

Note: Similar correlations were obtained when assessed using Spearman correlation.

Table 8. Pearson correlation between IL-6, CRP and leptin levels in serum (natural log transformed) and OSA severity in the Icelandic Sleep Apnea cohort and within the 3 different BMI categories. Significant p values ($p < 0.05$) are shown in bold. Adapted from Papers III and IV.

	Apnea-hypopnea index	**Oxygen desaturation index**	**Minimum SaO_2 (%)**	**Hypoxia time (minutes)**
IL-6 levels				
All subjects	0.03	**0.13**	**-0.22**	**0.26**
BMI < 30	-0.15	-0.09	0.10	-0.06
BMI 30- < 35	0.11	0.13	**-0.27**	**0.30**
BMI ≥ 35	0.05	0.16	**-0.26**	**0.30**
CRP levels				
All subjects	0.06	**0.12**	**-0.19**	**0.14**
BMI < 30	-0.02	-0.02	0.07	-0.12
BMI 30- < 35	0.06	0.06	**-0.20**	0.11
BMI ≥ 35	0.08	0.15	**-0.22**	**0.19**
Leptin levels				
All subjects	-0.04	0.05	**-0.17**	**0.20**
BMI < 30	0.01	0.02	-0.04	0.13
BMI 30- < 35	-0.14	-0.13	-0.12	0.08
BMI ≥ 35	-0.09	-0.07	-0.05	0.14

Note: Similar correlations were obtained when assessed using Spearman correlation. This analysis is not adjusted for other confounders. Abbreviations: BMI, body mass index; IL-6, interleukin-6; CRP, C-reactive protein; SaO_2, saturation of oxygen.

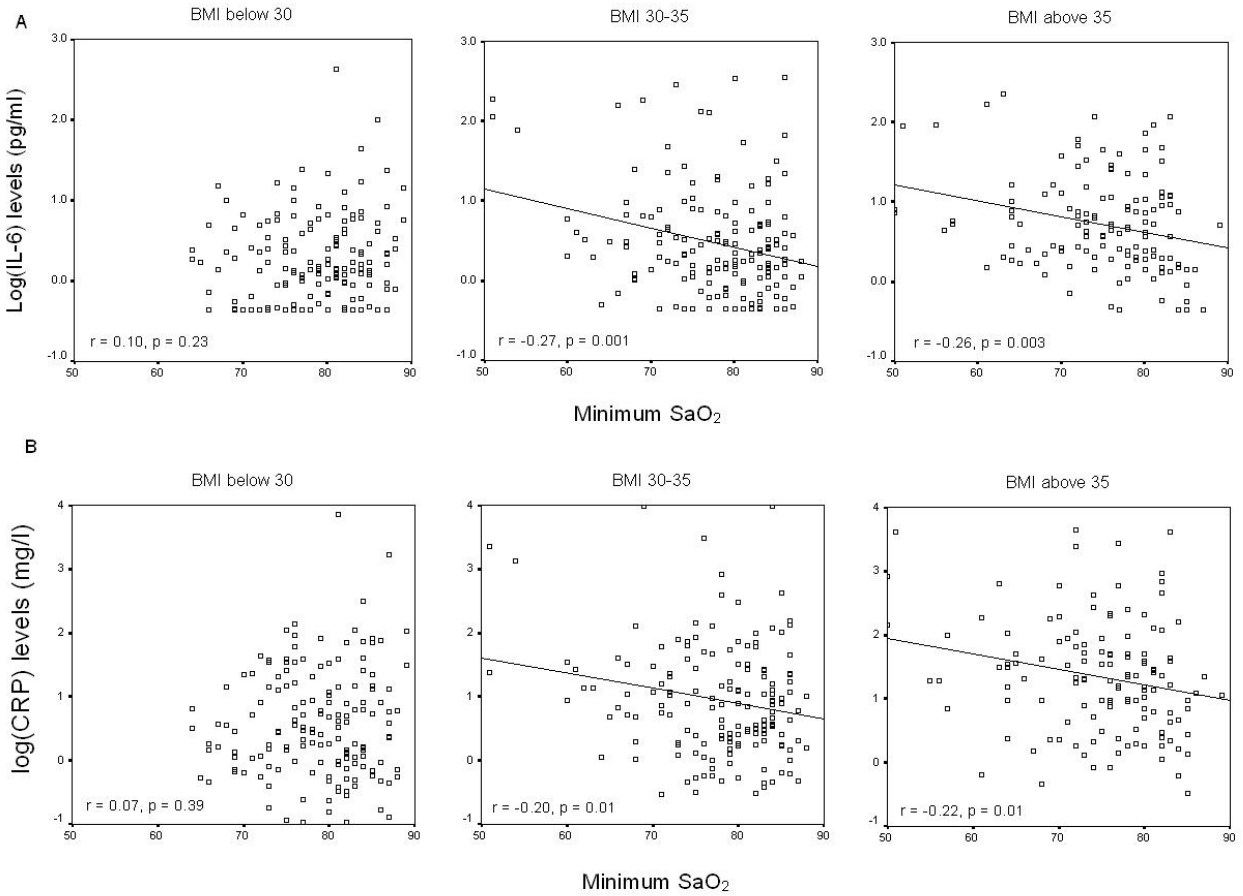

Figure 19. The relationship between severity of obstructive sleep apnea as assessed by the minimum oxygen saturation (SaO_2) across the night and inflammatory biomarkers a) interleukin-6 (IL-6) and b) C-reactive protein (CRP) for three body mass index (BMI) groups; BMI <30, BMI 30 – <35 and BMI ≥35. Reproduced from Paper III.

4.3.3 IL-6 levels: Does obesity affect the role of OSA?

4.3.3.1 Simultaneous assessment of BMI and OSA

To evaluate how the obesity level affects the relationship between OSA severity and the different biomarker levels, a multiple linear regression model for obesity and OSA severity, including interaction and quadratic terms (allowing for nonlinear effects of both obesity and OSA severity), was used. BMI was selected as the primary obesity variable as it showed the highest correlation with both IL-6 and CRP levels in univariate analysis as well as a high correlation with leptin levels, even after adjustment for gender (Table 7).

Both OSA severity and BMI level were independently associated with IL-6 levels in our cohort of OSA patients. This association was robust as it was found for all OSA severity indices assessed, i.e., AHI, ODI, hypoxia time, and minimum SaO2 ($p \leq 0.01$). Also a significant interaction between OSA severity and BMI was found, indicating a differential effect of OSA severity on IL-6 levels depending on obesity level (significant for AHI, hypoxia time, and minimum SaO2 and a trend for ODI, $p = 0.10$). A 3-dimensional plot was used to illustrate visually the combined role of OSA severity and obesity in IL-6 levels (Figure 20). This plot shows that among obese patients, IL-6 levels increased as OSA severity increased (especially evident for BMI ≥ 35). However, the effect of increased OSA severity was clearly attenuated in nonobese patients (BMI < 30 kg/m2). Please note that the apparent reversal of the role of OSA severity in IL-6 levels for the nonobese patients needs to be interpreted cautiously, as there is less statistical precision on the boundaries of the model. However, it is clear from Figure 20 that the marked increase in IL-6 levels for increasing OSA severity observed among the obese subjects was not found for the nonobese. The overall model with BMI and hypoxia time explained 18.2% of the variance in IL-6 levels ($p < 0.0001$). Models including other measures of OSA severity explained less of the variance (13.5-17.0%, Figure 20) but all showed a significant association of both OSA severity and obesity level with IL-6 levels. BMI explained the largest part of the variance in IL-6 levels or 12.0% and OSA, as assessed by hypoxia time, explained 8.6%. A significant part of the variance was explained by the interaction between OSA severity and BMI level (3.7%) and nonlinear associations (4.7%). The multivariate results therefore agreed with the simpler correlation analysis (Figure 19a) and confirmed that the relationship between OSA severity and IL-6 levels depended on the degree of obesity.

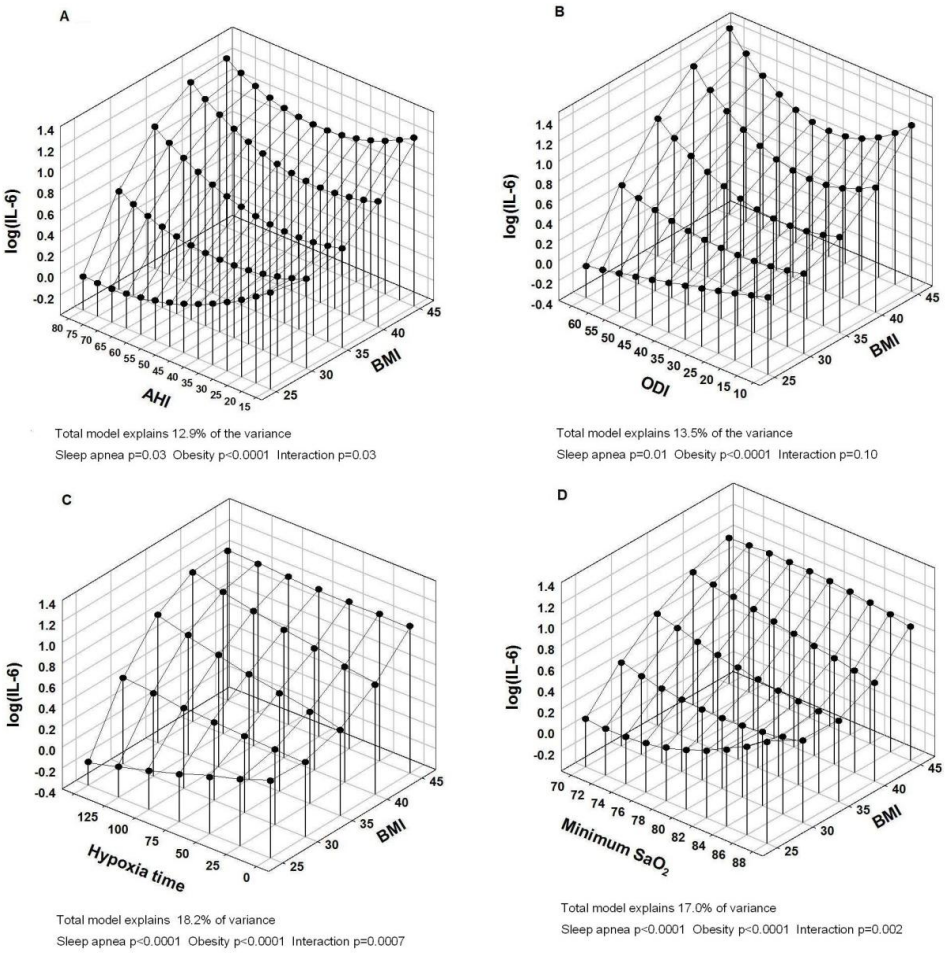

Figure 20. Three-dimensional plots assessing the role of obesity level (body mass index, BMI) and four different severity indices of obstructive sleep apnea (OSA) in interleukin-6 (IL-6) levels: a) apnea-hypopnea index (AHI); b) oxygen desaturation index (ODI); c) hypoxia time (minutes with SaO_2 < 90%), and d) minimum oxygen saturation (SaO_2). The significance of associations between IL-6 levels and OSA severity, obesity and the interaction between them are shown below the panels as well as the total variance in IL-6 explained by the model. These analyses are not adjusted for other potential confounders. Reproduced from Paper III.

4.3.3.2 Relationship of other obesity variables and IL-6

The role of visceral and total abdominal fat as the main obesity variable was compared with the role of BMI in IL-6 levels in patients who underwent an MRI

(n = 373) to assess whether this would be a more appropriate measure of obesity level and explain more of the variance in IL-6 levels. Hypoxia time as the OSA severity variable continued to explain the highest amount of variance in IL-6. The hypoxia time model with visceral fat explained 9.5% of the variance, while total fat explained 12.4%, but the BMI model explained the highest amount of the variance or 14.2% for this smaller sample of n=373 OSA patients. Finally, waist circumference was tested as the main obesity variable and found to explain less of the variance in IL-6 levels than did BMI (15.9% versus 18.2%, n = 454), similar to the simpler correlation analysis (Table 8 and Figure 19a). All of these analyses showed an independent association of OSA severity and IL-6 levels that depends on obesity level.

4.3.3.3 Role of confounders

Potential confounders that could contribute to increased inflammation were assessed separately for the IL-6 model with BMI and hypoxia time: higher age, current smoking, hypertension, cardiovascular disease, and diabetes, and higher visceral fat volume were all significantly associated with higher IL-6 levels independent of OSA severity and BMI level (Table 9). These covariates individually had a small, additive association with IL-6 levels (explaining an additional 0.9-3.0% of the variance in IL-6 levels), but importantly, did not affect the interaction between OSA severity and obesity. A significant interaction with a covariate would indicate that the relationship between OSA severity and obesity level with IL-6 levels changed depending on the presence or level of the covariate. Therefore, the variable association of OSA and IL-6 levels at different BMI levels, as shown in Figure 20, holds after adjusting for these covariates. *Gender* was not significantly associated with IL-6 levels.

Table 9. The effect of potential confounders on the model for interleukin-6 (IL-6) levels with OSA severity (hypoxia time) and obesity level (body mass index). The additional variance (partial R^2) in IL-6 levels explained by the covariate is shown (main effect) as well as the effect of the covariate on the interaction of OSA and obesity on IL-6 levels. Significant p values ($p < 0.05$) are shown in bold. Adapted from Paper III.

Covariate	**Main effect**			**Interaction[a]**
	β ± SE	p	Partial R^2	p
Male gender	-0.09 ± 0.07	0.22	0.3%	0.54
Age (10 years)	**0.09 ± 0.03**	**<0.001**	**2.5%**	0.62
Current smoking	**0.13 ± 0.06**	**0.04**	**0.9%**	0.72
Pack-years (quartiles)	**0.06 ± 0.03**	**0.01**	**1.7%**	0.62
Excessive alcohol use	0.15 ± 0.14	0.28	0.3%	0.98
Hypertension	**0.20 ± 0.06**	**<0.001**	**3.0%**	0.69
Cardiovascular disease	**0.24 ± 0.07**	**<0.001**	**2.6%**	0.22
Diabetes	**0.30 ± 0.09**	**0.008**	**2.5%**	0.30
Exercise	-0.07 ± 0.06	0.27	0.3%	0.99
Any statin use	0.09 ± 0.06	0.16	0.4%	0.32
Visceral fat (L)	**0.04 ± 0.02**	**0.03**	**1.3%**	0.86
Visceral/subcutaneous fat ratio	**0.25 ± 0.10**	**0.01**	**1.5%**	0.07

Note: Significant effects are shown in bold.[a.] A significant interaction would indicate that the shape of the association of OSA and obesity with the biomarker levels varies according to the level of the covariate. [b]β stands for change in log(biomarker) for an increase of 1 unit in the predictor covariate, when the other factors of the model are held constant. SE is the standard error of the β coefficient. OSA, obstructive sleep apnea; SaO_2, saturation of oxygen.

4.3.4 CRP levels: Does obesity affect the role of OSA?

4.3.4.1 Simultaneous assessment of BMI and OSA

The role of OSA severity and obesity level in CRP levels was assessed in the same manner as for IL-6. A multiple linear regression model with BMI was tested with a single OSA severity index at a time. BMI was significantly associated with CRP level in models using all 4 measures of OSA severity (Figure 21). The variance in CRP explained by OSA severity and BMI was highest for the minimum SaO_2 (15.6%), the only OSA severity index that had an independent association with CRP levels. AHI, ODI and hypoxia time were not independently associated with CRP levels. In addition, no significant interaction was found between OSA severity and obesity level. The relative importance of OSA severity measured as minimum SaO_2 was smaller for CRP than for IL-6 (partial R^2 3.7% versus 8.6%) and considerably smaller than the explained variance of BMI (11.3%) on CRP levels.

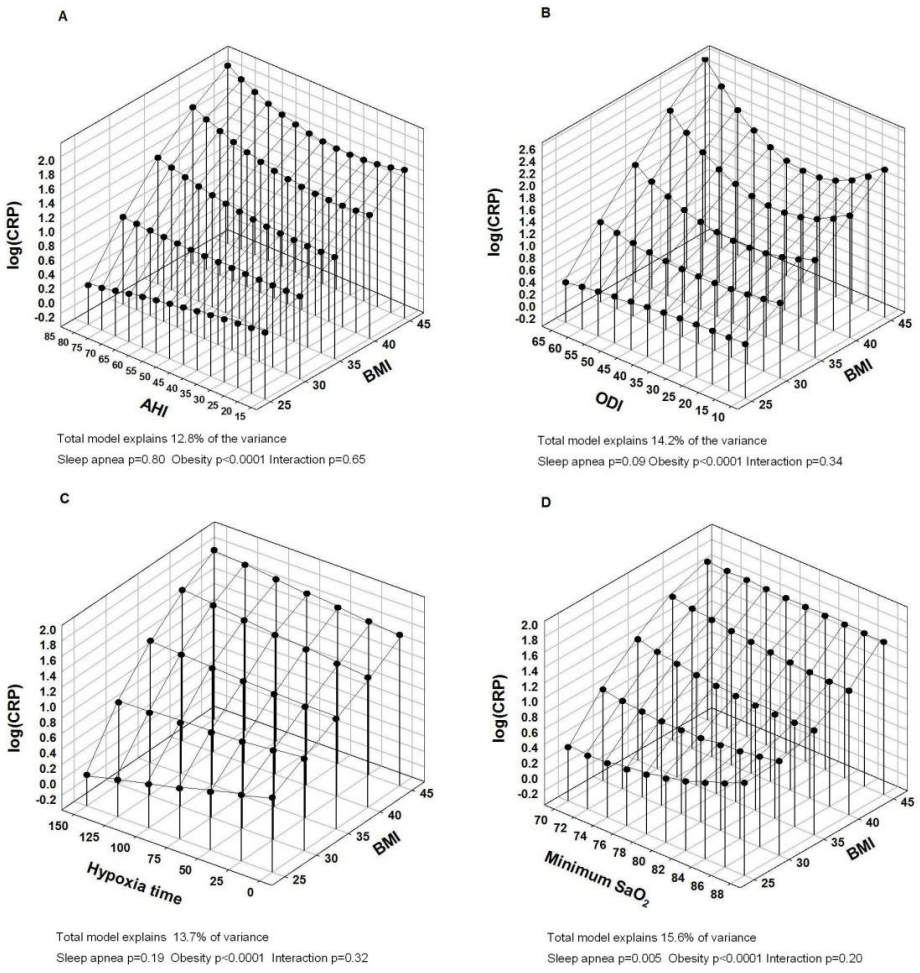

Figure 21. Three-dimensional plots assessing the role of obesity level (body mass index, BMI) and four different severity indices of obstructive sleep apnea (OSA) in C-reactive protein (CRP) levels: a) apnea-hypopnea index (AHI); b) oxygen desaturation index (ODI); c) hypoxia time (minutes with SaO2 < 90%), and d) minimum oxygen saturation (SaO2). The significance of associations between CRP levels and OSA severity, obesity and the interaction between them are shown below the panels as well as the total variance in CRP explained by the model. These analyses are not adjusted for other potential confounders. Reproduced from Paper III.

4.3.4.2 Relationship of other obesity variables and CRP

The role of visceral and total abdominal fat as the main obesity variable was compared with the role of BMI in CRP levels (n = 373, with MRI). The models with minimum SaO_2 continued to explain the highest amount of variance in CRP (again, the only OSA severity measure that was significant). Models including visceral fat as the obesity variable explained less of the variance in CRP levels than BMI did (6.9% versus 11.9%, respectively, n = 373) and total abdominal fat explained a similar amount as BMI or 12.5%. No additional association of visceral fat with CRP levels was found after adjusting for BMI. Also, the association of waist circumference and CRP was tested separately and found to explain less of the variance in CRP levels than BMI did (13.2% versus 15.6%, n = 454). Again, an independent association of sleep apnea severity and CRP levels was only found using minimum SaO_2 saturation as the OSA variable.

4.3.4.3 Role of confounders

Of the different covariates tested in the model with BMI level and minimum SaO_2, only exercise was significantly associated with CRP levels, decreasing the levels (Table 10). General use of statins was not associated with CRP levels. However, when looking separately at the 2 statins mainly used, i.e., atorvastatin and simvastatin, atorvastatin had a borderline association with decreased CRP levels ($p = 0.053$). Excluding participants on these medications affecting inflammatory levels did not change our findings (Paper III, Table S5). However, the relationship between OSA severity and BMI with CRP levels was not the same in those with and without cardiovascular disease ($p = 0.01$ for interaction with the OSA and obesity level). Borderline significance was also found for a gender difference ($p = 0.053$). Subgroup analysis by gender and cardiovascular disease, are shown in the next section.

Table 10. The effect of potential confounders on the model for C-reactive protein (CRP) levels with OSA severity (minimum oxygen saturation) and obesity level (body mass index, BMI). The additional variance (partial R^2) in CRP levels explained by the covariate is shown (main effect) as well as the effect of the covariate on the interaction of OSA and obesity on CRP levels. Significant p values ($p < 0.05$) are shown in bold. Adapted from Paper III.

Covariate	Main effect			Interaction[a]
	β ± SE[b]	p	Partial R^2	p
Male gender	-0.14 ± 0.11	0.21	0.3%	0.053
Age (10 years)	0.007 ± 0.04	0.86	0.01%	0.40
Current smoking	0.05 ± 0.10	0.57	0.07%	0.67
Pack-yr (quartiles)	0.007 ± 0.04	0.86	0.008%	0.36
Excessive alcohol use	0.20 ± 0.21	0.33	0.2%	0.82
Hypertension	0.07 ± 0.08	0.37	0.2%	0.41
Cardiovascular disease	0.13 ± 0.10	0.21	0.4%	**0.01**
Diabetes	0.02 ± 0.13	0.89	0.004%	0.58
Exercise	**-0.19 ± 0.09**	**0.03**	**1.1%**	0.81
Any statin use	-0.12 ± 0.10	0.20	0.4%	0.80
Visceral fat (L)	0.04 ± 0.03	0.18	0.5%	0.09
Visceral/subcutaneous fat ratio	0.15 ± 0.14	0.30	0.3%	0.07

Note: [a] A significant interaction would indicate that the shape of the association of OSA and obesity with the biomarker levels varies according to the level of the covariate. There was a significant interaction with the presence or not of cardiovascular disease (bolded) and a trend for an interaction with gender ($P = 0.053$). [b] β stands for change in log(biomarker) for an increase of 1 unit in the predictor covariate, when the other factors of the model are held constant. SE is the standard error of the β coefficient.

4.3.4.4 CRP : Post-hoc subgroup analyses

The cohort was divided into subgroups (with/without cardiovascular disease and males/females) to explore further the influence of these covariates on how obesity and sleep apnea are associated with CRP levels. For CRP the findings we had for IL-6 were replicated for males only, i.e., independent associations of OSA and obesity and a significant interaction (Paper III - Table S5). The analysis was repeated excluding premenopausal women and postmenopausal women on HRT (n = 17). Importantly, these results showed that postmenopausal women behave similarly to men, i.e., after excluding premenopausal women (n = 13) and women on HRT (n = 4) from the main analysis, there were independent associations of OSA and obesity on CRP levels and a trend toward an interaction between the two for males and postmenopausal women (borderline significant, P = 0.06). Gender did not significantly affect this relationship after excluding premenopausal women and women on HRT.

The findings we had for IL-6 were also largely replicated for CRP in those with cardiovascular disease, i.e., independent associations of OSA and obesity with CRP levels and a significant interaction between OSA and obesity (Paper III - Table S5). However, in those without cardiovascular disease, an independent association of OSA severity and CRP levels was found, but there was no significant interaction between obesity and OSA severity on CRP levels. Interestingly, in otherwise healthy patients with no cardiovascular disease, no hypertension or diabetes, the interaction between OSA severity and obesity was again significant. In all the analyses with a significant interaction, the interaction was driven by OSA severity not having a role in nonobese patients. The relationship of OSA severity and CRP levels depends therefore not only on obesity but may also be affected by other covariates such as menopausal status and the presence of cardiovascular disease.

4.3.5 Leptin levels: Does obesity affect the role of OSA?

4.3.5.1 Simultaneous assessment of BMI and OSA

The role of OSA severity and obesity level in leptin levels was assessed in the same manner as for IL-6 and CRP. All analyses were adjusted for gender as the gender main effect explained ~20% of the variance in leptin levels. The models with BMI, gender and the four different measures of OSA severity (AHI, ODI, hypoxia time and minSaO_2) all explained a similar amount of variance in leptin levels (R^2 = 60.0-60.6%, Figure 22), a considerably higher percentage than in the models for IL-6 and CRP. BMI was significantly associated with leptin levels in all analyses, accounting for over half of explained variance in

leptin, while gender explained ~20%. However, no measure of OSA severity was significantly associated with leptin levels and no interaction was found between BMI and OSA severity on leptin levels. Figure 22 shows a three-dimensional graph of the association of BMI and leptin (large effect) and different measures of OSA severity (no effect) and leptin levels, adjusted for gender. When the same analyses were performed for the complete cohort with no exclusions, AHI explained 1% of the variance in leptin levels, indicating a potential very minor independent association of OSA severity and leptin levels.

4.3.5.2 Relationship of other obesity variables and leptin

The model with waist circumference and AHI, instead of BMI and AHI explained a similar percentage of leptin levels (63.2% *vs*. 60.6%, difference not significant in bootstrap comparison). In addition, AHI significantly explained a very small percentage (1.1%) of the variance of leptin levels with waist circumference in the model, not found for any of the three hypoxia variables or when using BMI as the obesity variable.

To compare how well MRI measures of obesity predict leptin levels in multivariable models, in comparison to anthropometric measures, the n=371 subset with MRI measurements was assessed (gender and OSA severity included in model). The model with total abdominal fat explained the largest amount of variance in leptin levels, 69.0% (significantly highest by bootstrap analysis) while models with BMI and waist circumference explained 61.1% and 62.4%, respectively. The model with subcutaneous fat explained 66.5% but the model with visceral fat explained less than all the models above, i.e., 55.5% of the variance. The association of OSA and leptin levels remained nonsignificant in the models with total abdominal fat as the obesity variable, and there was no interaction between total fat volume and OSA severity. Gender continued to explain a large proportion of the variance in leptin levels in these models.

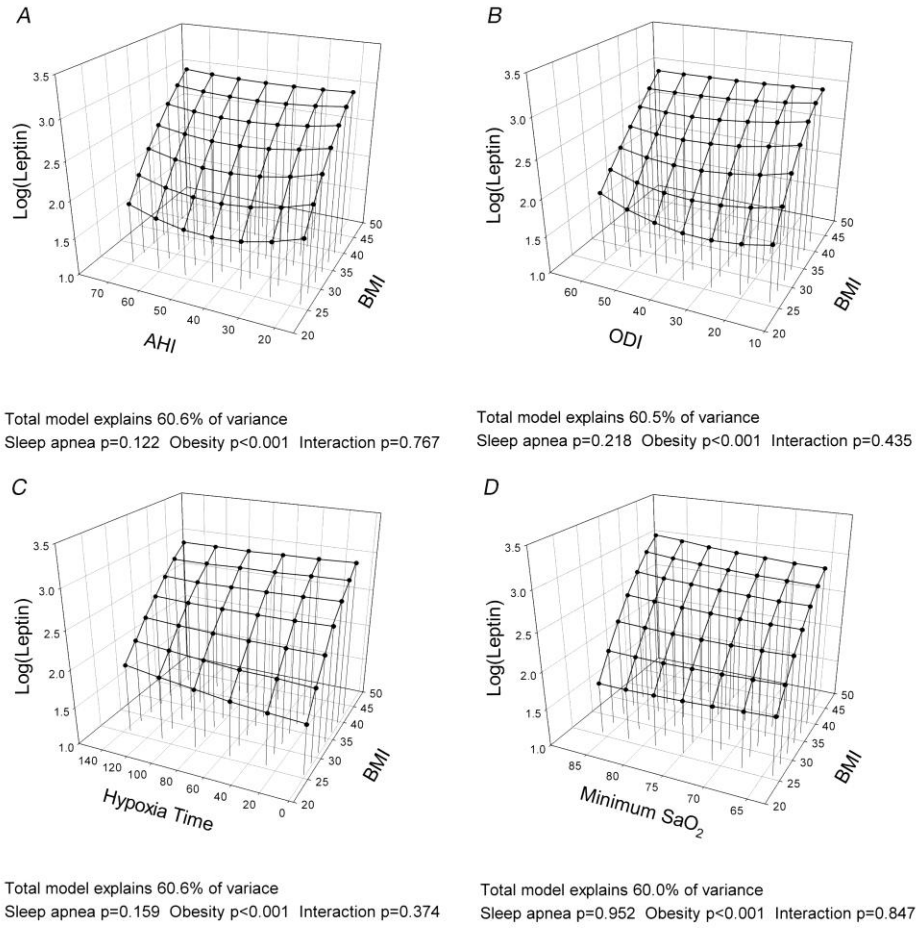

Figure 22. Three-dimensional plots assessing the role of obesity level (body mass index, BMI) and four different severity indices of obstructive sleep apnea (OSA) in leptin levels: a) apnea-hypopnea index (AHI); b) oxygen desaturation index (ODI); c) hypoxia time (minutes with SaO_2 < 90%), and d) minimum oxygen saturation (SaO_2). The significance of associations between leptin levels and OSA severity, obesity and the interaction between them are shown below the panels, as well as the total variance in leptin levels explained by the model. These analyses are not adjusted for other potential confounders. Reproduced from Paper IV.

4.3.5.3 Role of confounders

Because the gender main effect was so large, careful attention was paid to the potential for gender to act as a confounding factor and effect modifier (interaction). Although adding gender to the model increased explained variance substantially, its addition did not appreciably change model parameter estimates, indicating that gender is not a key confounding factor when estimating simultaneously the relationship between BMI and OSA severity with leptin levels. Moreover, when the set of gender interactions with BMI and OSA were added to the model, these were not statistically significant ($p = 0.10$) and the increase in explained variance was very small (1.0%). While the relatively small number of females makes estimating gender-specific parameters challenging and reduces power to detect interactions, results implied that controlling for gender as a main effect added power and did not distort the primary findings.

The associations of other potential confounders on leptin levels were tested separately for each confounder in the model with BMI, gender and AHI (Table 11). Age and cardiovascular disease had statistically significant but small associations with leptin levels and explained some additional variance in leptin levels beyond obesity and gender. Adding to the model an extra fat variable, visceral to subcutaneous fat volume ratio or visceral fat volume alone explained additional variance in leptin levels (6.1% and 2.8%, respectively), further emphasizing the importance of fat distribution in leptin levels. Total fat volume and subcutaneous fat volume could not be assessed as covariates as they were highly correlated with BMI (both $r = 0.85$, Table S2 in Paper III). Two covariates had a significant effect on the interaction between OSA severity and obesity; hypertension ($p = 0.04$) and age group ($p = 0.04$). Subgroup analyses for age category showed minor inconsistent associations of OSA severity and leptin levels. The subgroup analyses for hypertension status are described below.

Table 11. The effect of potential confounders on the model for leptin levels with apnea hypopnea index (AHI) and obesity level (body mass index) adjusted for gender. The additional variance (partial R^2) in leptin levels explained by the covariate is shown (main effect) as well as the effect of the covariate on the interaction of OSA and obesity on leptin levels. Significant p values ($p < 0.05$) are shown in bold. Adapted from Paper VI.

Covariate	**Main effect**			**Interaction[a]**
	β (SE)[b]	p	Partial R^2	p
Age (10 yrs)	**0.07 ± 0.02**	**0.001**	**1.0%**	**0.04**
Current smoking	-0.07 ±0.05	0.17	0.2%	0.97
Pack years (quartiles)	0.00 ± 0.00	0.25	0.1%	0.44
Excessive alcohol use	0.12 ± 0.11	0.28	0.1%	0.67
Hypertension	0.07 ± 0.04	0.12	0.2%	**0.04**
Cardiovascular disease	**0.11 ± 0.06**	**0.049**	**0.4%**	0.82
Diabetes	0.03 ± 0.07	0.65	0.02%	0.08
Premenopausal status	-0.24 ± 0.15	0.11	1.83	0.71
Visceral fat (L)	**0.09 ± 0.02**	**<0.0001**	**2.8%**	0.44
Visceral /subcutaneous fat ratio	**-0.59 ± 0.09**	**<0.0001**	**6.1%**	0.82

Note: [a] A significant p value indicates an interaction between the covariate with the variable role of sleep apnea depending on obesity level in determining leptin levels.

[b]β stands for change in log(leptin) for an increase of one unit in the predictor covariate, when the other factors of the model are held constant. SE is the standard error of the β coefficient.

4.3.5.4 Leptin: post-hoc subgroup analyses

Post-hoc subgroup analyses were performed separately for hypertensive (n = 199) and nonhypertensive (n = 249) subjects using the same model as above with BMI. Hypertensive subjects had higher leptin levels than the nonhypertensive (12.6 [11.4-14.0] ng/ml *vs*. 9.3 [8.5-10.1] ng/ml, p<0.0001). They were also 6 years older on average and more obese (by 2.3 BMI units). No gender differences were found. For nonhypertensive subjects, there was a minor but significant effect of OSA severity on leptin levels, explaining 3.2% of the variance in leptin levels as well as a significant interaction between OSA severity and BMI, explaining an additional 1.5% of the variance (Figure 23A). However, OSA severity did not have a significant effect on leptin levels in hypertensive subjects ((Figure 23B). For nonhypertensive subjects, the role of increased OSA severity was greatest for nonobese subjects (Figure 23A), i.e., a nonhypertensive OSA patient with a BMI of 25 and AHI 30 had expected leptin levels of 4.6 ng/ml in the model, whereas a nonhypertensive subject with the same BMI but an AHI 70 had expected leptin levels of 8.4 ng/ml. No such differences were found for obese nonhypertensive subjects. Models with waist circumference or total fat volume instead of BMI gave the same findings.

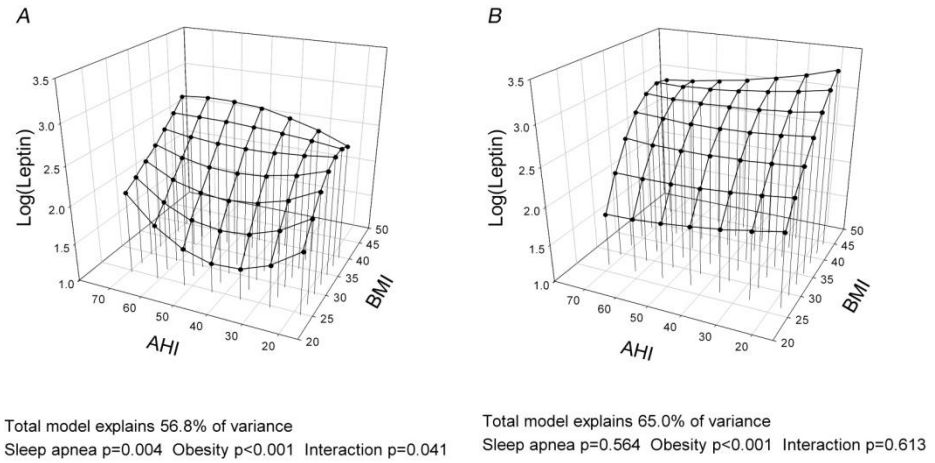

Figure 23. Three-dimensional plots assessing the role of obesity level (body mass index, BMI) and the apnea-hypopnea index (AHI) in leptin levels for a) hypertensive OSA patients and b) nonhypertensive OSA patients. The significance of associations between leptin levels and OSA severity, obesity and the interaction between them are shown below the panels, as well as the total variance in leptin levels explained by the model. Reproduced from Paper IV.

5 Discussion

Interindividual differences were found in the response to similar OSA severity with regards to both nocturnal sweating and biomarker levels. The prevalence of reported frequent nocturnal sweating was three-fold higher in untreated OSA patients than in the general population but decreased to general population levels with full PAP therapy. Objective measurements of sweating also showed a decrease with PAP treatment. Nocturnal sweating can be considered a symptom of OSA that at least a third of OSA patients experience and may perhaps be an indicator of increased cardiovascular risk and sleep-related symptoms in this group compared to other OSA patients. OSA severity is an independent predictor of levels of IL-6 and CRP but interacts with obesity such that this association is found only in obese patients. However, obesity and gender are the dominant determinants of leptin levels. OSA severity was not related to leptin levels except to a minor degree in nonhypertensive nonobese OSA subjects only, not likely to be clinically meaningful.

5.1 Nocturnal sweating and OSA

Our study (Paper I) was the first, to our knowledge, to perform objective measurements of sweating in OSA patients when untreated and again on successful PAP treatment. The OSA patients when untreated appeared to be able to assess their sweating quite accurately, but perhaps less accurately while on PAP treatment.

Previous studies have suggested a possible relationship between obstructive sleep apnea and frequent nocturnal sweating[277, 278, 292]. However until now, studies comparing the prevalence of frequent nocturnal sweating in untreated OSA compared to the prevalence in the general population as well as changes with PAP treatment have been lacking. Our larger study (Paper II) of subjective nocturnal sweating confirmed the findings of Cruz *et al*.[277] and Kiely *et al*.[278] who showed a decrease of subjective sweating with PAP treatment in smaller cohorts of OSA patients (n = 98 and n = 56, respectively). We have extended their findings by showing that the prevalence of nocturnal sweating in untreated OSA is three times greater than in a general population cohort and that it is reduced to general population levels with full PAP treatment. In Papers I and II, we did not find a relationship between traditional OSA severity indices and objective and subjective nocturnal sweating, respectively, in agreement with Mold *et al*.[292] but contrary to the findings of Cruz *et al*.[277] Most of the participants in our OSA cohorts had moderate to severe OSA, limiting our ability to assess relationships with OSA severity. However, an association was found between the change in objective nocturnal sweating and the RMI index, a

marker of paradoxical breathing, with PAP treatment (Paper I), which needs to be confirmed in larger studies.

The results of the current study (Paper I and II) that nocturnal sweating is related to daytime sleepiness is in agreement with Mold *et al*.[292]. Even in non-treated OSA patients, decreased complaints of nocturnal sweating were related to decreased sleepiness, possibly indicating a relationship with nocturnal sweating beyond the presence of OSA *per se*. Unlike the Mold *et al*.[292] study, we did not find a relationship with RLS symptoms. However, they used a less strict definition of RLS than we did. Interestingly, our findings are in agreement with a study of a general cohort of children (n = 6381) in whom their parents reported more sleep-related sweating in relation to both OSA and insomnia symptoms[334], similar to our general population cohort. Nocturnal sweating has also previously been found to be related to GER symptoms[335] and younger age[277] as found in this study. For the general population cohort, we found indications that having OSA symptoms (both snoring and higher MAP index) was related to reporting frequent nocturnal sweating, unlike Mold *et al*. in their sleep laboratory sample[292]. Our study also showed a relationship between decreased quality of life and frequent nocturnal sweating, both in the general population and OSA cohort, as has been shown previously in elderly primary care patients[336]. However, decreased sweating at the follow-up of OSA patients was not related to any improvement in quality of life.

Both in the objective and subjective measurements of sweating (Papers I and II), indications of a relationship between nocturnal sweating and hypertension were found in OSA patients. This relationship was stronger for the objective measurements (Paper I), where a strong correlation was found between sweating and systolic blood pressure in untreated patients. The patients who sweated the most had the highest systolic blood pressure and showed the greatest decrease in systolic blood pressure with treatment. The change in sweating with treatment was also associated with a simultaneous change in the diastolic blood pressure. Sweating and blood pressure, even though controlled by different branches of the sympathetic nervous system, therefore showed similar changes with treatment. Thus, OSA subjects with higher amounts of sweating appear to have increased sympathetic activity despite not finding an association with urinary sympathetic metabolites. No relationship was found between temperature measurements with nocturnal sweating or sleep disordered breathing events, further strengthening the role of sympathetic activity in nocturnal sweating. In Paper I, a negative association was found between REM sleep and sweating in OSA patients. Sympathetic activity has been found to have a role in REM sleep regulation as both norepinephrine agonists and blockage of reuptake have been shown to suppress REM sleep in animal models[337, 338]. Also, a negative relationship

between REM sleep and hot flushes in women has been found, strengthening these findings[339, 340].

5.2 Biomarkers and OSA severity

Our results, in a large cross-sectional clinical sample of OSA subjects, show that OSA severity independently contributes to explaining variance in IL-6 levels. For CRP levels the association among measures of OSA severity is with minimum SaO2 only, a measure of hypoxia. The association of OSA severity and both IL-6 and CRP levels depends on the obesity level of the patients and is only found in obese patients (BMI ≥ 30). Indeed, in nonobese patients, there is no association of IL-6 and CRP levels with OSA severity, assessed as the number of sleep-disordered breathing events (AHI) or different measures of hypoxia during sleep. However, obesity and gender have a dominant role in leptin levels, not OSA severity. Subgroup analyses indicate that OSA severity is independently related to leptin levels in nonhypertensive, nonobese OSA subjects only, but even in this group the role of OSA severity is small and not likely to be clinically meaningful.

5.2.1 The role of fat

Obesity and OSA commonly coexist as obesity is the most important risk factor for OSA (reviewed in Paper V), making studies assessing their independent effects more difficult.

Paper III demonstrates that total fat measures such as BMI and total abdominal fat volume were significantly more associated with IL-6 and CRP levels than visceral fat alone within an overweight and obese apneic population. Visceral fat volume was a significant predictor of IL-6 levels after adjusting for BMI, but explained only a small additional amount of the variance in IL-6 levels (1.3%). No additional association of visceral fat volume was found for CRP levels, after adjusting for BMI. Visceral fat has been shown to release more IL-6 than subcutaneous fat when measured directly [93, 94] but previous smaller human studies assessing the role of total fat versus visceral fat in circulating inflammatory levels show contradictory results[341-346]. The findings of our study are in agreement with findings from the large Framingham cohort[347], that total fat is more associated with inflammatory biomarkers than visceral fat alone. The strength of the associations between the inflammatory markers and different fat measures in the Framingham study are remarkably similar to the current study despite the fact that the patients in our current study were considerably more obese. The Framingham study also found, in agreement with the results of this study, that after accounting for BMI, that there is a small additional relationship between visceral fat and increased IL-6 levels; in addition, the same was noted for CRP levels, but not found in our study. The current study therefore extends

the results of the Framingham study, showing that the stronger association of IL-6 and CRP with total fat compared with visceral fat and the small additional role of visceral fat after adjustment for BMI is true, even for patients who are quite obese.

Paper IV showed that obesity level explains the majority of the variance in leptin levels. Our results are in agreement with previous studies showing that subcutaneous fat produces more leptin than visceral fat[201-203], as we found a higher correlation between leptin levels and subcutaneous fat volume than with visceral fat volume. However, our study also showed that total abdominal fat volume is the best indicator for leptin levels in sleep apneic subjects. Our results are in contrast to a study on nonobese Japanese OSA subjects (n = 96) which found a higher correlation between leptin and visceral fat than with subcutaneous fat and no effect of obesity after adjusting for OSA severity[12]. The differences in results are likely due to the large BMI differences between the two studies and the small BMI range in the previous study. Ethnic differences may have also played a role[348-352].

5.2.2 The role of gender

It is important to consider the role of gender in OSA studies as the majority of the literature has focused on studying males[353]. In our study the number of females was relatively small compared to males and the power to detect a gender difference may therefore have been limited. Nevertheless, our study included more females (n>70) than the majority of previously published studies on OSA (see Strengths and Limitations).

Menopausal status and HRT affected the relationship between sleep apnea and CRP levels, possibly due to their effects on fat distribution[354, 355]. However, gender *per se* did not have a significant independent effect on CRP or IL-6 levels. Our analysis did not allow us to compare directly the effects of menopause on biomarker levels because most of the women were postmenopausal. However, our results for the relationship of OSA severity and obesity on CRP levels showed a significant change in the results by excluding premenopausal women and women on HRT.

Females had, on average, more than twice as high leptin levels than males in this study, as previously found in other studies[353]. These differences are partly explained by higher fat mass and more subcutaneous fat in females compared to males but may also be due to gender steroids (bio-available estrogen and testosterone).

5.2.3 The role of sleep disordered breathing

Previous studies on the relationship between sleep disordered breathing in adults and the levels of biomarker IL-6, CRP and leptin have contradictory findings, as is summarized in the Introduction. It is difficult to reconcile the different results of these previous studies but many of these studies had a low number of patients (n ≤ 30 per group[13, 95-97, 99, 100, 105, 112, 114, 115, 118, 122-127, 131, 134, 145, 172, 174, 175, 178-182, 184-187, 190, 193-197, 199, 200, 356, 357]). In case-control studies, control patients were often less obese than those with apnea (average BMI difference of 2-16 kg/m^2[13, 96, 97, 105, 114, 116, 120, 121, 123, 126, 127, 173, 175, 178, 180, 189, 190, 356, 357]) or showed differences in other fat variables[12, 171, 173, 192]. Controls were also often younger (average age difference of 3-16 years between groups[13, 96, 103, 105, 120, 121, 126, 127, 173, 175, 178, 189, 190, 193, 194, 356, 357]), with fewer comorbidities[13, 121, 171, 194, 356, 357], smoked less[96, 115, 120, 356] or there were marked differences in gender percentages between compared groups[125]. In PAP studies[134, 172, 180-188, 190, 194-199], changes in weight[194, 195] and possibly fat distribution with PAP use[187] may also be of importance. Furthermore, ethnic differences may play an important role in the discrepancy, due to different etiologies in the onset of obesity-related morbidities, such as type 2 diabetes[348]. Asians may have a different susceptibility to OSA[349, 350] and a different leptin profile from Caucasians and other ethnicities[351, 352]. For example, many studies reporting effects of OSA on leptin levels have been performed in relatively lean Asians[12, 172, 174, 178, 181, 182, 186], while only two studies in Asians have shown no relationship between leptin levels and OSA[192, 194].

These contradictory findings in the earlier literature indicate the likely heterogeneity in the inflammatory response in patients with OSA and the need to address specifically whether obesity levels and the presence of comorbidities influence how OSA affects biomarker levels.

5.2.3.1 Inflammatory biomarkers

Some studies have started to address the issue of whether obesity level influences how OSA affects inflammatory levels. In two studies by Vgontzas *et al*.[13, 105] obese patients with OSA were compared with both obese and nonobese controls and the highest IL-6 levels found in the obese OSA patients. However, although there was an overall significant difference between the three groups in the levels of IL-6, the difference between obese control patients and obese patients with OSA was not significant and nonobese patients with OSA were not studied[13, 105]. Hargens *et al*. reported similar findings[129]. A study by Barcelo *et al*.[115] compared obese and nonobese OSA patients to nonobese control patients and found no difference in CRP levels between nonobese patients with OSA and nonobese control patients. However, obese control patients were not included in the study. Thus, all of these studies lack 1 cell of

the 2 × 2 factorial design (obese patients with OSA, obese control patients, nonobese patients with OSA, and nonobese control patients). The results are compatible with our findings, i.e., there is an independent role of OSA in obese patients but not in nonobese patients.

The current study (Paper III), the largest performed so far on IL-6 levels and sleep apnea, supports the independent role of OSA in IL-6 levels, i.e., above and beyond the role of obesity *per se*, as has been shown in some of the previous studies[13, 95, 97, 104, 105]. We have also demonstrated that the independent association of OSA and IL-6 levels depends on the degree of obesity, i.e., there is an obesity-by-OSA interaction such that OSA severity is associated with IL-6 levels in obese patients only, not in those with BMI < 30 kg/m^2. This is a novel finding. The association between IL-6 levels and OSA severity is strongest for hypoxic measures, not the number of apneas and hypopneas. An independent association of OSA severity with CRP levels, which depends on obesity level and menopausal status in women, is found but only for minimum SaO_2, not AHI, ODI or hypoxia time, making the evidence for a role of OSA in CRP levels less robust than for IL-6 levels. The presence of cardiovascular disease is also an important factor influencing the relationship between OSA severity, obesity, and CRP levels. This requires further study. Our results are in agreement with the largest study so far on the relationship between AHI level and CRP (n = 907), showing no independent associations of AHI and CRP levels after adjustment for BMI, age, and gender.[117] Our results are, however, in disagreement with another study[101] on Asian patients showing independent associations of AHI and CRP levels, adjusting for BMI and visceral fat. It is conceivable that there are ethnic differences that affect this relationship and these need to be studied in depth[348-350, 358, 359].

The role of OSA severity appears to be more pronounced for IL-6 levels than CRP levels. The reason for this difference is likely that CRP is much more stable than IL-6 with a half-life of 19 hrs and does not have a circadian rhythm, unlike IL-6[91, 360, 361]. The immediate effect of respiratory events during the night in patients with OSA will increase IL-6 levels, when assessed in the morning after sleep. In contrast, there is a much longer time constant for changes in CRP. Thus, the immediate effect of events during sleep will be less marked for CRP and the levels will reflect not only what happened during sleep but across the entire 24-hr period.

The variable association of OSA severity with IL-6 levels and CRP levels, depending on obesity, may be mediated by a difference in the inflammatory response to events occurring in sleep apnea between nonobese and obese patients. The likely reason for this difference is the increased numbers of nonfat cells such as macrophages, secreting IL-6, that are found in adipose tissue of obese patients[94, 139, 362]. This will allow for more production of IL-6 from adipose

tissue in response to the hypoxic insult that occurs in OSA. Recent studies have shown that there is already hypoxia within adipose tissue in obesity[40-43], meaning that sleep apneic events in obese subjects add further hypoxic insult to the fat tissue, while the changes in nonobese OSA subjects will be of lower magnitude. In support of our findings, a study administering intermittent hypoxia to healthy, lean humans for 14 nights, showed no change in inflammatory blood biomarkers[47]. Another potential explanation is the decreased lung volume in those who are more obese[10], causing comparatively larger desaturations in more obese than in leaner patients with the same durations of apneas[363]. However, the strongest relationships were with the hypoxia variables themselves, making this explanation of our results less likely.

5.2.3.2 Leptin

There was no or only a very minor association of OSA severity and leptin levels after adjusting for obesity and gender in the whole cohort, in contrast with the finding for IL-6 and CRP levels. However, a post-hoc subgroup analysis on nonhypertensive subjects showed a significant, albeit very small, association of OSA severity and leptin levels. This association was found in nonobese subjects. It may be that the oxidative stress and inflammation associated with OSA (reviewed in Paper V) only contribute to an increase in leptin levels in subjects who are not already chronically exposed to these physiological stressors. The obese and hypertensive OSA subjects may already have maximal stimulation of leptin levels. It is important to remember though that there is strong evidence that OSA itself causes hypertension, complicating research when looking at hypertension simply as a confounder to the independent effects of OSA. Those more vulnerable to pathological cardiometabolic effects of OSA, likely due to genetic predisposition, will therefore already have hypertension and be on hypertensive medication (reviewed in Paper V and [227]).

In support of our results, other studies comparing obese and nonobese subjects have shown differential effects of OSA on leptin levels depending on the degree of obesity[190, 199, 200]. Two of these studies have results comparable to those of our study; showing a difference in leptin levels between nonobese OSA subjects and controls only, as well as a decrease in leptin levels with CPAP in nonobese subjects only[199, 200]. A recent study in mice exposed to intermittent hypoxia also supports these findings, showing increased leptin levels in lean but not obese mice[39] and a human study administering intermittent hypoxia to healthy, lean subjects for 14 nights found no changes in leptin levels[47]. However, in two other studies, no difference in leptin levels between OSA subjects and controls was found [129, 190] and a decrease in leptin levels with CPAP was found in the obese OSA subjects only[190]. The discrepancy in the

earlier OSA literature on leptin levels, as discussed above, is likely partly due to the heterogeneity of leptin levels for different obesity levels and hypertension status in OSA subjects as found in our study.

5.2.4 Strengths and limitations

The strength of the clinical OSA cohort was the longitudinal follow-up, the detailed measurements performed in each patient with a full PSG and additional measurements of sweating and temperature, never performed before in OSA patients to our knowledge. This study was, however, a pilot study and is limited by its small sample size. A larger study is needed that includes women with OSA, OSA patients with hypertension, diabetes and other comorbidities, both before and after PAP treatment, as well as a healthy control group to examine differences in objective sweating. Another limitation was the possible first-night effect on measurements as an adaptation night was not included in the study and the overnight sampling of urine instead of a 24-hour assessment of sympathetic metabolites.

The strengths of the ISAC study (Papers II – IV) include the large number of participants, the broad distribution of obesity, and the high proportion of severely obese participants and the detailed analysis performed in each subject (extensive questionnaire, anthropometric measurements, sleep studies, fasting blood samples and MRI measurements). The large sample size of ISAC was such that it was possible to assess associations separately in overweight, obese, and severely obese patient and investigate the complex interaction between OSA and obesity (Papers III and IV) and hence provide new insights. The ISAC cohort is a clinical cohort, with no exclusion of the relevant OSA comorbidities. We believe this to be a major strength of the study as by investigating only otherwise “healthy” patients with OSA as done in Paper I, the study results may be biased by studying a subpopulation of patients with OSA with some sort of genetic or environmental protective mechanism that is not generally present. Our results suggest, for example, a differential response in CRP levels to sleep apnea depending on the presence or absence of cardiovascular disease. These results require further study. Including patients with comorbidities does, however, complicate the analysis as the presence of cardiometabolic disease will affect, for example, inflammatory levels. In the ISAC study, different medical conditions and medications were assessed as potential confounders and sensitivity analyses were performed when needed, e.g. excluding patients on anti-inflammatory medications as well as all patients with known hypertension, diabetes, and cardiovascular disease to confirm our main findings.

Another strength of the study was the state-of-the-art MRI technology for measuring visceral and subcutaneous abdominal fat. The MRI and sleep

studies were all read by centralized laboratories with very high reliability of measurement (see Paper III, online supplement). Other strengths specific to paper II were the comparison with a general population cohort as well as the extensive longitudinal follow-up of the ISAC cohort. This study was an observational study, not a randomized controlled trial (RCT), which may be considered a limitation. However, an RCT study with such long-term follow-up of severely affected OSA patients would be difficult to perform for ethical reasons. The importance of observational studies was highlighted in a recent NIH workshop report on comparative effectiveness research[364].

A limitation of our ISAC cohort includes the smaller number of females than males as a consequence of the "gender-bias" of patients in Iceland in whom OSA was diagnosed. We did, however, have >70 females in Papers III and IV and a total of 156 females in the final cohort in Paper II. This was still quite a high number, especially in comparison with many earlier studies in the field, where only 8 of 27 studies on CRP levels[95-103, 108-111, 113-122, 130, 131, 356, 357] included a significant total number of females (n > 40). In addition and interestingly, only 2 of these studies specifically looked at gender differences[108, 116], none adjusted for menopausal status and only 1 study adjusted for HRT in women[117]. For IL-6, the largest study so far in the literature (n = 155 patients[123]) does not report whether females are included in their study and all other studies either do not include females[13, 97, 105, 112, 114, 124, 130, 132] or have a negligible number of them in their analysis (n = 1-20[103, 104, 122, 123, 125-127, 131]). One published study even compared a mixed group of OSA males and females with a female-only control group[125]. The same is found for leptin levels where most studies have focused on males only[12, 13, 145, 173, 174, 177-179, 181, 184, 186-188, 190, 191, 194, 199] and many studies include a very low number of females (≤5 per group).[172, 175, 180, 182, 185, 195, 196] Studies addressing specifically the role of OSA in inflammatory biomarkers in women taking into account their menopausal status are needed, although sleep apnea is much less common in premenopausal women[365].

In both the ISAC and the clinical OSA cohort there is a lack of controls and patients with mild OSA. Therefore, a potential effect of OSA severity on biomarker levels or nocturnal sweating from mild to moderate disease, with a plateau above this level of severity, as has been described for other biomarkers[366], cannot be excluded. The lack of control patients without OSA in the biomarker assessment of the ISAC study (Paper III and IV) is, in our view, not a major limitation as comparing patients with OSA to control patients was not the intent of this study. Rather, we sought to evaluate within a clinical cohort of patients with OSA the relative role of obesity and sleep apnea. Previous studies that included a control group have shown that visceral fat volume is more associated with the presence of OSA than other measures of obesity, such as BMI or subcutaneous fat[6, 12, 13, 105, 367, 368]. However, many of these

studies share the same limitation regarding control groups as mentioned previously (differences in BMI, age, etc. between cases and control patients). Finding control patients who do not have OSA, with the obesity levels found in this study and matched for comorbidities and other factors, is very challenging as the OSA literature shows but would be very valuable. Instead, our analysis made use of the wide range of obesity and OSA severity within a clinical cohort of individuals with sleep apnea to address our primary question, i.e., the independent roles of obesity and OSA severity and their interaction on biomarker levels. Also, our analyses assessed the role of various confounders that may influence how sleep apnea is related to biomarker levels. Our sample size is sufficient to do so. We would, however, like to emphasize that this part of our study was cross-sectional; therefore, findings of significant associations are not sufficient to infer causality.

In the ISAC study, unlike the clinical OSA study, portable type 3 monitors were used. The study therefore lacks electroencephalographic recording and assessment of arousals. However, the validity of the type 3 portable monitoring system for assessing sleep disordered breathing has been attested when compared with polysomnography[369] and the recommended American Academy of Sleep Medicine 2007 scoring rules do not require arousals to assess hypopneas, as some scoring rules do[370]. Additionally, it has been shown that subjects spend more time in the supine position during PSG than portable monitoring[371], likely due to the discomfort of the patient, which is reflected in falsely higher OSA severity levels measured in the laboratory than in home studies, as at least some OSA subjects have positional OSA with much higher indices while in the supine position[372]. The fact that people may sleep worse in the laboratory, as has been shown be comparing laboratory PSG to home PSG in the same subjects, finding increased sleep efficiency and longer sleep duration in the home studies[373], should also be taken into account.

Another potential limitation is that the blood for biomarker assessment, while drawn in untreated patients, was not drawn the morning after the sleep study. Therefore, night-to-night variability in OSA severity added some noise to the data. However, this variability has been found to be smaller when assessed in home studies and in patients with severe OSA[374, 375]. The assessment of biomarkers is made from a single blood draw, not overnight measurements performed before and after sleep (reviewed in Paper V). However, three small OSA studies performed using more than a single blood draw have indicated that if a difference in, for example, IL-6 levels is found because of OSA, these changes can be found in the morning blood sampling, as was done in this study[13, 105, 112].

The highest dropout rate for the MRIs was in the most obese category, as these patients had more problems with MRI assessment than those who were

less obese. However, even in the most obese group, MRI assessments of abdominal fat were obtained in 87 of 133 patients, permitting meaningful analyses. Furthermore, our study did not include a whole body fat percentage assessment, such as dual energy X-ray absorptiometry (DEXA)[376] in the otherwise detailed assessment of obesity level.

Other limitations specific to Paper II were found as the ISAC subjects were more obese and had a higher prevalence of hypertension, cardiovascular and diabetes than the general population subjects. Therefore we adjusted for these co-morbidities as covariates. Again, the number of females was substantially lower in the ISAC cohort, a consequence of the "gender bias" of patients in Iceland in whom OSA has been diagnosed and the consequent lower prevalence of OSA in females than males[3, 365]. However, no gender differences were found in the prevalence of sweating and no statistical interactions were found between gender and the other tested variables related to nocturnal sweating.

Other weaknesses include the use of subjective measures of sweating. However, as shown in Paper I, at least in untreated OSA patients, the correlation between subjective and objective symptoms was high and many of the relationships found with subjective sweating were also found for objective sweating, strengthening the significance of assessing subjective sweating. It should be noted that the reporting of subjective sweating is three-fold higher in untreated OSA patients than in the general population, which may be used as a clinical indicator of OSA.

A sleep study was not performed in the general population cohort. Therefore we do not know how many subjects in the general population cohort had treated or untreated OSA. Instead, we assessed their OSA risk by calculating their MAP index. Finally, some subjects in the OSA cohort reported an improvement in frequent nocturnal sweating despite receiving no PAP treatment at follow-up. These subjects also described less sleepiness. This may be related to other life style changes during the 2 year interim between baseline and follow up, the Hawthorne effect (improvement due to observation alone[377]) or regression to the mean as most subjects described severe symptoms at baseline. Other treatment use may also be a factor. We did, however, remove all subjects using a mandibular advancement device from our follow-up analysis in the OSA cohort and repeated the analyses excluding all non-PAP users with significant weight loss and upper airway surgery (uvula/soft palate) between the baseline and follow-up assessments, and this did not affect the results.

Finally, we would like to mention that heating in Icelandic houses is relatively cheap and year-round outdoor temperatures in Reykjavik rather low (monthly average of -1°C to 11°C). Therefore ambient temperatures in houses should be stable across the year and not affect the current findings. Additionally, data collection was year-round, both for baseline and follow-up data.

6 Conclusions

One-third of adults with sleep apnea experience frequent nocturnal sweating and they are three times more likely to report it compared to adults in the general population. A good relationship was found between objective sweating and subjective symptoms of nocturnal sweating in untreated OSA patients. The symptom is responsive to treatment in the majority of sleep apnea patients. Similarly, objective sweating is reduced with treatment despite no correlation between nocturnal sweating and OSA severity *per se*. Also nocturnal sweating was found to be related to sympathetic activity but not with temperature changes. Our studies indicated a possible role of frequent nocturnal sweating as a marker for untreated sleep apnea. Therefore, clinicians should include OSA in the differential diagnosis of patients presenting with a complaint of nocturnal sweating and further evaluate that possibility by performing a more complete sleep evaluation. It should be noted that OSA patients describing nocturnal sweating may be at a higher cardiovascular risk than other OSA patients as well as be younger, sleepier and have more insomnia symptoms than other OSA patients. Future research that increases the understanding of the link between OSA and nocturnal sweating is important, both by subjective and objective measures. These studies should assess whether the subgroup of OSA subjects with frequent nocturnal sweating have a different cardiovascular risk profile and worse sleep quality despite similar OSA severity.

Our studies, to our knowledge, have also been the first to show that the association of OSA severity and increased levels of inflammatory biomarkers depends on obesity level. Our results therefore show a more pronounced role of OSA as a cardiovascular risk factor in patients who are also obese. Also for CRP, this relationship was affected by the presence of cardiovascular disease and menopausal status of women. In contrast the role of OSA in leptin levels was minor and only found in nonobese, nonhypertensive subjects. These results emphasize the complicated relationship between OSA and obesity and their differential effects on various biomarkers.

More studies looking at well-defined groups of OSA patients and controls stratified based on gender, body mass index and other comorbidities are needed. Most current studies either do not include females, have a very low number of females or do not analyze gender differences as discussed above and studies addressing specifically the role of OSA in women taking into account their menopausal status are needed. Understanding interindividual differences in symptoms such as sleep-related sweating and cardiovascular risk biomarkers in OSA patients and relating the differences to OSA pathophysiology and comorbidities is a step towards much needed personalized medicine[378, 379] for OSA patients.

References

1. Pack AI. Advances in sleep-disordered breathing. Am J Respir Crit Care Med 2006;173:7-15.
2. Caples SM, Gami AS, Somers VK. Obstructive sleep apnea. Ann Intern Med 2005;142:187-97.
3. Young T, Palta M, Dempsey J, Skatrud J, Weber S, Badr S. The occurrence of sleep-disordered breathing among middle-aged adults. New England Journal of Medicine 1993;328:1230-5.
4. Frey WC, Pilcher J. Obstructive sleep-related breathing disorders in patients evaluated for bariatric surgery. Obes Surg 2003;13:676-83.
5. Bassiri AG, Guilleminault C. Clinical features and evaluation of obstructive sleep apnea-hypopnea syndrome. In: Kryger MH, Roth T, Dement WC, ed. Principles and practise of sleep medicine. 3. ed. Philadelphia: W.B. Saunders Company, 2000:870-1.
6. Shinohara E, Kihara S, Yamashita S, et al. Visceral fat accumulation as an important risk factor for obstructive sleep apnoea syndrome in obese subjects. J Intern Med 1997;241:11-8.
7. Grunstein R, Wilcox I, Yang TS, Gould Y, Hedner J. Snoring and sleep apnoea in men: association with central obesity and hypertension. Int J Obes Relat Metab Disord 1993;17:533-40.
8. Hallowell PT, Stellato TA, Schuster M, et al. Potentially life-threatening sleep apnea is unrecognized without aggressive evaluation. Am J Surg 2007;193:364-7.
9. Daltro C, Gregorio PB, Alves E, et al. Prevalence and severity of sleep apnea in a group of morbidly obese patients. Obes Surg 2007;17:809-14.
10. Schwartz AR, Patil SP, Laffan AM, Polotsky V, Schneider H, Smith PL. Obesity and obstructive sleep apnea: pathogenic mechanisms and therapeutic approaches. Proc Am Thorac Soc 2008;5:185-92.
11. Kershaw EE, Flier JS. Adipose tissue as an endocrine organ. J Clin Endocrinol Metab 2004;89:2548-56.
12. Tatsumi K, Kasahara Y, Kurosu K, Tanabe N, Takiguchi Y, Kuriyama T. Sleep oxygen desaturation and circulating leptin in obstructive sleep apnea-hypopnea syndrome. Chest 2005;127:716-21.
13. Vgontzas AN, Papanicolaou DA, Bixler EO, et al. Sleep apnea and daytime sleepiness and fatigue: relation to visceral obesity, insulin resistance, and hypercytokinemia. J Clin Endocrinol Metab 2000;85:1151-8.
14. Schwab RJ. Genetic determinants of upper airway structures that predispose to obstructive sleep apnea. Respir Physiol Neurobiol 2005;147:289-98.
15. Giles TL, Lasserson TJ, Smith BJ, White J, Wright J, Cates CJ. Continuous positive airways pressure for obstructive sleep apnoea in adults. Cochrane Database Syst Rev 2006:CD001106.
16. Meurice JC, Dore P, Paquereau J, et al. Predictive factors of long-term compliance with nasal continuous positive airway pressure treatment in sleep apnea syndrome. Chest 1994;105:429-33.
17. Weaver TE, Maislin G, Dinges DF, et al. Relationship between hours of CPAP use and achieving normal levels of sleepiness and daily functioning. Sleep 2007;30:711-9.
18. Pépin JL, Krieger J, Rodenstein D, et al. Effective compliance during the first 3 months of continuous positive airway pressure. A European prospective study of 121 patients. Am J Respir Crit Care Med 1999;160:1124-9.
19. Marin JM, Carrizo SJ, Vicente E, Agusti AG. Long-term cardiovascular outcomes in men with obstructive sleep apnoea-hypopnoea with or without treatment with continuous positive airway pressure: an observational study. Lancet 2005;365:1046-53.
20. Campos-Rodriguez F, Martinez-Garcia MA, de la Cruz-Moron I, Almeida-Gonzalez C, Catalan-Serra P, Montserrat JM. Cardiovascular mortality in women with obstructive sleep apnea with or without continuous positive airway pressure treatment: a cohort study. Ann Intern Med 2012;156:115-22.

21. Martínez-García MA, Campos-Rodríguez F, Catalán-Serra P, et al. Cardiovascular mortality in obstructive sleep apnea in the elderly: role of long-term continuous positive airway pressure treatment: a prospective observational study. Am J Respir Crit Care Med 2012;186:909-16.
22. Semenza GL, Prabhakar NR. HIF-1-dependent respiratory, cardiovascular, and redox responses to chronic intermittent hypoxia. Antioxid Redox Signal 2007;9:1391-6.
23. Lavie L. Oxidative stress inflammation and endothelial dysfunction in obstructive sleep apnea. Front Biosci (Elite Ed) 2012;4:1391-403.
24. Babior BM. Phagocytes and oxidative stress. Am J Med 2000;109:33-44.
25. Dematteis M, Julien C, Guillermet C, et al. Intermittent hypoxia induces early functional cardiovascular remodeling in mice. Am J Respir Crit Care Med 2008;177:227-35.
26. Phillips SA, Olson EB, Lombard JH, Morgan BJ. Chronic intermittent hypoxia alters NE reactivity and mechanics of skeletal muscle resistance arteries. J Appl Physiol 2006;100:1117-23.
27. Savransky V, Nanayakkara A, Li J, et al. Chronic intermittent hypoxia induces atherosclerosis. Am J Respir Crit Care Med 2007;175:1290-7.
28. Zoccal DB, Bonagamba LG, Oliveira FR, Antunes-Rodrigues J, Machado BH. Increased sympathetic activity in rats submitted to chronic intermittent hypoxia. Exp Physiol 2007;92:79-85.
29. Veasey SC, Davis CW, Fenik P, et al. Long-term intermittent hypoxia in mice: protracted hypersomnolence with oxidative injury to sleep-wake brain regions. Sleep 2004;27:194-201.
30. Xu W, Chi L, Row BW, et al. Increased oxidative stress is associated with chronic intermittent hypoxia-mediated brain cortical neuronal cell apoptosis in a mouse model of sleep apnea. Neuroscience 2004;126:313-23.
31. Zhu Y, Fenik P, Zhan G, et al. Selective loss of catecholaminergic wake active neurons in a murine sleep apnea model. J Neurosci 2007;27:10060-71.
32. Polotsky VY, Li J, Punjabi NM, et al. Intermittent hypoxia increases insulin resistance in genetically obese mice. Journal of Physiology 2003;552:253-64.
33. Iiyori N, Alonso LC, Li J, et al. Intermittent hypoxia causes insulin resistance in lean mice independent of autonomic activity. Am J Respir Crit Care Med 2007;175:851-7.
34. Toffoli S, Feron O, Raes M, Michiels C. Intermittent hypoxia changes HIF-1alpha phosphorylation pattern in endothelial cells: unravelling of a new PKA-dependent regulation of HIF-1alpha. Biochim Biophys Acta 2007;1773:1558-71.
35. Peng YJ, Yuan G, Ramakrishnan D, et al. Heterozygous HIF-1alpha deficiency impairs carotid body-mediated systemic responses and reactive oxygen species generation in mice exposed to intermittent hypoxia. J Physiol 2006;577:705-16.
36. Li J, Bosch-Marce M, Nanayakkara A, et al. Altered metabolic responses to intermittent hypoxia in mice with partial deficiency of hypoxia-inducible factor-1alpha. Physiol Genomics 2006;25:450-7.
37. Rius J, Guma M, Schachtrup C, et al. NF-kappaB links innate immunity to the hypoxic response through transcriptional regulation of HIF-1alpha. Nature 2008;453:807-11.
38. Lee EJ, Woodske ME, Zou B, O'Donnell CP. Dynamic arterial blood gas analysis in conscious, unrestrained C57BL/6J mice during exposure to intermittent hypoxia. J Appl Physiol 2009;107:290-4.
39. Reinke C, Bevans-Fonti S, Drager LF, Shin MK, Polotsky VY. Effects of different acute hypoxic regimens on tissue oxygen profiles and metabolic outcomes. J Appl Physiol 2011;111:881-90.
40. Ye J, Gao Z, Yin J, He Q. Hypoxia is a potential risk factor for chronic inflammation and adiponectin reduction in adipose tissue of ob/ob and dietary obese mice. Am J Physiol Endocrinol Metab 2007;293:E1118-28.
41. Pasarica M, Sereda OR, Redman LM, et al. Reduced adipose tissue oxygenation in human obesity: evidence for rarefaction, macrophage chemotaxis, and inflammation without an angiogenic response. Diabetes 2009;58:718-25.
42. Rausch ME, Weisberg S, Vardhana P, Tortoriello DV. Obesity in C57BL/6J mice is characterized by adipose tissue hypoxia and cytotoxic T-cell infiltration. Int J Obes (Lond) 2007.

43. Hosogai N, Fukuhara A, Oshima K, et al. Adipose tissue hypoxia in obesity and its impact on adipocytokine dysregulation. Diabetes 2007;56:901-11.
44. Somers VK, Mark AL, Zavala DC, Abboud FM. Contrasting effects of hypoxia and hypercapnia on ventilation and sympathetic activity in humans. J Appl Physiol 1989;67:2101-6.
45. Somers VK, Mark AL, Zavala DC, Abboud FM. Influence of ventilation and hypocapnia on sympathetic nerve responses to hypoxia in normal humans. J Appl Physiol 1989;67:2095-100.
46. Tamisier R, Gilmartin GS, Launois SH, et al. A new model of chronic intermittent hypoxia in humans: effect on ventilation, sleep, and blood pressure. J Appl Physiol 2009;107:17-24.
47. Tamisier R, Pépin JL, Rémy J, et al. 14 nights of intermittent hypoxia elevate daytime blood pressure and sympathetic activity in healthy humans. Eur Respir J 2011;37:119-28.
48. Gilmartin GS, Lynch M, Tamisier R, Weiss JW. Chronic intermittent hypoxia in humans during 28 nights results in blood pressure elevation and increased muscle sympathetic nerve activity. Am J Physiol Heart Circ Physiol 2010;299:H925-31.
49. Leuenberger U, Jacob E, Sweer L, Waravdekar N, Zwillich C, Sinoway L. Surges of muscle sympathetic nerve activity during obstructive apnea are linked to hypoxemia. Journal of Applied Physiology 1995;79:581-8.
50. Horner RL, Brooks D, Kozar LF, Tse S, Phillipson EA. Immediate effects of arousal from sleep on cardiac autonomic outflow in the absence of breathing in dogs. J Appl Physiol 1995;79:151-62.
51. Blasi A, Jo J, Valladares E, Morgan BJ, Skatrud JB, Khoo MC. Cardiovascular variability after arousal from sleep: time-varying spectral analysis. J Appl Physiol 2003;95:1394-404.
52. Blasi A, Jo JA, Valladares E, Juarez R, Baydur A, Khoo MC. Autonomic cardiovascular control following transient arousal from sleep: a time-varying closed-loop model. IEEE Trans Biomed Eng 2006;53:74-82.
53. Catcheside PG, Chiong SC, Mercer J, Saunders NA, McEvoy RD. Noninvasive cardiovascular markers of acoustically induced arousal from non-rapid-eye-movement sleep. Sleep 2002;25:797-804.
54. Nalivaiko E, Catcheside PG, Adams A, Jordan AS, Eckert DJ, McEvoy RD. Cardiac changes during arousals from non-REM sleep in healthy volunteers. Am J Physiol Regul Integr Comp Physiol 2007;292:R1320-7.
55. Ekstedt M, Akerstedt T, Soderstrom M. Microarousals during sleep are associated with increased levels of lipids, cortisol, and blood pressure. Psychosom Med 2004;66:925-31.
56. Bonnet MH, Berry RB, Arand DL. Metabolism during normal, fragmented, and recovery sleep. J Appl Physiol 1991;71:1112-8.
57. Bonnet MH. Effect of sleep disruption on sleep, performance, and mood. Sleep 1985;8:11-9.
58. Luckhaupt SE, Tak S, Calvert GM. The prevalence of short sleep duration by industry and occupation in the National Health Interview Survey. Sleep 2010;33:149-59.
59. Goel N, Rao H, Durmer JS, Dinges DF. Neurocognitive consequences of sleep deprivation. Semin Neurol 2009;29:320-39.
60. Wang Q, Xi B, Liu M, Zhang Y, Fu M. Short sleep duration is associated with hypertension risk among adults: a systematic review and meta-analysis. Hypertens Res 2012;35:1012-8.
61. Cappuccio FP, Taggart FM, Kandala NB, et al. Meta-analysis of short sleep duration and obesity in children and adults. Sleep 2008;31:619-26.
62. Cappuccio FP, D'Elia L, Strazzullo P, Miller MA. Quantity and quality of sleep and incidence of type 2 diabetes: a systematic review and meta-analysis. Diabetes Care 2010;33:414-20.
63. Gallicchio L, Kalesan B. Sleep duration and mortality: a systematic review and meta-analysis. J Sleep Res 2009;18:148-58.
64. Vgontzas AN, Zoumakis E, Bixler EO, et al. Adverse effects of modest sleep restriction on sleepiness, performance, and inflammatory cytokines. J Clin Endocrinol Metab 2004;89:2119-26.
65. Irwin MR, Wang M, Campomayor CO, Collado-Hidalgo A, Cole S. Sleep deprivation and activation of morning levels of cellular and genomic markers of inflammation. Archives of internal medicine 2006;166:1756-62.

66. Meier-Ewert HK, Ridker PM, Rifai N, et al. Effect of sleep loss on C-reactive protein, an inflammatory marker of cardiovascular risk. Journal of the American College of Cardiology 2004;43:678-83.
67. Del Ben M, Fabiani M, Loffredo L, et al. Oxidative stress mediated arterial dysfunction in patients with obstructive sleep apnoea and the effect of continuous positive airway pressure treatment. BMC Pulm Med 2012;12:36.
68. Jurado-Gámez B, Fernandez-Marin MC, Gómez-Chaparro JL, et al. Relationship of oxidative stress and endothelial dysfunction in sleep apnoea. Eur Respir J 2011;37:873-9.
69. Lavie L, Dyugovskaya L, Golan-Shany O, Lavie P. Heat-shock protein 70: expression in monocytes of patients with sleep apnoea and association with oxidative stress and tumour necrosis factor-alpha. J Sleep Res 2010;19:139-47.
70. Alzoghaibi MA, Bahammam AS. The effect of one night of continuous positive airway pressure therapy on oxidative stress and antioxidant defense in hypertensive patients with severe obstructive sleep apnea. Sleep Breath 2012;16:499-504.
71. Barcelo A, Miralles C, Barbe F, Vila M, Pons S, Agusti AG. Abnormal lipid peroxidation in patients with sleep apnoea. Eur Respir J 2000;16:644-7.
72. Minoguchi K, Yokoe T, Tanaka A, et al. Association between lipid peroxidation and inflammation in obstructive sleep apnoea. Eur Respir J 2006;28:378-85.
73. Murri M, Alcázar-Ramírez J, Garrido-Sánchez L, et al. Oxidative stress and metabolic changes after continuous positive airway pressure treatment according to previous metabolic disorders in sleep apnea-hypopnea syndrome patients. Transl Res 2009;154:111-21.
74. de Lima AM, Franco CM, de Castro CM, Bezerra Ade A, Ataide L, Jr., Halpern A. Effects of nasal continuous positive airway pressure treatment on oxidative stress and adiponectin levels in obese patients with obstructive sleep apnea. Respiration 2010;79:370-6.
75. Schulz R, Mahmoudi S, Hattar K, et al. Enhanced release of superoxide from polymorphonuclear neutrophils in obstructive sleep apnea. Impact of continuous positive airway pressure therapy. Am J Respir Crit Care Med 2000;162:566-70.
76. Dyugovskaya L, Lavie P, Lavie L. Increased adhesion molecules expression and production of reactive oxygen species in leukocytes of sleep apnea patients. Am J Respir Crit Care Med 2002;165:934-9.
77. Carpagnano GE, Kharitonov SA, Resta O, Foschino-Barbaro MP, Gramiccioni E, Barnes PJ. 8-Isoprostane, a marker of oxidative stress, is increased in exhaled breath condensate of patients with obstructive sleep apnea after night and is reduced by continuous positive airway pressure therapy. Chest 2003;124:1386-92.
78. Lavie L, Vishnevsky A, Lavie P. Evidence for lipid peroxidation in obstructive sleep apnea. Sleep 2004;27:123-8.
79. Yamauchi M, Nakano H, Maekawa J, et al. Oxidative stress in obstructive sleep apnea. Chest 2005;127:1674-9.
80. Jordan W, Cohrs S, Degner D, et al. Evaluation of oxidative stress measurements in obstructive sleep apnea syndrome. J Neural Transm 2006;113:239-54.
81. Wysocka E, Cofta S, Piorunek T, Dziegielewska-Gesiak S, Bryl W, Torlinski L. Blood antioxidant status, dysglycemia and obstructive sleep apnea. Adv Exp Med Biol 2013;756:121-9.
82. Wysocka E, Cofta S, Cymerys M, Gozdzik J, Torlinski L, Batura-Gabryel H. The impact of the sleep apnea syndrome on oxidant-antioxidant balance in the blood of overweight and obese patients. J Physiol Pharmacol 2008;59 Suppl 6:761-9.
83. Okur HK, Pelin Z, Yuksel M, Yosunkaya S. Lipid peroxidation and paraoxonase activity in nocturnal cyclic and sustained intermittent hypoxia. Sleep Breath 2013;17:365-71.
84. Guo Q, Wang Y, Li QY, Li M, Wan HY. Levels of thioredoxin are related to the severity of obstructive sleep apnea: based on oxidative stress concept. Sleep Breath 2013;17:365-71.
85. Jurado-Gamez B, Bujalance Cabrera C, Caballero Ballesteros L, et al. Association of cellular adhesion molecules and oxidative stress with endothelial function in obstructive sleep apnea. Intern Med 2012;51:363-8.
86. Monneret D, Pepin JL, Godin-Ribuot D, et al. Association of urinary 15-F2t-isoprostane level with oxygen desaturation and carotid intima-media thickness in nonobese sleep apnea patients. Free Radic Biol Med 2010;48:619-25.

87. Svatikova A, Wolk R, Lerman LO, et al. Oxidative stress in obstructive sleep apnoea. Eur Heart J 2005;26:2435-9.
88. Ozturk L, Mansour B, Yuksel M, Yalcin AS, Celikoglu F, Gokhan N. Lipid peroxidation and osmotic fragility of red blood cells in sleep-apnea patients. Clinica chimica acta; international journal of clinical chemistry 2003;332:83-8.
89. Alzoghaibi MA, Bahammam AS. Lipid peroxides, superoxide dismutase and circulating IL-8 and GCP-2 in patients with severe obstructive sleep apnea: a pilot study. Sleep Breath 2005;9:119-26.
90. Lee SD, Ju G, Choi JA, Kim JW, Yoon IY. The association of oxidative stress with central obesity in obstructive sleep apnea. Sleep Breath 2012;16:511-7.
91. Ikonomidis I, Stamatelopoulos K, Lekakis J, Vamvakou GD, Kremastinos DT. Inflammatory and non-invasive vascular markers: the multimarker approach for risk stratification in coronary artery disease. Atherosclerosis 2008;199:3-11.
92. Bastard JP, Maachi M, Lagathu C, et al. Recent advances in the relationship between obesity, inflammation, and insulin resistance. Eur Cytokine Netw 2006;17:4-12.
93. Fried SK, Bunkin DA, Greenberg AS. Omental and subcutaneous adipose tissues of obese subjects release interleukin-6: depot difference and regulation by glucocorticoid. J Clin Endocrinol Metab 1998;83:847-50.
94. Fain JN, Madan AK, Hiler ML, Cheema P, Bahouth SW. Comparison of the release of adipokines by adipose tissue, adipose tissue matrix, and adipocytes from visceral and subcutaneous abdominal adipose tissues of obese humans. Endocrinology 2004;145:2273-82.
95. Minoguchi K, Yokoe T, Tazaki T, et al. Increased carotid intima-media thickness and serum inflammatory markers in obstructive sleep apnea. Am J Respir Crit Care Med 2005;172:625-30.
96. Shamsuzzaman AS, Winnicki M, Lanfranchi P, et al. Elevated C-reactive protein in patients with obstructive sleep apnea. Circulation 2002;105:2462-4.
97. Yokoe T, Minoguchi K, Matsuo H, et al. Elevated levels of C-reactive protein and interleukin-6 in patients with obstructive sleep apnea syndrome are decreased by nasal continuous positive airway pressure. Circulation 2003;107:1129-34.
98. Kokturk O, Ciftci TU, Mollarecep E, Ciftci B. Elevated C-reactive protein levels and increased cardiovascular risk in patients with obstructive sleep apnea syndrome. Int Heart J 2005;46:801-9.
99. Can M, Acikgoz S, Mungan G, et al. Serum cardiovascular risk factors in obstructive sleep apnea. Chest 2006;129:233-7.
100. Punjabi NM, Beamer BA. C-reactive protein is associated with sleep disordered breathing independent of adiposity. Sleep 2007;30:29-34.
101. Lui MM, Lam JC, Mak HK, et al. C-reactive protein is associated with obstructive sleep apnea independent of visceral obesity. Chest 2009;135:950-6.
102. Drager LF, Lopes HF, Maki-Nunes C, et al. The impact of obstructive sleep apnea on metabolic and inflammatory markers in consecutive patients with metabolic syndrome. PLoS ONE 2010;5:e12065.
103. Sahlman J, Miettinen K, Peuhkurinen K, et al. The activation of the inflammatory cytokines in overweight patients with mild obstructive sleep apnoea. J Sleep Res 2010;19:341-8.
104. Thomopoulos C, Tsioufis C, Dimitriadis K, et al. Obstructive sleep apnoea syndrome is associated with enhanced sub-clinical inflammation and asymmetric dimethyl-arginine levels in hypertensives. J Hum Hypertens 2009;23:65-7.
105. Vgontzas AN, Zoumakis E, Bixler EO, et al. Selective effects of CPAP on sleep apnoea-associated manifestations. Eur J Clin Invest 2008;38:585-95.
106. Panoutsopoulos A, Kallianos A, Kostopoulos K, et al. Effect of CPAP treatment on endothelial function and plasma CRP levels in patients with sleep apnea. Med Sci Monit 2012;18:CR747-51.
107. Guven SF, Turkkani MH, Ciftci B, Ciftci TU, Erdogan Y. The relationship between high-sensitivity C-reactive protein levels and the severity of obstructive sleep apnea. Sleep Breath 2012;16:217-21.
108. Mermigkis C, Bouloukaki I, Mermigkis D, et al. CRP evolution pattern in CPAP-treated obstructive sleep apnea patients. Does gender play a role? Sleep Breath 2012;16:813-9.

109. Schiza SE, Mermigkis C, Panagiotis P, et al. C-reactive protein evolution in obstructive sleep apnoea patients under CPAP therapy. Eur J Clin Invest 2010;40:968-75.
110. Ishida K, Kato M, Kato Y, et al. Appropriate use of nasal continuous positive airway pressure decreases elevated C-reactive protein in patients with obstructive sleep apnea. Chest 2009;136:125-9.
111. Steiropoulos P, Tsara V, Nena E, et al. Effect of continuous positive airway pressure treatment on serum cardiovascular risk factors in patients with obstructive sleep apnea-hypopnea syndrome. Chest 2007;132:843-51.
112. Burioka N, Miyata M, Fukuoka Y, Endo M, Shimizu E. Day-night variations of serum interleukin-6 in patients with severe obstructive sleep apnea syndrome before and after continuous positive airway pressure (CPAP). Chronobiol Int 2008;25:827-34.
113. Drager LF, Bortolotto LA, Figueiredo AC, Krieger EM, Lorenzi GF. Effects of continuous positive airway pressure on early signs of atherosclerosis in obstructive sleep apnea. Am J Respir Crit Care Med 2007;176:706-12.
114. Carneiro G, Togeiro SM, Ribeiro-Filho FF, et al. Continuous Positive Airway Pressure Therapy Improves Hypoadiponectinemia in Severe Obese Men with Obstructive Sleep Apnea without Changes in Insulin Resistance. Metab Syndr Relat Disord 2009; 7(6): 537-42.
115. Barcelo A, Barbe F, Llompart E, et al. Effects of obesity on C-reactive protein level and metabolic disturbances in male patients with obstructive sleep apnea. Am J Med 2004;117:118-21.
116. Guilleminault C, Kirisoglu C, Ohayon MM. C-reactive protein and sleep-disordered breathing. Sleep 2004;27:1507-11.
117. Taheri S, Austin D, Lin L, Nieto FJ, Young T, Mignot E. Correlates of serum C-reactive protein (CRP)--no association with sleep duration or sleep disordered breathing. Sleep 2007;30:991-6.
118. Ryan S, Nolan GM, Hannigan E, Cunningham S, Taylor C, McNicholas WT. Cardiovascular risk markers in obstructive sleep apnoea syndrome and correlation with obesity. Thorax 2007;62:509-14.
119. Akashiba T, Akahoshi T, Kawahara S, Majima T, Horie T. Effects of long-term nasal continuous positive airway pressure on C-reactive protein in patients with obstructive sleep apnea syndrome. Intern Med 2005;44:899-900.
120. Aihara K, Oga T, Harada Y, et al. Comparison of biomarkers of subclinical lung injury in obstructive sleep apnea. Respir Med 2011;105:939-45.
121. Kanbay A, Kaya E, Buyukoglan H, et al. Serum gamma-glutamyl transferase activity is an independent predictor for cardiovascular disease in obstructive sleep apnea syndrome. Respir Med 2011;105:637-42.
122. Steiropoulos P, Papanas N, Nena E, et al. Inflammatory markers in middle-aged obese subjects: does obstructive sleep apnea syndrome play a role? Mediators Inflamm 2010:675320.
123. Imagawa S, Yamaguchi Y, Ogawa K, et al. Interleukin-6 and tumor necrosis factor-alpha in patients with obstructive sleep apnea-hypopnea syndrome. Respiration 2004;71:24-9.
124. Ryan S, Taylor CT, McNicholas WT. Predictors of elevated nuclear factor-kappaB-dependent genes in obstructive sleep apnea syndrome. Am J Respir Crit Care Med 2006;174:824-30.
125. Roytblat L, Rachinsky M, Fisher A, et al. Raised interleukin-6 levels in obese patients. Obes Res 2000;8:673-5.
126. Kim J, Lee CH, Park CS, Kim BG, Kim SW, Cho JH. Plasma levels of MCP-1 and adiponectin in obstructive sleep apnea syndrome. Arch Otolaryngol Head Neck Surg 2010;136:896-9.
127. Vgontzas AN, Papanicolaou DA, Bixler EO, Kales A, Tyson K, Chrousos GP. Elevation of plasma cytokines in disorders of excessive daytime sleepiness: role of sleep disturbance and obesity. J Clin Endocrinol Metab 1997;82:1313-6.
128. Chung S, Yoon IY, Shin YK, Lee CH, Kim JW, Ahn HJ. Endothelial dysfunction and inflammatory reactions of elderly and middle-aged men with obstructive sleep apnea syndrome. Sleep Breath 2009;13:11-7.
129. Hargens TA, Guill SG, Kaleth AS, et al. Insulin resistance and adipose-derived hormones in young men with untreated obstructive sleep apnea. Sleep Breath 2013;17:403-9.

130. Kohler M, Ayers L, Pepperell JC, et al. Effects of continuous positive airway pressure on systemic inflammation in patients with moderate to severe obstructive sleep apnoea: a randomised controlled trial. Thorax 2009;64:67-73.
131. Phillips CL, Yang Q, Williams A, et al. The effect of short-term withdrawal from continuous positive airway pressure therapy on sympathetic activity and markers of vascular inflammation in subjects with obstructive sleep apnoea. J Sleep Res 2007;16:217-25.
132. Steiropoulos P, Kotsianidis I, Nena E, et al. Long-term effect of continuous positive airway pressure therapy on inflammation markers of patients with obstructive sleep apnea syndrome. Sleep 2009;32:537-43.
133. Colish J, Walker JR, Elmayergi N, et al. Obstructive sleep apnea: effects of continuous positive airway pressure on cardiac remodeling as assessed by cardiac biomarkers, echocardiography, and cardiac MRI. Chest 2012;141:674-81.
134. Dorkova Z, Petrasova D, Molcanyiova A, Popovnakova M, Tkacova R. Effects of continuous positive airway pressure on cardiovascular risk profile in patients with severe obstructive sleep apnea and metabolic syndrome. Chest 2008;134:686-92.
135. Karamanlı H, Ozol D, Ugur KS, et al. Influence of CPAP treatment on airway and systemic inflammation in OSAS patients. Sleep Breath 2012. Sep 4 [Epub ahead of print].
136. Steiropoulos P, Papanas N, Nena E, et al. Inflammatory markers in middle-aged obese subjects: does obstructive sleep apnea syndrome play a role? Mediators Inflamm 2010;Article ID 675320, 6 pages.
137. Guo Y, Pan L, Ren D, Xie X. Impact of continuous positive airway pressure on C-reactive protein in patients with obstructive sleep apnea: a meta-analysis. Sleep Breath 2013;17:495-503.
138. Friedman M, Samuelson CG, Hamilton C, et al. Effect of continuous positive airway pressure on C-reactive protein levels in sleep apnea: a meta-analysis. Otolaryngol Head Neck Surg 2012;147:423-33.
139. Hajer GR, van Haeften TW, Visseren FL. Adipose tissue dysfunction in obesity, diabetes, and vascular diseases. Eur Heart J 2008;29:2959-71.
140. Donadio V, Liguori R, Vetrugno R, et al. Daytime sympathetic hyperactivity in OSAS is related to excessive daytime sleepiness. J Sleep Res 2007;16:327-32.
141. Hedner J, Ejnell H, Sellgren J, Hedner T, Wallin G. Is high and fluctuating muscle nerve sympathetic activity in the sleep apnoea syndrome of pathogenetic importance for the development of hypertension? J Hypertens Suppl 1988;6:S529-31.
142. Goldstein DS, Eisenhofer G, Kopin IJ. Sources and significance of plasma levels of catechols and their metabolites in humans. J Pharmacol Exp Ther 2003;305:800-11.
143. Carlson JT, Hedner J, Elam M, Ejnell H, Sellgren J, Wallin BG. Augmented resting sympathetic activity in awake patients with obstructive sleep apnea. Chest 1993;103:1763-8.
144. Fletcher EC, Miller J, Schaaf JW, Fletcher JG. Urinary catecholamines before and after tracheostomy in patients with obstructive sleep apnea and hypertension. Sleep 1987;10:35-44.
145. McArdle N, Hillman D, Beilin L, Watts G. Metabolic risk factors for vascular disease in obstructive sleep apnea: a matched controlled study. Am J Respir Crit Care Med 2007;175:190-5.
146. Hedner J, Darpo B, Ejnell H, Carlson J, Caidahl K. Reduction in sympathetic activity after long-term CPAP treatment in sleep apnoea: cardiovascular implications. Eur Respir J 1995;8:222-9.
147. Grassi G, Facchini A, Trevano FQ, et al. Obstructive sleep apnea-dependent and -independent adrenergic activation in obesity. Hypertension 2005;46:321-5.
148. Ziegler MG, Mills PJ, Loredo JS, Ancoli-Israel S, Dimsdale JE. Effect of continuous positive airway pressure and placebo treatment on sympathetic nervous activity in patients with obstructive sleep apnea. Chest 2001;120:887-93.
149. Sukegawa M, Noda A, Sugiura T, et al. Assessment of continuous positive airway pressure treatment in obstructive sleep apnea syndrome using 24-hour urinary catecholamines. Clin Cardiol 2005;28:519-22.
150. Marrone O, Riccobono L, Salvaggio A, Mirabella A, Bonanno A, Bonsignore MR. Catecholamines and blood pressure in obstructive sleep apnea syndrome. Chest 1993;103:722-7.

151. Minemura H, Akashiba T, Yamamoto H, Akahoshi T, Kosaka N, Horie T. Acute effects of nasal continuous positive airway pressure on 24-hour blood pressure and catecholamines in patients with obstructive sleep apnea. Intern Med 1998;37:1009-13.
152. Waradekar NV, Sinoway LI, Zwillich CW, Leuenberger UA. Influence of treatment on muscle sympathetic nerve activity in sleep apnea. Am J Respir Crit Care Med 1996;153:1333-8.
153. Narkiewicz K, Kato M, Phillips BG, Pesek CA, Davison DE, Somers VK. Nocturnal continuous positive airway pressure decreases daytime sympathetic traffic in obstructive sleep apnea. Circulation 1999;100:2332-5.
154. Heitmann J, Ehlenz K, Penzel T, et al. Sympathetic activity is reduced by nCPAP in hypertensive obstructive sleep apnoea patients. Eur Respir J 2004;23:255-62.
155. Somers VK, Dyken ME, Clary MP, Abboud FM. Sympathetic neural mechanisms in obstructive sleep apnea. J Clin Invest 1995;96:1897-904.
156. Wieber SJ. The cardiac consequences of the obstructive sleep apnea-hypopnea syndrome. Mt Sinai J Med 2005;72:10-2.
157. Lago F, Dieguez C, Gomez-Reino J, Gualillo O. The emerging role of adipokines as mediators of inflammation and immune responses. Cytokine Growth Factor Rev 2007;18:313-25.
158. Koerner A, Kratzsch J, Kiess W. Adipocytokines: leptin--the classical, resistin--the controversical, adiponectin--the promising, and more to come. Best Pract Res Clin Endocrinol Metab 2005;19:525-46.
159. Bravo PE, Morse S, Borne DM, Aguilar EA, Reisin E. Leptin and hypertension in obesity. Vasc Health Risk Manag 2006;2:163-9.
160. Sanna V, Di Giacomo A, La Cava A, et al. Leptin surge precedes onset of autoimmune encephalomyelitis and correlates with development of pathogenic T cell responses. J Clin Invest 2003;111:241-50.
161. Farooqi IS, Matarese G, Lord GM, et al. Beneficial effects of leptin on obesity, T cell hyporesponsiveness, and neuroendocrine/metabolic dysfunction of human congenital leptin deficiency. J Clin Invest 2002;110:1093-103.
162. Zhao Y, Sun R, You L, Gao C, Tian Z. Expression of leptin receptors and response to leptin stimulation of human natural killer cell lines. Biochem Biophys Res Commun 2003;300:247-52.
163. Raso GM, Pacilio M, Esposito E, Coppola A, Di Carlo R, Meli R. Leptin potentiates IFN-gamma-induced expression of nitric oxide synthase and cyclo-oxygenase-2 in murine macrophage J774A.1. Br J Pharmacol 2002;137:799-804.
164. Sarraf P, Frederich RC, Turner EM, et al. Multiple cytokines and acute inflammation raise mouse leptin levels: potential role in inflammatory anorexia. J Exp Med 1997;185:171-5.
165. Grunfeld C, Zhao C, Fuller J, et al. Endotoxin and cytokines induce expression of leptin, the ob gene product, in hamsters. J Clin Invest 1996;97:2152-7.
166. Faggioni R, Fantuzzi G, Fuller J, Dinarello CA, Feingold KR, Grunfeld C. IL-1 beta mediates leptin induction during inflammation. Am J Physiol 1998;274:R204-8.
167. Zarkesh-Esfahani H, Pockley G, Metcalfe RA, et al. High-dose leptin activates human leukocytes via receptor expression on monocytes. J Immunol 2001;167:4593-9.
168. Ciccone M, Vettor R, Pannacciulli N, et al. Plasma leptin is independently associated with the intima-media thickness of the common carotid artery. Int J Obes Relat Metab Disord 2001;25:805-10.
169. Paolisso G, Tagliamonte MR, Galderisi M, et al. Plasma leptin level is associated with myocardial wall thickness in hypertensive insulin-resistant men. Hypertension 1999;34:1047-52.
170. Al Lawati N, Mulgrew A, Cheema R, et al. Pro-atherogenic cytokine profile of patients with suspected obstructive sleep apnea. Sleep Breath 2009;13:391-5.
171. Basoglu OK, Sarac F, Sarac S, Uluer H, Yilmaz C. Metabolic syndrome, insulin resistance, fibrinogen, homocysteine, leptin, and C-reactive protein in obese patients with obstructive sleep apnea syndrome. Ann Thorac Med 2011;6:120-5.
172. Ip MS, Lam KS, Ho C, Tsang KW, Lam W. Serum leptin and vascular risk factors in obstructive sleep apnea. Chest 2000;118:580-6.

173. Kapsimalis F, Varouchakis G, Manousaki A, et al. Association of sleep apnea severity and obesity with insulin resistance, C-reactive protein, and leptin levels in male patients with obstructive sleep apnea. Lung 2008;186:209-17.
174. Makinodan K, Yoshikawa M, Fukuoka A, et al. Effect of serum leptin levels on hypercapnic ventilatory response in obstructive sleep apnea. Respiration 2008;75:257-64.
175. Ozturk L, Unal M, Tamer L, Celikoglu F. The association of the severity of obstructive sleep apnea with plasma leptin levels. Arch Otolaryngol Head Neck Surg 2003;129:538-40.
176. Patel SR, Palmer LJ, Larkin EK, Jenny NS, White DP, Redline S. Relationship between obstructive sleep apnea and diurnal leptin rhythms. Sleep 2004;27:235-9.
177. Phillips BG, Kato M, Narkiewicz K, Choe I, Somers VK. Increases in leptin levels, sympathetic drive, and weight gain in obstructive sleep apnea. Am J Physiol Heart Circ Physiol 2000;279:H234-7.
178. Tokuda F, Sando Y, Matsui H, Koike H, Yokoyama T. Serum levels of adipocytokines, adiponectin and leptin, in patients with obstructive sleep apnea syndrome. Intern Med 2008;47:1843-9.
179. Ulukavak Ciftci T, Kokturk O, Bukan N, Bilgihan A. Leptin and ghrelin levels in patients with obstructive sleep apnea syndrome. Respiration 2005;72:395-401.
180. Zirlik S, Hauck T, Fuchs FS, Neurath MF, Konturek PC, Harsch IA. Leptin, obestatin and apelin levels in patients with obstructive sleep apnoea syndrome. Med Sci Monit 2011;17:CR159-64.
181. Chin K, Shimizu K, Nakamura T, et al. Changes in intra-abdominal visceral fat and serum leptin levels in patients with obstructive sleep apnea syndrome following nasal continuous positive airway pressure therapy. Circulation 1999;100:706-12.
182. Chin K, Nakamura T, Takahashi K, et al. Effects of obstructive sleep apnea syndrome on serum aminotransferase levels in obese patients. Am J Med 2003;114:370-6.
183. Cuhadaroglu C, Utkusavas A, Ozturk L, Salman S, Ece T. Effects of nasal CPAP treatment on insulin resistance, lipid profile, and plasma leptin in sleep apnea. Lung 2009;187:75-81.
184. Harsch IA, Konturek PC, Koebnick C, et al. Leptin and ghrelin levels in patients with obstructive sleep apnoea: effect of CPAP treatment. Eur Respir J 2003;22:251-7.
185. Saarelainen S, Lahtela J, Kallonen E. Effect of nasal CPAP treatment on insulin sensitivity and plasma leptin. J Sleep Res 1997;6:146-7.
186. Shimizu K, Chin K, Nakamura T, et al. Plasma leptin levels and cardiac sympathetic function in patients with obstructive sleep apnoea-hypopnoea syndrome. Thorax 2002;57:429-34.
187. Trenell MI, Ward JA, Yee BJ, et al. Influence of constant positive airway pressure therapy on lipid storage, muscle metabolism and insulin action in obese patients with severe obstructive sleep apnoea syndrome. Diabetes Obes Metab 2007;9:679-87.
188. Drummond M, Winck JC, Guimaraes JT, Santos AC, Almeida J, Marques JA. Autoadjusting-CPAP effect on serum leptin concentrations in obstructive sleep apnoea patients. BMC Pulm Med 2008;8:21.
189. Papaioannou I, Patterson M, Twigg GL, et al. Lack of association between impaired glucose tolerance and appetite regulating hormones in patients with obstructive sleep apnea. J Clin Sleep Med 2011;7:486-92B.
190. Sanchez-de-la-Torre M, Mediano O, Barcelo A, et al. The influence of obesity and obstructive sleep apnea on metabolic hormones. Sleep Breath 2012;16:649-56.
191. Schafer H, Pauleit D, Sudhop T, Gouni-Berthold I, Ewig S, Berthold HK. Body fat distribution, serum leptin, and cardiovascular risk factors in men with obstructive sleep apnea. Chest 2002;122:829-39.
192. Sharma SK, Kumpawat S, Goel A, Banga A, Ramakrishnan L, Chaturvedi P. Obesity, and not obstructive sleep apnea, is responsible for metabolic abnormalities in a cohort with sleep-disordered breathing. Sleep Med 2007;8:12-7.
193. Ursavas A, Ilcol YO, Nalci N, Karadag M, Ege E. Ghrelin, leptin, adiponectin, and resistin levels in sleep apnea syndrome: Role of obesity. Ann Thorac Med 2010;5:161-5.
194. Yamamoto Y, Fujiuchi S, Hiramatsu M, et al. Resistin Is Closely Related to Systemic Inflammation in Obstructive Sleep Apnea. Respiration 2008;76:377-85.

195. Garcia JM, Sharafkhaneh H, Hirshkowitz M, Elkhatib R, Sharafkhaneh A. Weight and metabolic effects of CPAP in obstructive sleep apnea patients with obesity. Respir Res 2011;12:80.
196. Harsch IA, Schahin SP, Bruckner K, et al. The effect of continuous positive airway pressure treatment on insulin sensitivity in patients with obstructive sleep apnoea syndrome and type 2 diabetes. Respiration 2004;71:252-9.
197. Harsch IA, Koebnick C, Wallaschofski H, et al. Resistin levels in patients with obstructive sleep apnoea syndrome--the link to subclinical inflammation? Med Sci Monit 2004;10:CR510-5.
198. Sanner BM, Kollhosser P, Buechner N, Zidek W, Tepel M. Influence of treatment on leptin levels in patients with obstructive sleep apnoea. Eur Respir J 2004;23:601-4.
199. Barcelo A, Barbe F, Llompart E, et al. Neuropeptide Y and leptin in patients with obstructive sleep apnea syndrome: role of obesity. Am J Respir Crit Care Med 2005;171:183-7.
200. Carpagnano GE, Resta O, Pergola GD, Sabato R, Foschino Barbaro MP. The role of obstructive sleep apnea syndrome and obesity in determining leptin in the exhaled breath condensate. J Breath Res 2010;4:036003.
201. Lefebvre AM, Laville M, Vega N, et al. Depot-specific differences in adipose tissue gene expression in lean and obese subjects. Diabetes 1998;47:98-103.
202. Minocci A, Savia G, Lucantoni R, et al. Leptin plasma concentrations are dependent on body fat distribution in obese patients. Int J Obes Relat Metab Disord 2000;24:1139-44.
203. Montague CT, Prins JB, Sanders L, Digby JE, O'Rahilly S. Depot- and sex-specific differences in human leptin mRNA expression: implications for the control of regional fat distribution. Diabetes 1997;46:342-7.
204. Nieto FJ, Young TB, Lind BK, et al. Association of sleep-disordered breathing, sleep apnea, and hypertension in a large community-based study. Sleep Heart Health Study.[see comment][erratum appears in JAMA 2002 Oct 23-30;288(16):1985]. JAMA 2000;283:1829-36.
205. Lavie P, Herer P, Hoffstein V. Obstructive sleep apnoea syndrome as a risk factor for hypertension: population study. BMJ 2000;320:479-82.
206. Bixler EO, Vgontzas AN, Lin HM, et al. Association of hypertension and sleep-disordered breathing. Arch Intern Med 2000;160:2289-95.
207. Peppard PE, Young T, Palta M, Skatrud J. Prospective study of the association between sleep-disordered breathing and hypertension. New England Journal of Medicine 2000;342:1378-84.
208. Grote L, Hedner J, Peter JH. Mean blood pressure, pulse pressure and grade of hypertension in untreated hypertensive patients with sleep-related breathing disorder. J Hypertens 2001;19:683-90.
209. Bazzano LA, Khan Z, Reynolds K, He J. Effect of nocturnal nasal continuous positive airway pressure on blood pressure in obstructive sleep apnea. Hypertension 2007;50:417-23.
210. Haentjens P, Van Meerhaeghe A, Moscariello A, et al. The impact of continuous positive airway pressure on blood pressure in patients with obstructive sleep apnea syndrome: evidence from a meta-analysis of placebo-controlled randomized trials. Arch Intern Med 2007;167:757-64.
211. Malik J, Drake CL, Hudgel DW. Variables affecting the change in systemic blood pressure in response to nasal CPAP in obstructive sleep apnea patients. Sleep Breath 2008;12:47-52.
212. Campos-Rodriguez F, Perez-Ronchel J, Grilo-Reina A, Lima-Alvarez J, Benitez MA, Almeida-Gonzalez C. Long-term effect of continuous positive airway pressure on BP in patients with hypertension and sleep apnea. Chest 2007;132:1847-52.
213. Robinson GV, Smith DM, Langford BA, Davies RJ, Stradling JR. Continuous positive airway pressure does not reduce blood pressure in nonsleepy hypertensive OSA patients. Eur Respir J 2006;27:1229-35.
214. Barbe F, Mayoralas LR, Duran J, et al. Treatment with continuous positive airway pressure is not effective in patients with sleep apnea but no daytime sleepiness. a randomized, controlled trial. Ann Intern Med 2001;134:1015-23.

215. Brooks D, Horner RL, Kozar LF, Render-Teixeira CL, Phillipson EA. Obstructive sleep apnea as a cause of systemic hypertension. Evidence from a canine model. J Clin Invest 1997;99:106-9.
216. Sharabi Y, Scope A, Chorney N, Grotto I, Dagan Y. Diastolic blood pressure is the first to rise in association with early subclinical obstructive sleep apnea: lessons from periodic examination screening. Am J Hypertens 2003;16:236-9.
217. Baguet JP, Levy P, Barone-Rochette G, et al. Masked hypertension in obstructive sleep apnea syndrome. J Hypertens 2008;26:885-92.
218. Baguet JP, Hammer L, Levy P, et al. Night-time and diastolic hypertension are common and underestimated conditions in newly diagnosed apnoeic patients. J Hypertens 2005;23:521-7.
219. Arias MA, Garcia-Rio F, Alonso-Fernandez A, Martinez I, Villamor J. Pulmonary hypertension in obstructive sleep apnoea: effects of continuous positive airway pressure: a randomized, controlled cross-over study. Eur Heart J 2006;27:1106-13.
220. Sjostrom C, Lindberg E, Elmasry A, Hagg A, Svardsudd K, Janson C. Prevalence of sleep apnoea and snoring in hypertensive men: a population based study. Thorax 2002;57:602-7.
221. Logan AG, Perlikowski SM, Mente A, et al. High prevalence of unrecognized sleep apnoea in drug-resistant hypertension. J Hypertens 2001;19:2271-7.
222. Goncalves SC, Martinez D, Gus M, et al. Obstructive sleep apnea and resistant hypertension: a case-control study. Chest 2007;132:1858-62.
223. Broström A, Sunnergren O, Årestedt K, et al. Factors associated with undiagnosed obstructive sleep apnoea in hypertensive primary care patients. Scand J Prim Health Care 2012;30:107-13.
224. Grote L, Hedner J, Peter JH. Sleep-related breathing disorder is an independent risk factor for uncontrolled hypertension. J Hypertens 2000;18:679-85.
225. Logan AG, Tkacova R, Perlikowski SM, et al. Refractory hypertension and sleep apnoea: effect of CPAP on blood pressure and baroreflex. Eur Respir J 2003;21:241-7.
226. Cui R, Tanigawa T, Sakurai S, et al. Associations of sleep-disordered breathing with excessive daytime sleepiness and blood pressure in Japanese women. Hypertens Res 2008;31:501-6.
227. Pack AI, Gislason T. Obstructive sleep apnea and cardiovascular disease: a perspective and future directions. Prog Cardiovasc Dis 2009;51:434-51.
228. Kathiresan S, Melander O, Guiducci C, et al. Six new loci associated with blood low-density lipoprotein cholesterol, high-density lipoprotein cholesterol or triglycerides in humans. Nat Genet 2008.
229. Samani NJ, Erdmann J, Hall AS, et al. Genomewide association analysis of coronary artery disease. N Engl J Med 2007;357:443-53.
230. Scott LJ, Mohlke KL, Bonnycastle LL, et al. A genome-wide association study of type 2 diabetes in Finns detects multiple susceptibility variants. Science 2007;316:1341-5.
231. Wang SS, Schadt EE, Wang H, et al. Identification of pathways for atherosclerosis in mice: integration of quantitative trait locus analysis and global gene expression data. Circ Res 2007;101:e11-30.
232. Zeggini E, Weedon MN, Lindgren CM, et al. Replication of genome-wide association signals in UK samples reveals risk loci for type 2 diabetes. Science 2007;316:1336-41.
233. Peker Y, Hedner J, Norum J, Kraiczi H, Carlson J. Increased incidence of cardiovascular disease in middle-aged men with obstructive sleep apnea: a 7-year follow-up. Am J Respir Crit Care Med 2002;166:159-65.
234. Yaggi HK, Concato J, Kernan WN, Lichtman JH, Brass LM, Mohsenin V. Obstructive sleep apnea as a risk factor for stroke and death. N Engl J Med 2005;353:2034-41.
235. Marti S, Sampol G, Munoz X, et al. Mortality in severe sleep apnoea/hypopnoea syndrome patients: impact of treatment. Eur Respir J 2002;20:1511-8.
236. Lavie P, Lavie L, Herer P. All-cause mortality in males with sleep apnoea syndrome: declining mortality rates with age. Eur Respir J 2005;25:514-20.
237. Campos-Rodriguez F, Pena-Grinan N, Reyes-Nunez N, et al. Mortality in obstructive sleep apnea-hypopnea patients treated with positive airway pressure. Chest 2005;128:624-33.

238. Munoz R, Duran-Cantolla J, Martinez-Vila E, et al. Severe sleep apnea and risk of ischemic stroke in the elderly. Stroke 2006;37:2317-21.
239. Milleron O, Pilliere R, Foucher A, et al. Benefits of obstructive sleep apnoea treatment in coronary artery disease: a long-term follow-up study. Eur Heart J 2004;25:728-34.
240. Peker Y, Kraiczi H, Hedner J, Loth S, Johansson A, Bende M. An independent association between obstructive sleep apnoea and coronary artery disease. Eur Respir J 1999;14:179-84.
241. Lavie P, Herer P, Lavie L. Mortality risk factors in sleep apnoea: a matched case-control study. J Sleep Res 2007;16:128-34.
242. Gami AS, Howard DE, Olson EJ, Somers VK. Day-night pattern of sudden death in obstructive sleep apnea. N Engl J Med 2005;352:1206-14.
243. Ip MS, Tse HF, Lam B, Tsang KW, Lam WK. Endothelial function in obstructive sleep apnea and response to treatment. Am J Respir Crit Care Med 2004;169:348-53.
244. Oflaz H, Cuhadaroglu C, Pamukcu B, et al. Endothelial function in patients with obstructive sleep apnea syndrome but without hypertension. Respiration 2006;73:751-6.
245. Itzhaki S, Lavie L, Pillar G, Tal G, Lavie P. Endothelial dysfunction in obstructive sleep apnea measured by peripheral arterial tone response in the finger to reactive hyperemia. Sleep 2005;28:594-600.
246. Kohler M, Craig S, Nicoll D, Leeson P, Davies RJ, Stradling JR. Endothelial Function and Arterial Stiffness in Minimally Symptomatic Obstructive Sleep Apnea. Am J Respir Crit Care Med 2008.
247. Drager LF, Bortolotto LA, Figueiredo AC, Silva BC, Krieger EM, Lorenzi-Filho G. Obstructive sleep apnea, hypertension, and their interaction on arterial stiffness and heart remodeling. Chest 2007;131:1379-86.
248. Protogerou AD, Laaban JP, Czernichow S, et al. Structural and functional arterial properties in patients with obstructive sleep apnoea syndrome and cardiovascular comorbidities. J Hum Hypertens 2008;22:415-22.
249. Phillips C, Hedner J, Berend N, Grunstein R. Diurnal and obstructive sleep apnea influences on arterial stiffness and central blood pressure in men. Sleep 2005;28:604-9.
250. Kraiczi H, Caidahl K, Samuelsson A, Peker Y, Hedner J. Impairment of vascular endothelial function and left ventricular filling : association with the severity of apnea-induced hypoxemia during sleep. Chest 2001;119:1085-91.
251. Phillips CL, Yee B, Yang Q, et al. Effects of continuous positive airway pressure treatment and withdrawal in patients with obstructive sleep apnea on arterial stiffness and central BP. Chest 2008;134:94-100.
252. El Solh AA, Akinnusi ME, Baddoura FH, Mankowski CR. Endothelial cell apoptosis in obstructive sleep apnea: a link to endothelial dysfunction. Am J Respir Crit Care Med 2007;175:1186-91.
253. Davignon J, Ganz P. Role of endothelial dysfunction in atherosclerosis. Circulation 2004;109:III27-32.
254. Barcelo A, Barbe F, de la Pena M, et al. Insulin resistance and daytime sleepiness In patients with sleep apnea. Thorax 2008;63:946-50.
255. Hecht L, Mohler R, Meyer G. Effects of CPAP-respiration on markers of glucose metabolism in patients with obstructive sleep apnoea syndrome: a systematic review and meta-analysis. Ger Med Sci 2011;9:Doc20.
256. Iftikhar IH, Blankfield RP. Effect of continuous positive airway pressure on hemoglobin A(1c) in patients with obstructive sleep apnea: a systematic review and meta-analysis. Lung 2012;190:605-11.
257. Hoyos CM, Killick R, Yee BJ, Phillips CL, Grunstein RR, Liu PY. Cardiometabolic changes after continuous positive airway pressure for obstructive sleep apnoea: a randomised sham-controlled study. Thorax 2012;67:1081-9.
258. Sleep-related breathing disorders in adults: recommendations for syndrome definition and measurement techniques in clinical research. The Report of an American Academy of Sleep Medicine Task Force. Sleep 1999;22:667-89.
259. Mazza S, Pepin JL, Naegele B, Plante J, Deschaux C, Levy P. Most obstructive sleep apnoea patients exhibit vigilance and attention deficits on an extended battery of tests. Eur Respir J 2005;25:75-80.

260. Johns MW. A new method for measuring daytime sleepiness: the Epworth sleepiness scale. Sleep 1991;14:540-5.
261. Sauter C, Asenbaum S, Popovic R, et al. Excessive daytime sleepiness in patients suffering from different levels of obstructive sleep apnoea syndrome. J Sleep Res 2000;9:293-301.
262. Sforza E, Haba-Rubio J, De Bilbao F, Rochat T, Ibanez V. Performance vigilance task and sleepiness in patients with sleep-disordered breathing. European Respiratory Journal 2004;24:279-85.
263. Jenkinson C, Davies RJ, Mullins R, Stradling JR. Comparison of therapeutic and subtherapeutic nasal continuous positive airway pressure for obstructive sleep apnoea: a randomised prospective parallel trial. Lancet 1999;353:2100-5.
264. Johns MW. Reliability and factor analysis of the Epworth Sleepiness Scale. Sleep 1992;15:376-81.
265. Sforza E, Lugaresi E. Daytime sleepiness and nasal continuous positive airway pressure therapy in obstructive sleep apnea syndrome patients: effects of chronic treatment and 1-night therapy withdrawal. Sleep 1995;18:195-201.
266. Engleman HM, Cheshire KE, Deary IJ, Douglas NJ. Daytime sleepiness, cognitive performance and mood after continuous positive airway pressure for the sleep apnoea/hypopnoea syndrome. Thorax 1993;48:911-4.
267. Marshall NS, Barnes M, Travier N, et al. Continuous positive airway pressure reduces daytime sleepiness in mild to moderate obstructive sleep apnoea: a meta-analysis. Thorax 2006;61:430-4.
268. Krieger J, Meslier N, Lebrun T, et al. Accidents in obstructive sleep apnea patients treated with nasal continuous positive airway pressure: a prospective study. The Working Group ANTADIR, Paris and CRESGE, Lille, France. Association Nationale de Traitement a Domicile des Insuffisants Respiratoires. Chest 1997;112:1561-6.
269. Cassel W, Ploch T, Becker C, Dugnus D, Peter JH, von Wichert P. Risk of traffic accidents in patients with sleep-disordered breathing: reduction with nasal CPAP. Eur Respir J 1996;9:2606-11.
270. Chee MW, Chuah LY, Venkatraman V, Chan WY, Philip P, Dinges DF. Functional imaging of working memory following normal sleep and after 24 and 35 h of sleep deprivation: Correlations of fronto-parietal activation with performance. NeuroImage 2006;31:419-28.
271. Van Dongen HP, Vitellaro KM, Dinges DF. Individual differences in adult human sleep and wakefulness: Leitmotif for a research agenda. Sleep 2005;28:479-96.
272. Chuah YM, Venkatraman V, Dinges DF, Chee MW. The neural basis of interindividual variability in inhibitory efficiency after sleep deprivation. J Neurosci 2006;26:7156-62.
273. Brin YS, Reuveni H, Greenberg S, Tal A, Tarasiuk A. Determinants affecting initiation of continuous positive airway pressure treatment. Isr Med Assoc J 2005;7:13-8.
274. Barbé F, Durán-Cantolla J, Sánchez-de-la-Torre M, et al. Effect of continuous positive airway pressure on the incidence of hypertension and cardiovascular events in nonsleepy patients with obstructive sleep apnea: a randomized controlled trial. JAMA 2012;307:2161-8.
275. Kasiakogias A, Tsioufis C, Thomopoulos C, et al. Effects of continuous positive airway pressure on blood pressure in hypertensive patients with obstructive sleep apnea: a 3-year follow-up. J Hypertens 2013;31:352-60.
276. Guilleminault C, Bassiri, A. Clinical features and evaluation of obstructive sleep apnea-hypopnea syndrome and upper airway resistance syndrome. In: Kryger MH, Roth, T., Dement, W.C., ed. Principles and Practice of Sleep Medicine, 4th ed. Philadelphia, PA: Elsevier Sanders, 2005.
277. Cruz IA, Drummond M, Winck JC. Obstructive sleep apnea symptoms beyond sleepiness and snoring: effects of nasal APAP therapy. Sleep Breath 2012;16:361-6.
278. Kiely JL, Murphy M, McNicholas WT. Subjective efficacy of nasal CPAP therapy in obstructive sleep apnoea syndrome: a prospective controlled study. Eur Respir J 1999;13:1086-90.
279. Krauchi K. The human sleep-wake cycle reconsidered from a thermoregulatory point of view. Physiol Behav 2007;90:236-45.
280. Sewitch DE, Kittrell EM, Kupfer DJ, Reynolds CF, 3rd. Body temperature and sleep architecture in response to a mild cold stress in women. Physiol Behav 1986;36:951-7.

281. Fletcher A, van den Heuvel C, Dawson D. Sleeping with an electric blanket: effects on core temperature, sleep, and melatonin in young adults. Sleep 1999;22:313-8.
282. Bouscein W. Methodological issues in electrodermal measurement. In: Roy JC, Bouscein W, Fowles D.C., Gruzelier, J.H., ed. Progress in electrodermal research. New York: Plenum Press, 1993:31-41.
283. Kobayashi R, Koike Y, Hirayama M, Ito H, Sobue G. Skin sympathetic nerve function during sleep - a study with effector responses. Auton Neurosci 2003;103:121-6.
284. Smetana GW. Approach to the patient with night sweats. In: UpToDate, Sokol HN and Aronson MD (Ed), UpToDate. Waltham, MA., 2012.
285. Mold JW, Woolley JH, Nagykaldi Z. Associations between night sweats and other sleep disturbances: An OKPRN study. Ann Fam Med 2006;4:423-6.
286. Mold JW, Mathew MK, Belgore S, DeHaven M. Prevalence of night sweats in primary care patients: an OKPRN and TAFP-Net collaborative study. J Fam Pract 2002;51:452-6.
287. Hartz A, Ross JJ, Noyes R, Williams P. Somatic symptoms and psychological characteristics associated with insomnia in postmenopausal women. Sleep Med 2013;14:71-78.
288. Lea MJ, Aber RC. Descriptive epidemiology of night sweats upon admission to a university hospital. South Med J 1985;78:1065-7.
289. Viera AJ, Bond MM, Yates SW. Diagnosing night sweats. Am Fam Physician 2003;67:1019-24.
290. Su CW, Gaskie S, Hitchcock K, Johnson L. Clinical inquiries. What's the best diagnostic evaluation of night sweats? J Fam Pract 2007;56:493-5.
291. Mold JW, Holtzclaw BJ, McCarthy L. Night sweats: a systematic review of the literature. J Am Board Fam Med 2012;25:878-93.
292. Mold JW, Goodrich S, Orr W. Associations between subjective night sweats and sleep study findings. J Am Board Fam Med 2008;21:96-100.
293. McNicholas WT, Bonsigore MR. Sleep apnoea as an independent risk factor for cardiovascular disease: current evidence, basic mechanisms and research priorities. Eur Respir J 2007;29:156-78.
294. Rader DJ. Effect of insulin resistance, dyslipidemia, and intra-abdominal adiposity on the development of cardiovascular disease and diabetes mellitus. Am J Med 2007;120:S12-8.
295. Van Gaal LF, Mertens IL, De Block CE. Mechanisms linking obesity with cardiovascular disease. Nature 2006;444:875-80.
296. Eikelis N, Esler M. The neurobiology of human obesity. Exp Physiol 2005;90:673-82.
297. Tentolouris N, Liatis S, Katsilambros N. Sympathetic system activity in obesity and metabolic syndrome. Ann N Y Acad Sci 2006;1083:129-52.
298. Vincent HK, Taylor AG. Biomarkers and potential mechanisms of obesity-induced oxidant stress in humans. Int J Obes (Lond) 2006;30:400-18.
299. Clement K, Langin D. Regulation of inflammation-related genes in human adipose tissue. J Intern Med 2007;262:422-30.
300. Haffner SM. Abdominal adiposity and cardiometabolic risk: do we have all the answers? Am J Med 2007;120:S10-6; discussion S6-7.
301. Romero-Corral A, Montori VM, Somers VK, et al. Association of bodyweight with total mortality and with cardiovascular events in coronary artery disease: a systematic review of cohort studies. Lancet 2006;368:666-78.
302. Carmelli D, Swan GE, Bliwise DL. Relationship of 30-year changes in obesity to sleep-disordered breathing in the Western Collaborative Group Study. Obes Res 2000;8:632-7.
303. Kim SK, Kim HJ, Hur KY, et al. Visceral fat thickness measured by ultrasonography can estimate not only visceral obesity but also risks of cardiovascular and metabolic diseases. Am J Clin Nutr 2004;79:593-9.
304. Fox CS, Massaro JM, Hoffmann U, et al. Abdominal visceral and subcutaneous adipose tissue compartments: association with metabolic risk factors in the Framingham Heart Study. Circulation 2007;116:39-48.
305. Rosito GA, Massaro JM, Hoffmann U, et al. Pericardial fat, visceral abdominal fat, cardiovascular disease risk factors, and vascular calcification in a community-based sample: the Framingham Heart Study. Circulation 2008;117:605-13.

306. Onat A, Avci GS, Barlan MM, Uyarel H, Uzunlar B, Sansoy V. Measures of abdominal obesity assessed for visceral adiposity and relation to coronary risk. Int J Obes Relat Metab Disord 2004;28:1018-25.
307. Pascot A, Despres JP, Lemieux I, et al. Contribution of visceral obesity to the deterioration of the metabolic risk profile in men with impaired glucose tolerance. Diabetologia 2000;43:1126-35.
308. Barcelo A, Barbe F, de la Pena M, et al. Antioxidant status in patients with sleep apnoea and impact of continuous positive airway pressure treatment. Eur Respir J 2006;27:756-60.
309. Gharib SA, Hayes AL, Rosen MJ, Patel SR. A pathway-based analysis on the effects of obstructive sleep apnea in modulating visceral fat transcriptome. Sleep 2013;36:23-30.
310. Santamaria J, Iranzo A, Ma Montserrat J, de Pablo J. Persistent sleepiness in CPAP treated obstructive sleep apnea patients: evaluation and treatment. Sleep Med Rev 2007;11:195-207.
311. Peppard PE, Young T, Palta M, Dempsey J, Skatrud J. Longitudinal study of moderate weight change and sleep-disordered breathing. JAMA 2000;284:3015-21.
312. Young T, Evans L, Finn L, Palta M. Estimation of the clinically diagnosed proportion of sleep apnea syndrome in middle-aged men and women. Sleep 1997;20:705-6.
313. Margretardottir OB, Thorleifsson SJ, Gudmundsson G, et al. Hypertension, systemic inflammation and body weight in relation to lung function impairment-an epidemiological study. Copd 2009;6:250-5.
314. Benediktsdottir B, Janson C, Lindberg E, et al. Prevalence of restless legs syndrome among adults in Iceland and Sweden: Lung function, comorbidity, ferritin, biomarkers and quality of life. Sleep Med 2010;11:1043-8.
315. Partinen M, Gislason T. Basic Nordic Sleep Questionnaire (BNSQ): a quantitated measure of subjective sleep complaints. J Sleep Res 1995;4:150-5.
316. Bjornsdottir E, Janson C, Gislason T, et al. Insomnia in untreated sleep apnea patients compared to controls. J Sleep Res 2012;21:131-8.
317. Gislason T, Janson C, Vermeire P, et al. Respiratory symptoms and nocturnal gastroesophageal reflux: a population-based study of young adults in three European countries. Chest 2002;121:158-63.
318. Emilsson OI, Janson C, Benediktsdóttir B, Júlíusson S, Gíslason T. Nocturnal gastroesophageal reflux, lung function and symptoms of obstructive sleep apnea: Results from an epidemiological survey. Respir Med 2012;106:459-66.
319. Ware J, Jr., Kosinski M, Keller SD. A 12-Item Short-Form Health Survey: construction of scales and preliminary tests of reliability and validity. Med Care 1996;34:220-33.
320. Allen RP, Picchietti D, Hening WA, et al. Restless legs syndrome: diagnostic criteria, special considerations, and epidemiology. A report from the restless legs syndrome diagnosis and epidemiology workshop at the National Institutes of Health. Sleep Med 2003;4:101-19.
321. Maislin G, Pack AI, Kribbs NB, et al. A survey screen for prediction of apnea. Sleep 1995;18:158-66.
322. Rechtschaffen A, Kales A. A manual of standardized terminology, techniques and scoring system for sleep stages of human subjects: U.S. Departhment of Health, Education and Welfare Public Health Service - National Institute of Health, 1968.
323. EEG arousals: scoring rules and examples: a preliminary report from the Sleep Disorders Atlas Task Force of the American Sleep Disorders Association. Sleep 1992;15:173-84.
324. Recording and scoring leg movements. The Atlas Task Force. Sleep 1993;16:748-59.
325. Andreassi JL. Psychophysiology: human behavior and physiological response. 3rd ed. Hillsdale, New Jersey: Lawrence Erlbaum Associates, Publishers, 1995.
326. Fowles DC, Christie MJ, Edelberg R, Grings WW, Lykken DT, Venables PH. Committee report. Publication recommendations for electrodermal measurements. Psychophysiology 1981;18:232-9.
327. Lajos LE. The relationship between electrodermal activity in sleep, negative effect and stress in patients referred for nocturnal polysomnography: Louisiana State University, 2002.

328. Krauchi K, Cajochen C, Werth E, Wirz-Justice A. Functional link between distal vasodilation and sleep-onset latency? Am J Physiol Regul Integr Comp Physiol 2000;278:R741-8.
329. Barratt J, Topham P. Urine proteomics: the present and future of measuring urinary protein components in disease. CMAJ 2007;177:361-8.
330. Moyer TP, Jiang NS, Tyce GM, Sheps SG. Analysis for urinary catecholamines by liquid chromatography with amperometric detection: methodology and clinical interpretation of results. Clin Chem 1979;25:256-63.
331. Maislin G, Ahmed MM, Gooneratne N, et al. Single slice vs. volumetric MR assessment of visceral adipose tissue: reliability and validity among the overweight and obese. Obesity (Silver Spring) 2012;20:2124-32.
332. Efron B, Tibshirani RJ. An Introduction to the Bootstrap, (Monographs on Statistics and Applied Probability, 57). New York: Chapman and Hall, 1993.
333. Myers RH, Montgomery DC. Response Surface Methodology, Process and Product Optimization Using Designed Experiments. New York: John Wiley & Sons, Inc., 1995.
334. So HK, Li AM, Au CT, et al. Night sweats in children: prevalence and associated factors. Arch Dis Child 2012;97:470-3.
335. Reynolds WA. Are night sweats a sign of esophageal reflux? J Clin Gastroenterol 1989;11:590-1.
336. Mold JW, Roberts M, Aboshady HM. Prevalence and predictors of night sweats, day sweats, and hot flashes in older primary care patients: an OKPRN study. Ann Fam Med 2004;2:391-7.
337. Ross RJ, Gresch PJ, Ball WA, Sanford LD, Morrison AR. REM sleep inhibition by desipramine: evidence for an alpha-1 adrenergic mechanism. Brain Res 1995;701:129-34.
338. Pal D, Mallick BN. Role of noradrenergic and GABA-ergic inputs in pedunculopontine tegmentum for regulation of rapid eye movement sleep in rats. Neuropharmacology 2006;51:1-11.
339. Woodward S, Freedman RR. The thermoregulatory effects of menopausal hot flashes on sleep. Sleep 1994;17:497-501.
340. Freedman RR, Roehrs TA. Effects of REM sleep and ambient temperature on hot flash-induced sleep disturbance. Menopause 2006;13:576-83.
341. Forouhi NG, Sattar N, McKeigue PM. Relation of C-reactive protein to body fat distribution and features of the metabolic syndrome in Europeans and South Asians. Int J Obes Relat Metab Disord 2001;25:1327-31.
342. Fenkci S, Rota S, Sabir N, Sermez Y, Guclu A, Akdag B. Relationship of serum interleukin-6 and tumor necrosis factor alpha levels with abdominal fat distribution evaluated by ultrasonography in overweight or obese postmenopausal women. J Investig Med 2006;54:455-60.
343. Piche ME, Lemieux S, Weisnagel SJ, Corneau L, Nadeau A, Bergeron J. Relation of high-sensitivity C-reactive protein, interleukin-6, tumor necrosis factor-alpha, and fibrinogen to abdominal adipose tissue, blood pressure, and cholesterol and triglyceride levels in healthy postmenopausal women. Am J Cardiol 2005;96:92-7.
344. Park HS, Park JY, Yu R. Relationship of obesity and visceral adiposity with serum concentrations of CRP, TNF-alpha and IL-6. Diabetes Res Clin Pract 2005;69:29-35.
345. Cartier A, Cote M, Lemieux I, et al. Sex differences in inflammatory markers: what is the contribution of visceral adiposity? Am J Clin Nutr 2009;89:1307-14.
346. Beasley LE, Koster A, Newman AB, et al. Inflammation and race and gender differences in computerized tomography-measured adipose depots. Obesity (Silver Spring) 2009;17:1062-9.
347. Pou KM, Massaro JM, Hoffmann U, et al. Visceral and subcutaneous adipose tissue volumes are cross-sectionally related to markers of inflammation and oxidative stress: the Framingham Heart Study. Circulation 2007;116:1234-41.
348. Chiu M, Austin PC, Manuel DG, Shah BR, Tu JV. Deriving ethnic-specific BMI cutoff points for assessing diabetes risk. Diabetes Care 2011;34:1741-8.
349. Genta PR, Lorenzi-Filho G. Sleep apnoea in Asians and Caucasians: comparing apples and oranges. Eur Respir J 2011;37:1537-8; author reply 8-9.

350. Lee RW, Vasudavan S, Hui DS, et al. Differences in craniofacial structures and obesity in Caucasian and Chinese patients with obstructive sleep apnea. Sleep 2010;33:1075-80.
351. Conroy SM, Chai W, Lim U, Franke AA, Cooney RV, Maskarinec G. Leptin, adiponectin, and obesity among Caucasian and Asian women. Mediators Inflamm 2011:253580.
352. Mente A, Razak F, Blankenberg S, et al. Ethnic variation in adiponectin and leptin levels and their association with adiposity and insulin resistance. Diabetes Care 2010;33:1629-34.
353. Thomas T, Burguera B, Melton LJ, 3rd, et al. Relationship of serum leptin levels with body composition and sex steroid and insulin levels in men and women. Metabolism 2000;49:1278-84.
354. Franklin RM, Ploutz-Snyder L, Kanaley JA. Longitudinal changes in abdominal fat distribution with menopause. Metabolism 2009;58:311-5.
355. Sites CK, Brochu M, Tchernof A, Poehlman ET. Relationship between hormone replacement therapy use with body fat distribution and insulin sensitivity in obese postmenopausal women. Metabolism 2001;50:835-40.
356. Saletu M, Nosiska D, Kapfhammer G, et al. Structural and serum surrogate markers of cerebrovascular disease in obstructive sleep apnea (OSA): association of mild OSA with early atherosclerosis. J Neurol 2006;253:746-52.
357. Hayashi M, Fujimoto K, Urushibata K, Takamizawa A, Kinoshita O, Kubo K. Hypoxia-sensitive molecules may modulate the development of atherosclerosis in sleep apnoea syndrome. Respirology 2006;11:24-31.
358. Lee JW, Brancati FL, Yeh HC. Trends in the prevalence of type 2 diabetes in Asians versus whites: results from the United States National Health Interview Survey, 1997-2008. Diabetes Care 2011;34:353-7.
359. Coe CL, Love GD, Karasawa M, et al. Population differences in proinflammatory biology: Japanese have healthier profiles than Americans. Brain Behav Immun 2011;25:494-502.
360. Sothern RB, Roitman-Johnson B, Kanabrocki EL, et al. Circadian characteristics of circulating interleukin-6 in men. J Allergy Clin Immunol 1995;95:1029-35.
361. Kao PC, Shiesh SC, Wu TJ. Serum C-reactive protein as a marker for wellness assessment. Ann Clin Lab Sci 2006;36:163-9.
362. Fain JN. Release of interleukins and other inflammatory cytokines by human adipose tissue is enhanced in obesity and primarily due to the nonfat cells. Vitam Horm 2006;74:443-77.
363. Peppard PE, Ward NR, Morrell MJ. The impact of obesity on oxygen desaturation during sleep-disordered breathing. Am J Respir Crit Care Med 2009;180:788-93.
364. Lieu TA, Au D, Krishnan JA, et al. Comparative effectiveness research in lung diseases and sleep disorders: recommendations from the National Heart, Lung, and Blood Institute workshop. Am J Respir Crit Care Med 2011;184:848-56.
365. Bixler EO, Vgontzas AN, Lin HM, et al. Prevalence of sleep-disordered breathing in women: effects of gender. Am J Respir Crit Care Med 2001;163:608-13.
366. Mehra R, Xu F, Babineau DC, et al. Sleep-disordered breathing and prothrombotic biomarkers: cross-sectional results of the Cleveland Family Study. Am J Respir Crit Care Med 2010;182:826-33.
367. Tatsumi K, Saibara T. Effects of obstructive sleep apnea syndrome on hepatic steatosis and nonalcoholic steatohepatitis. Hepatol Res 2005;33:100-4.
368. Ogretmenoglu O, Suslu AE, Yucel OT, Onerci TM, Sahin A. Body fat composition: a predictive factor for obstructive sleep apnea. Laryngoscope 2005;115:1493-8.
369. Dingli K, Coleman EL, Vennelle M, et al. Evaluation of a portable device for diagnosing the sleep apnoea/hypopnoea syndrome. Eur Respir J 2003;21:253-9.
370. Iber C A-IS, Chesson A, Quan SF, editors. The AASM manual for the scoring of sleep and associated events: rules, terminology, and technical specification. 1st ed. Westchester, IL: American Academy of Sleep Medicine, 2007.
371. Smith LA, Chong DW, Vennelle M, Denvir MA, Newby DE, Douglas NJ. Diagnosis of sleep-disordered breathing in patients with chronic heart failure: evaluation of a portable limited sleep study system. J Sleep Res 2007;16:428-35.
372. Sunnergren O, Broström A, Svanborg E. Positional sensitivity as a confounder in diagnosis of severity of obstructive sleep apnea. Sleep Breath 2013;17:173-9.

373. Bruyneel M, Sanida C, Art G, et al. Sleep efficiency during sleep studies: results of a prospective study comparing home-based and in-hospital polysomnography. J Sleep Res 2011;20:201-6.
374. Levendowski D, Steward D, Woodson BT, Olmstead R, Popovic D, Westbrook P. The impact of obstructive sleep apnea variability measured in-lab versus in-home on sample size calculations. Int Arch Med 2009;2:2.
375. Wittig RM, Romaker A, Zorick FJ, Roehrs TA, Conway WA, Roth T. Night-to-night consistency of apneas during sleep. Am Rev Respir Dis 1984;129:244-6.
376. Laskey MA. Dual-energy X-ray absorptiometry and body composition. Nutrition 1996;12:45-51.
377. McCarney R, Warner J, Iliffe S, van Haselen R, Griffin M, Fisher P. The Hawthorne Effect: a randomised, controlled trial. BMC Med Res Methodol 2007;7:30.
378. Bell J. Predicting disease using genomics. Nature 2004;429:453-6.
379. Ginsburg GS, Donahue MP, Newby LK. Prospects for personalized cardiovascular medicine: the impact of genomics. Journal of the American College of Cardiology 2005;46:1615-27.

Original publications