Title: Evolution of sleep-disordered breathing and blood pressure during menopausal transition

Running head: SDB and blood pressure during menopause

Authors and affliations:

Ville Rimpilä 1,#*; Laura Lampio 1,2 #; Nea Kalleinen 1,3; Tero Vahlberg 4; Arho Virkki 5,6; Tarja Saaresranta 1,7**; Olli Polo 8**

1. Sleep Research Centre, Department of Pulmonary Diseases and Clinical Allergology, University of Turku, Turku, Finland

2. Department of Obstetrics and Gynecology, University of Helsinki and Helsinki University Hospital, Helsinki, Finland

3. Heart Center, Turku University Hospital and University of Turku, Turku , Finland

4. Department of Clinical Medicine, Biostatistics, University of Turku, Turku, Finland.

5. Department of Mathematics and Statistics, University of Turku, Turku, Finland.

6. Auria Clinical Informatics, Turku University Hospital, Turku, Finland.

7. Division of Medicine, Department of Pulmonary Diseases, Turku University Hospital, Turku, Finland

8. Bragée ME/CFS Center, Bragée Kliniker, Stockholm, Sweden

# The first two authors have contributed equally to the manuscript and have a dual co-first authorship.

** The last two authors contributed equally to the manuscript and have dual co-last authorship

*Corresponding author: Ville Rimpilä, Sleep Research Centre, Department of Pulmonary Diseases and Clinical Allergology, University of Turku, Lemminkäisenkatu 3B, FI-20520 Turku, Finland. Email: ville.rimpila@utu.fi Tel: +358405944358

Word count: 4617

Number of references: 49

Conflict of interest: None of the authors reports financial conflict of interest.

V.R., L.L., A.V.,T.S. and O.P. designed the study. V.R. and L.L. performed the study. All authors participated to the analysis of data and writing of the article.

Abstract

Purpose of this study was to investigate how blood pressure increase observed during menopausal transition is affected by sleep-disordered breathing and the menopause itself. Further, we aimed to find new sleep-disordered breathing related markers that would predict the development of hypertension. Community-dwelling 64 premenopausal women aged 45-47 years were studied. Polysomnography, serum follicle stimulating hormone, forced expiratory volume in one second and physical examination were performed at baseline and again after ten years of follow-up. Indices for sleep apnea/hypopnea and inspiratory flow-limitation were determined. Regression models were used to study the relationships between variables. Changes in apnea-hypopnea-index or serum follicle stimulating hormone were not significant for blood pressure change. Increase in morning blood pressure during the follow-up period was associated with body mass-index increase. Increase in evening blood pressure was associated with increase in inspiratory flow-limitation during non-rapid eye movement sleep. Incident hypertension during the follow-up was associated with hypopnea (median hypopnea-index 7.6/h, $p = 0.048$) during rapid eye movement sleep at baseline. Users of menopausal hormone therapy had lower rapid eye movement sleep apnea-hypopnea-index (1.6/h vs. 6.9/h, $p = 0.026$) at baseline whereas at follow-up users and non-users did not differ in any way. Progression of menopause or the use of menopausal hormone therapy had minimal effect on blood pressure in our population. The effects of inspiratory flow-limitation on blood pressure profile should be studied further.

Keywords: obstructive sleep apnea, upper airway, prolonged partial upper airway obstruction, REM, NREM

Introduction

Upper airway patency is reduced during sleep, which may result in snoring, episodes of inspiratory flow-limitation, hypopnea and apnea. Sleep-disordered breathing (SDB), especially obstructive sleep apnea (OSA) is an established risk factor for cardiovascular disease and hypertension (Campos-Rodriguez et al., 2012; Hou et al., 2018; Mokhlesi et al., 2016; Peppard et al., 2000; Sánchez-de-la-Torre et al., 2013). Even mild OSA and snoring have been connected to increased risk of hypertension (Bixler et al., 2000; Bouloukaki et al., 2020; Hou et al., 2018). Especially REM-related OSA has been connected to hypertension (Mokhlesi et al., 2014) and non-dipping of BP (Mokhlesi et al., 2015). These findings are relevant in women in whom sleep apnea is more commonly observed during REM sleep compared to men (Conwell et al., 2012; Koo et al., 2008; O'Connor et al., 2000).

Number of community-based studies have shown that SDB increases in women after menopause (Bixler et al., 2001; Heinzer et al., 2018; Mirer et al., 2017; Young et al., 2003). Our previous study beyond menopausal transition did not support the role of menopause in SDB (Kalleinen et al., 2021). Improvement of AHI with menopausal hormone therapy (MHT) is not consistent either (Bixler et al., 2001; Shahar et al., 2003; Young et al., 2003). Other possible contributing factors in the development of SDB after menopause are loss of the protective effect of progesterone (Bixler et al., 2001; Young et al., 2003), and changes in fat distribution (Polesel et al., 2015). Whether SDB increase has a pure menopausal component is still an open question (Lindberg et al., 2020).

Relationship between BP and menopause is complex. Higher BP is commonly observed in postmenopausal women in comparison to premenopausal women (Heinzer et al., 2018). However, when premenopausal and postmenopausal women are matched by age and BMI the BP difference may even disappear (Casiglia et al., 2008). The possible benefits of MHT in terms of cardiovascular prevention are limited to healthy women, if MHT is initiated before the age of 60 or within 10 years of menopause (Boardman et al., 2015; de Villiers et al., 2016).

While most studies on SDB report only AHI, partial upper airway obstruction with inspiratory flow-limitation is probably still the most common form of SDB in women (Anttalainen et al., 2007a, 2007b). The interactions between inspiratory flow-limitation and blood pressure are of interest, as flow-limitation is associated with increased respiratory efforts and carbon dioxide levels (Rimpilä et al., 2014). Therefore, our objective for this study was to investigate how the increasing blood pressure is affected by the SDB and the menopause itself. Hence, evolution of SDB from

premenopause to postmenopause was of interest as well as the role of different sleep stages and MHT. Aim was also to find threshold values for SDB that could be used for screening guidelines in midlife women.

Methods

This study is a part of the larger study "Woman 46" conducted between 2001-2017 for investigating sleep and cardiovascular risk factors in middle-aged women as they progress through menopause. The study protocol was approved by the Ethics Committee of the Hospital District of Southwest Finland.

Participants

Participants were recruited with announcements in the local newspaper seeking women aged 45 -47 for sleep and cardiovascular study. Women with known coronary heart disease, respiratory insufficiency, sleep apnea, neurological disease, liver disease, malignancies or alcohol abuse were excluded. In total, 147 women were enrolled after written informed consent was obtained. Participants were studied three times, at baseline, 6 years and 10 years after the baseline study (Kalleinen et al., 2021; Lampio et al., 2017). Data from the 6-year follow-up was not used, because perimenopause was not the focus of the present study. Premenopausal state was confirmed in 116 women, with serum follicle-stimulating hormone concentration (S-FSH) ≤ 20 IU/L. Finally, 64 women were included to the present study after excluding 52 women for different reasons (Figure 1). Weight, height, BP, S-FSH, and forced expiratory volume in one second (FEV1) were measured and polysomnography (PSG) performed at each study visit, and participants completed a battery of questionnaires. At baseline, all women were premenopausal and none used MHT. At follow-up, 13 women used MHT based on choice of them and their gynecologist and from one woman the data was missing. Anti-hypertensive medication was used by 4 women at baseline and by 14 women at follow-up.

[Figure 1 should be placed here]

Serum follicle-stimulating hormone measurement

S-FSH was measured as previously described in the morning prior to PSG (Kalleinen et al., 2021). Time-resolved immunofluorometric assay (AutoDELFIA; PerkinElmer Life and Analytical Sciences, Wallac Oy, Turku, Finland) was used to analyse the samples using direct sandwich technique.

Polysomnography

Sleep studies were carried out at the Turku Sleep Research Centre during baseline and follow-up using the same device. PSGs comprised of four channels of electroencephalograms (EEG; C3/A2, C4/A1, O1/A2 and O2/A1), two channels of electrooculograms (EOG) and electromyogram (EMG) from the mandible, nasal flow with prongs connected to pressure transducer and arterial oxyhemoglobin saturation (SpO2) with finger pulse oximeter (integrated Nonin oximeter) recorded with Somnologica system (Embla, MedcareFlaga hf, Reykjavik, Iceland). Sleep was visually scored using the Rechtschaffen – Kales criteria (Rechtschaffen & Kales, 1968). Arousals were scored using the American Sleep Disorders Association criteria ('EEG Arousals', 1992).

Episodes of apnea and hypopnea were scored according to AASM guideline (Berry et al., 2012) and AHI, apnea-index (AI), hypopnea-index (HI), and oxygen desaturation-index for 3% decrease (ODI_3) was determined for each participant. Periods of insufficient flow signal were marked and omitted from AHI, HI, AI and inspiratory flow-limitation index (IFL) calculations in order to compare the baseline and follow-up. At baseline thoraco-abdominal belts were not used and therefore apnea classification was not possible. Scoring for IFL was done visually by labelling the breaths as normal with smooth, round appearance or flow-limited with flattened, scooped, spiked appearance with or without high frequency oscillations as a marker for snoring. Minimal IFL event length was one minute to distinguish flow-limitation events from hypopnea events and to ensure the consistency of flow limitation. Periods of breathing where flow-limited breaths and normal breaths alternated were not included to the analysis as this behaviour indicates that the airway obstruction can be resolved with minimal effort and varies greatly between breaths. In addition, periods of breathing instability with waxing and waning pattern were not included as this would lead to mixture of central and obstructive events because the airway may collapse due to the decrease in ventilatory drive. Figure 2 shows a representative IFL event. All of the aforementioned indices were determined separately for non-rapid eye movement (NREM) sleep and rapid eye movement (REM) sleep as well as for the whole night. Superscripts; ^{NREM} and ^{REM} are used with SDB-indices when necessary.

[Figure 2 here]

Blood pressure and FEV1 measurements

Blood pressure was measured in the evening before the sleep study and again in the morning (Model M3, Omron, Japan). On each occasion the measurement was taken in seated position three times and the average values were used in the study. Participants were considered to have hypertension if systolic pressure was ≥ 140 mmHg and/or diastolic was ≥ 90 mmHg, or the participant was using anti-hypertensive medication. Abbreviations Sys_E Sys_M, Dia_E and Dia_M are used to indicate systolic/diastolic BP and the time of day (evening/morning) the measurement was made.

Surrogate of lung function was determined with FEV1. Measurement was performed three times and the average result was used in the study (One FLOW tester ATS 94, STI Medical).

Statistical analyses

Participant characteristics between the baseline and 10-year follow-up were studied with paired samples T-test or Wilcoxon signed rank test. Characteristics of SDB between baseline and 10-year follow-up were studied with Wilcoxon signed rank test. Variables that measure the difference between the baseline study and 10-year follow-up study have delta (Δ) symbol in the variable name. Progression of SDB during the follow-up was studied using Spearman correlation between baseline and 10-year visits using the SDB-indices. Pearson and Spearman correlations were used to determine how change in body habitus (BMI, weight, waist, neck, waist-to-hip ratio), BP and FEV1 were related to ΔIFL during follow-up. Linear regression was used to study the effects of body habitus, MHT, FEV1 and S-FSH on all of the SDB variables; ΔIFL (ΔIFL, ΔIFL^{NREM}, ΔIFL^{REM}), ΔAHI (ΔAHI, ΔAHI^{NREM}, ΔAHI^{REM}), ΔHI (ΔHI, ΔHI^{NREM}, ΔHI^{REM}). Selection of body habitus variable used in the model was based on highest correlation. Two models were built for each dependent variable; full model included all of the parameters and the second model used backward algorithm to detect the significant independent variables in model (exclusion criteria $p > 0.1$). The normality of residuals was checked. Effects of SDB on BP were studied using linear regression. Change in blood pressure (ΔBP) during the follow-up was used as a dependent variable and ΔAHI (or ΔAI, or ΔHI) and ΔIFL were entered as explanatory variables in the unadjusted model. In the adjusted model, ΔBMI and ΔS-FSH were entered as continuous variables and use of MHT as a dichotomic variable. For incident HTN during the follow-up, women without HTN at baseline were stratified to two groups according to HTN status at follow-up and comparisons between the SDB variables, FEV1, S-FSH and body habitus were performed with Mann-Whitney U test using the values from baseline, 10-year follow-up and change during the follow-up (delta). Significant parameters from Mann-Whitney U test were used to construct a logistic regression model for HTN. The effects of SDB, lung function (FEV1), hormonal status (S-FSH), BMI and BP between MHT users and non-users were studied with Mann-Whitney U test. The p – value < 0.05 was considered statistically significant. The statistical analyses were performed with SPSS 26 (IBM SPSS Statistics for Windows, Version 26.0. IBM Corp., Armonk, NY).

Results

The participants characteristics are presented in Table 1. At the age of 46 years both the systolic and diastolic blood pressures were higher in the evening compared to those in the morning. The median SDB events were low at baseline, in line with the purpose to recruit a healthy population without previously diagnosed diseases. Inspiratory flow-limitation during NREM was the most frequent form

of SDB. Episodes of apnea/hypopnea were most common during REM. During the 10-year follow-up, the incidence of AHI ≥ 5 increased from 12 to 40 subjects, AHI ≥ 15 from 2 to 13 and AHI ≥ 30 from 1 to 7 (Table 2).

[Tables 1 and 2 here]

SDB events between the corresponding baseline and 10-year follow-up indices were positively correlated (Table 3). Although relatively infrequent at baseline, both IFL^{NREM} and AHI^{REM} increase during follow-up independently (r = -0,08, p = 0,532). A negative correlation between ΔIFL^{NREM} and ΔAHI^{NREM} (r = -0.298, p = 0.017) suggests a ceiling effect during NREM sleep (additional correlations in supplement).

[table 3 here]

In a full model multivariate regression analysis only ΔHI was explained by ΔS-FSH (β = -0.058, p = 0.041). This was unexpected and further analysis revealed that there was negative correlation between ΔS-FSH and neck circumference. Backward models showed that ΔAHI (β = 1.223, p = 0.036) and ΔAHI^{REM} (β = 2.315, p = 0.030) were explained by ΔBMI. ΔIFL^{NREM} could not be explained by any single variable (see supplement table 1 for details).

Change in body habitus had a very strong correlation with morning ΔBP. BMI (ΔBMI vs. ΔSys_M, r_s = 0.712, p < 0.001) or weight were the most important contributors, followed by change in waist circumference. Change in neck circumference or WHR did not affect ΔBP. While ΔIFL^{NREM} did not correlate with body habitus, a correlation was found between ΔIFL^{NREM} and evening ΔBP. Correlation was stronger for diastolic (ΔIFL^{NREM} vs. ΔDia_E, r= 0.468, p < 0.001) than systolic blood pressure (ΔIFL^{NREM} vs. ΔSys_E, r = 0.362 p = 0.004). None of the ΔIFL parameters correlated with morning ΔBP variables.

Unadjusted linear regression model showed that ΔIFL was associated with ΔBP in the evening (Figure 3) but not in the morning (Table 4). The effect of ΔIFL^{NREM} persisted after adjusting the model for ΔBMI, ΔS-FSH and MHT use. In the adjusted model, increase in ΔIFL^{NREM} was associated with ΔBP (β = 0.266 for Sys_E), and β = 0.240 for Dia_E). This means that 4% - unit increase (approx. 11 minutes) in IFL during NREM sleep would lead to one mmHg increase in evening BP after ten years. In the adjusted models, ΔBMI explains most of the variation in the morning BP, especially the ΔSys_M as shown with prominent increase in the adjusted R^2-values (Table 4). Regression models that used ΔAI or ΔHI as an independent factor instead of ΔAHI were not significant (data not shown). Distribution of ΔAHI and ΔBMI were skewed. Replacing the ΔAHI with logΔAHI, reduced the adjusted R^2 for the evening ΔBP

(0.117/0.191 vs. 0.113/0.189) but increased the adjusted R^2 for the morning ΔBP (0.321/0.175 vs. 0.340/0.205). Transformation failed to normalize the ΔBMI distribution.

[Table 4 and figure 3 here]

Incident hypertension during the follow-up was studied in the subset of initially nonhypertensive (41 out of 64) participants. These were stratified to hypertensive (N = 14) and non-hypertensive (N = 27) according to incident HTN at the follow-up. Baseline median ODI_3 (3.3 vs. 1.1, p = 0.008), ODI_3^{NREM} (1.8 vs. 0.6, p = 0.03), ODI_3^{REM} (6.5 vs. 2.5, p = 0.050) and median HI^{REM} (7.6 vs. 2.5, p = 0.048) were higher in the hypertensive group vs. non-hypertensive. Anthropometric values (BMI, weight, neck or hip or waist circumference and waist to hip ratio) showed no difference. Delta or 10-year follow-up values did not show difference between hypertensive and non-hypertensive participants in any of the tested variables. Post-hoc logistic regression model for HTN, which included ODI_3 and HI^{REM}, suggested a role for HI^{REM} in the development of HTN (OR 1.235, p = 0.039), but not for ODI_3 (OR 0.841, p = 0.131).

During the study, 13 women had started and continued to use MHT at follow-up. Users and non-users did not differ in any tested variable at the point of follow-up. However, differences were found at baseline. Users had had smaller median neck circumference (33.0 cm vs. 34.8, p = 0.026), lower AHI^{REM} and HI^{REM} (1.6/h vs. 6.9/h, p = 0.026 and 0.8/h vs. 4.9/h, p = 0.039, respectively) and lower evening BP (Sys_E 114 vs 125.5 mmHg, p = 0.01 and Dia_E 76 vs. 85 mmHg, p = 0.001). However, compared to baseline, MHT users had a greater ΔDia_E (6.5 mmHg vs. 1.0 mmHg, p = 0.034).

The evolution of body composition, SDB and blood pressures was further tested in subgroups more homogenic in terms of IFL. 14 women with IFL below 10 % of TST at both baseline and at follow-up were compared with, 14 women, who presented with IFL more than 20 % of TST at both occasions. It was found that women in the IFL10 group had lower BMI at baseline (24.8 vs. 28.4, p = 0.035), smaller ΔAHI^{REM} (0.1 vs. 18.6, p = 0.002) and smaller waist-to-hip ratio (0.87 vs. 0.91, p = 0.009) at follow-up (see supplement text and Table 2 for details).

Discussion

While inspiratory flow-limitation (IFL) during NREM sleep is recognized as common in women, few studies have explored its impact on health outcomes, including blood pressure. We had the possibility to investigate natural evolution of IFL and blood pressure in a 10-year follow-up study in women during the period of menopausal transition. The major novel finding was while increasing BMI was linked to increasing morning blood pressure, increase of IFL over time was associated with

higher evening blood pressure. Presence of hypopnea during REM sleep at premenopause was identified as a risk factor for incident hypertension during 10 years.

Development of SDB during the 10-year follow-up

There was an overall increase in SDB, both in apneic/hypopneic events as well as flow-limitation during the follow-up. Negative correlation between ΔIFL and ΔAI/AHI supports inverse proportionality between the event types so that increased IFL may not lead to increased AHI or vice versa. This seems contradictory, but one possible explanation for this finding is that the women in our study were at different phases of SDB development. Those who were in the early phase, were more likely to develop more flow-limitation (and less apnea), whereas those with more advanced condition were more likely to develop more apnea. If IFL and episodes of hypopnea and apnea were to develop in parallel as suggested by Table 2, a positive correlation would be expected between the delta-variables of these different SDB event types. Since this was not observed, gradual development of SDB through different stages is supported. Sleep stage had a major role for SDB type in our study; the increase in AHI and HI occurred mostly during REM sleep, which is in line with previous studies that show REM predominance of SDB in women (O'Connor et al., 2000). On the other hand, increase in IFL occurred during NREM sleep, which is also in line with previous findings (Anttalainen et al., 2007b). Increased ΔAHI associated with weight gain as expected, whereas ΔIFL was not affected with any single independent parameter, underlining the importance of scoring this variable. Aforementioned finding is in agreement with study by Bixler (Bixler et al., 2000), which showed that snoring had no interaction with BMI but two more severe categories of SDB did. Our study showed that SDB increased during menopausal transition with NREM sleep characterized with IFL and REM sleep with episodes of hypopnea and apnea.

Sleep-disordered breathing, BMI and blood pressure

Linear regression models confirmed that the effects of ΔAHI, ΔIFL and ΔBMI on ΔBP vary greatly according to the sleep stage and time of the day. Unexpectedly, ΔBMI was not significant for evening BP change in NREM models and the overall effect on evening BP was clearly smaller than for morning BP (Table 4.). Our results cast further light on how IFL and snoring increase have on BP, extending the previous observations regarding the relationship between hypertension and self-reported snoring frequency (Hu et al., 1999) and low levels (0 to 4.9/h) of AHI (Peppard et al., 2000). Developing IFL seems to gradually increase BP as indicated by our results, and the phenomenon is also supported by the 24 h BP profiles showed by Young (Young et al., 1996). Using the log-transformed ΔAHI variable in the regression models had an opposite effect on evening and morning BP, which could be interpreted as a relatively bigger role for episodes of apnea and hypopnea in the

morning BP. This could be related to our relatively healthy sample of women (skewed distribution of AHI towards low values) and the fact that most new events of apnea and hypopnea developed in REM sleep. However, somewhat similar observation was made by Bixler et al. who did not find an association between hypertension and mild to severe SDB in premenopausal and postmenopausal women with MHT (Bixler et al., 2000). We did not detect any significant effect for ΔS-FSH and MHT status.

It is unclear why the increase in IFL leads to increase specifically in the evening BP, while AHI shows a very minor effect. The minor effect of AHI can be explained by only minor increase of apnea/hypopnea events during the follow-up period. But one would expect that the major effects of any SDB would be seen at morning and this was the trend for apnea/hypopnea events (Table 4). It cannot be excluded that IFL also has a morning effect, but this effect is masked by BMI and AHI. Prolonged periods of partial upper airway obstruction are characterized by increased inspiratory time, decreased SaO_2, increased levels of end-tidal CO_2 and increased esophageal pressure, as measured during CPAP-titration (Calero et al., 2006). Experimental studies show that hypercapnic exposure leads to immediate increased ventilation and further increase over time, and the effect is augmented with hypoxia (Griffin et al., 2012). Furthermore, hypoxia produces long-lasting sympathetic activity that is sustained even though ventilation returns to baseline (Xie et al., 2001). Yet another study showed that a phenomenon where ventilation remains elevated after the hypoxic stimulus has been removed, was present only in snorers during sleep (M. A. Babcock & Badr, 1998). Later study revealed that minute ventilation during the post-stimulus recovery period was positively correlated with the percentage of IFL (M. Babcock et al., 2003). Based on these findings it seems plausible that IFL could have long-term stimulatory effect. In addition, there was not significant increase in Dia_E on group level during the follow-up. This also supports the idea that Dia_E increases when IFL increases.

Increasing AHI has been associated with generally increased BP (Wang et al., 2016), and early studies suggested that there could be a shift from higher evening BP towards higher morning BP as the SDB progresses (Hoffstein & Mateika, 1992). Several studies have shown that AHI associates with morning BP (Han et al., 2019; Lee & Jeong, 2014; Mokros et al., 2017; Wang et al., 2016). The role of gender is however unclear, as some of these recent studies did not find an association between AHI and BP in women (Han et al., 2019; Lee & Jeong, 2014). In non-hypertensive patients, high AHI associated with elevated morning diastolic BP, independent of obesity, age or gender (Mokros et al., 2017). Although the AHI increase was clear in our study, it was not a significant variable for BP increase. In contrast, incident HTN during the follow-up was connected to increased HI^{REM} at baseline, but not with any of the follow-up or delta variables. Unexpectedly, even BMI was not

significantly different between the groups (HTN, non-HTN), which might be explained by our relatively small population. Half of those who developed HTN during the follow up had HI^{REM} of 7.6/h or more at baseline, so this value could serve as a simple threshold or screening value to pinpoint those at risk at the age of 46. Although IFL is not synonymous to snoring, post-hoc results showed that having IFL above 20% of total sleep time continuously is a marker for undesirable condition. This is in line with other population studies showing that REM specific obstructive sleep apnea (OSA) is associated with "non-dipping" of BP (Mokhlesi et al., 2015) and early signs of atherosclerosis in women (Ljunggren et al., 2018). It was also shown previously that women with partial upper airway obstruction were less likely to use reimbursed medication for hypertension, compared to those with OSA and adjusted for BMI and age (Anttalainen et al., 2010). Our results support the early detection of SDB and the association of REM specific SDB with the evolution of HTN and encourage more detailed analysis of SDB. Whether long-term exposure to IFL is directly responsible for the increase in evening BP in women or is a surrogate for some underlying phenomenon remains to be studied in detail.

Role of S-FSH and MHT for SDB and BP during menopausal transition

S-FSH turned out not to be a significant parameter for BP change. Neither did we observe an effect for S-FSH in incident HTN, implying that the progression of menopause per se does not seem to have an effect on blood pressure or HTN. Nevertheless, our results indicate that smaller increase in S-FSH during the follow-up was associated with greater increase in HI which is counter-intuitive, and not in agreement with previous study by Mirer, which showed consistent increase of AHI from pre- to peri- to post-menopause (Mirer et al., 2017). It is possible that our finding relates to negative correlation between S-FSH and neck circumference. Also, the effects of MHT were marginal. The only significant finding was that MHT users had lower BP at baseline, but the difference diminished during the follow-up. Therefore, according to our study, MHT did not bring any benefit in terms of BP. A recent study showed lower AHI (10.2/h vs. 12.4/h) in post-menopausal MHT-users vs. non-users (Heinzer et al., 2018), but the difference was quite small and population much larger, which could explain the different outcome compared to our study. Our results indicate a minor role of the physiological events occurring during menopausal transition for the development of SDB or HTN.

Strengths and limitations

Our study has inherent strengths and also relevant limitations that need to be considered when interpreting the results. A particular strength of our study is the prospective design with long follow-up period which allowed us to follow the progression of SDB and menopause. Women in our study were recruited from the community, and major diseases were excluded, which improves

generalizability of our results. Unfortunately, our sample size turned out to be smaller than expected due to loss to follow-up and issues with data. On the other hand, evaluation of IFL requires good quality data and we did not want to compromise this aspect. Manual scoring is prone to errors and with only one scorer for SDB (VR), the variability between scorers cannot be determined. In order to avoid false positive breaths to be included to the study, only longer periods of IFL were included to ensure that presence of the IFL was consistent. Correlation between IFL and BP in this relatively small sample adds to our confidence that the selected approach was valid. It is acknowledged that one minute cut-off time for IFL was arbitrary, but based on the experience that respiratory events commonly addressed as hypopneas are usually shorter than the chosen limit. 24-hour ambulatory blood pressure measurement would have given more detailed view to the acute effects of SDB on BP as well as the BP profile during sleep, but unfortunately the measurement was not available. This study can be criticized for not being adjusted for age, but since all the women were practically same age at baseline and follow-up, the effect of age was similar between participants. Number of MHT-users was limited (N = 13) so the statistical power in this regard was reduced. Therefore, conclusions drawn from this sub-population must be made with caution.

Future perspectives

Our findings support the idea that the amount of IFL should be controlled for in the future studies aiming to determine the relationship between SDB and hypertension because: 1) the amount of IFL varies greatly between individuals and was not directly related to the number of apneic/hypopneic events and 2) even small increases in the duration of flow-limitation associated with evening BP increase over time. The link between SDB events and physiological load to the cardiovascular system is still incompletely understood. We speculate that by including IFL to the analysis, the role of SDB spectrum in the pathogenesis of cardiovascular disease can be understood in more detail, allowing efficient and timely prevention. The relationship between various types of SDB and ambulatory BP, as well as nocturnal dipping of BP, should be further explored in order to determine SDB specific effects. While the role of menopause for blood pressure increase or HTN was not supported by our study, the effect of IFL on evening BP was a novel finding in the dynamics between SDB and BP that warrants further investigation.

Conclusions

SDB in women is characterized by sleep apnea during REM and IFL during NREM sleep. These findings increase during 10-year follow-up over menopausal transition. Initially high HI during REM sleep was associated with incident hypertension at 10-year follow-up. While increase in BMI was associated with increase in morning blood pressures, increase in IFL was linked with higher evening

blood pressures. This finding underlines the importance of IFL in the evolution of blood pressure over time in relatively healthy female population during menopausal transition.

Acknowledgements

Study nurse Marjo Sunnari is acknowledged for her valuable help in the data management for the study. Study was supported by grants from Tampere Tuberculosis Foundation, Foundation of the Finnish Anti-Tuberculosis Association and Finnish Research Foundation of Pulmonary Diseases, Väinö and Laina Kivi Foundation, and Governmental VTR Grant of the Turku University Hospital.

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Figure legends

Figure 1. Flowchart of the study. S-FSH, serum follicle-stimulating hormone; IU/L, international units per litre.

Figure 2. IFL event from S2 sleep. Frame from PSG shows hypnogram on top and the selected area (blue rectangle) at the bottom. Signals from the top down are: EOG, EEG, nasal flow and SpO_2. Black bar on the flow channel shows the IFL event. Frame width is six minutes and the IFL event length is 157 seconds. Reduction of the peak flow is seen at the beginning of the event and the event terminates with arousal. SpO_2 stays very stable at 97% during the IFL event. EEG, electroencephalogram; EOG, electro-oculogram; IFL, inspiratory flow-limitation, PSG, polysomnography; SpO2, Arterial oxyhemoglobin saturation from pulse oximetry.

Figure 3. Scatter plot between evening diastolic BP change and IFL change during the 10 years of follow-up. Regression line and equation from unadjusted model. BP, blood pressure; IFL, inspiratory flow-limitation.

Supplement for: Evolution of sleep-disordered breathing during menopause

Correlations

Negative correlation between SDB delta variables:

for NREM, ΔIFL^{NREM} vs. ΔAHI^{NREM} ($r = -0.298$, $p = 0.017$)

and REM, ΔIFL^{REM} vs. ΔAI^{REM} ($r_s = -0.286$, $p = 0.023$)

Increase in correlation between HI and IFL:

for HI vs. IFL (from baseline $r_s = 0.042$, $p = 0.742$ to $r_s = 0.240$, $p = 0.056$),

HI^{NREM} vs. IFL^{NREM} (from baseline $r_s = -0.081$, $p = 0.526$ to $r_s = 0.194$, $p = 0.125$).

Correlations between ΔIFL and ΔBP:

ΔIFL vs. Sys_E/Dia_E, $r = 0.343/0.444$, $p = 0.006/<0.001$ and

ΔIFL^{NREM} vs. Sys_E/Dia_E, $r = 0.362/0.468$, $p = 0.004/<0.001$.

Correlations between ΔBMI/Δwaist and ΔBP:

ΔBMI vs. Sys_M, $r_s = 0.712$, $p < 0.001$ and ΔBMI vs. Dia_M, $r_s = 0.612$, $p < 0.001$

Δwaist vs. ΔSys_M, $r_s = 0.422$, $p < 0.023$ and Δwaist vs. ΔDia_M, $r_s = 0.410$, $p < 0.027$

Regression models for ΔIFL, ΔHI and ΔAHI

Supplement table 1.

Model	dependent	p*	R^2	Variable**	β (95%, CI)	p#
Full	ΔAHI	**0.046**	0.109	ΔFSH	-0.89 (-0.184 - 0.005)	0.064
	ΔHI	**0.039**	0.116	**ΔFSH**	-0.058 (-0.114 - (-0.002))	**0.041**
	ΔIFL	0.297	0.020	ΔFEV	- 7.587 (-18.511 - 3.337)	0.169
	ΔAHI^{NREM}	0.066	0.093	ΔFSH	-0.084 (-0.172 - 0.003)	0.059
	ΔHI^{NREM}	0.084	0.082	ΔFSH	-0.059 (-0.119 - 0.000)	0.051
	ΔIFL^{NREM}	0.166	0.050	ΔFEV	-10.857 (-22.737 - 1.023)	0.072
	ΔAHI^{REM}	0.139	0.059	ΔBMI	2.057 (-0.075 - 4.189)	0.058
	ΔHI^{REM}	0.511	-0.013	ΔBMI	0.853 (-0.464 - 2.170)	0.199
	ΔIFL^{REM}	0.757	-0.042	ΔFSH	0.122 (-0.075 - 0.318)	0.220
Backward	ΔAHI	**0.023**	0.104	**ΔBMI**	1.223 (0.084 - 2.361)	**0.036**
	ΔHI	**0.020**	0.108	ΔBMI	0.670 (-0.002 - 1.342)	0.051
	ΔIFL	0.091	0.036	ΔFEV	-9.094 (-19.694 - 1.505)	0.091
	ΔAHI^{NREM}	**0.039**	0.085	ΔBMI	0.971 (-0.082 - 2.023)	0.070
	ΔHI^{NREM}	0.062	0.048	ΔFSH	-0.057 (-0.117 - 0.003)	0.062
	ΔIFL^{NREM}	**0.044**	0.081	ΔFEV	-11.161 (-22.769 - 0.446)	0.059
	ΔAHI^{REM}	**0.030**	0.070	**ΔBMI**	2.315 (0.238 - 4.393)	**0.030**
	ΔHI^{REM}	-	-	-	-	-
	ΔIFL^{REM}	-	-	-	-	-

All models included ΔFEV, ΔFSH and MHT variables. In addition, ΔBMI was included to ΔAHI and ΔHI models, ΔWHR to IFL models and ΔNeck to ΔIFL^{NREM} and ΔIFL^{REM} models. p*, model p-value; ** best explanatory variable; p#, variable p-value.

Coefficients for only significant ΔIFL model:

ΔIFL^{NREM}: β (ΔFEV1) = -11.2 (CI, - 22.8 – 0.45), p = 0.059) and β (ΔNeck) = 1.93 (CI, -0.37 – 4.22), p = 0.098, adj. R^2 = 0.081, p = 0.044.

Adjusted odds ratios for HTN post-hoc logistic regression model:

OR HI^{REM} = 1.235, 95% CI: 1.011 – 1.509, p = 0.039,

OR ODI_3 = 0.841, 95% CI: 0.672 – 1.053, p = 0.131.

Post-hoc IFL10 and IFL20

In order to better understand the relation of IFL with other factors, post -hoc tests were performed. Women were divided into two groups; women with IFL below 10 (IFL10) and women with IFL above 20 (IFL20). This rule was applied for both baseline and follow-up in order to create groups with constantly low vs. high IFL. Thus, two groups of 14 women were formed. The ILF20 group had consistently worse indices compared to ILF10 group. At baseline the body habitus was less favorable in the IFL20 group, during the follow-up IFL20 group had greater increase in SDB and at the end of the study, IFL20 group had higher HI^{REM}, WHR, SWS (min.) and tendency for higher BP. Detailed results are presented in the table below. The following table 2 presents the parameters with significant difference between the ILF10 and IFL20 groups at baseline, during the follow-up (delta), and at the end of the study **and** the (non-significant) difference in BP at the end of the study.

Supplement Table 2.

		Group		
time	variable	IFL10 (IQR)	IFL20 (IQR)	p
baseline	BMI	24.8 (23.4 - 26.4)	28.4 (24.8 - 32.9)	0.035
	waist	81.8 (79.9 - 87.0)	90.8 (85.5 - 104.8)	0.027
	hip	98.0 (94.8 - 106.8)	109.5 (101.5 - 119.8)	0.050
	S1 (min.)	21.0 (9.5 - 28.0)	33.3 (16.9 - 42.4	0.027
	S1 (%)	5.6 (2.6 - 7.9)	9.0 (7.4 - 11.1)	0.044
delta	HI	0.2 (-0.9 - 3.7)	5.0 (1.7 - 8.7)	0.011
	HI^{NREM}	0 (-0.9 - 3.8)	3.1 (0.5 - 6.8)	0.039
	HI^{REM}	0 (-2.8 - 1.2)	8.5 (2.6 - 26.5)	0.002
	AHI^{REM}	0.1 (-5.5 - 8.5)	18.6 (6.4 - 39.7)	0.002
Follow-up	WHR	0.87 (0.84 - 0.90)	0.91 (0.90 - 0.93)	0.009
	HI^{REM}	2.4 (0 - 8.4)	15.0 (4.2 - 36.9)	0.014
	SWS (min.)	75.0 (65.5 - 99.9)	101.0 (81.9 - 122.9)	0.027
	Sys_E	126 (119 - 140)	141 (127 - 158)	0.076
	Dia_E	84 (80 - 91)	92 (86 - 97)	0.054
	Sys_M	116 (107 - 139)	128 (116 - 140)	0.239
	Dia_M	76 (73 - 88)	87 (80 - 92)	0.155

Table 1. Participant characteristics at baseline and follow-up (N = 64)

	Baseline (SD)	10-year follow-up (SD)	Difference	p
Age, y	46 (0.9)	56.8 (0.9)	10.8 (0.8)	< 0.001
S-FSH, IU/L	7.4 (3.5)	66.7 (30.0)	59.3 (29.9)	< 0.001
BMI, kg/m^2	26.4 (4.7)	28.9 (6.1)	2.49 (2.47)	< 0.001
Neck, cm	*34.0 (3.5)*	36.3 (3.2)	1.4 (1.9)	*< 0.001*
WHR	0.84 (0.07)	0.90 (0.06)	0.06 (0.07)	< 0.001
FEV1, L/s *	2.78 (0.38)	2.62 (0.41)	- 0.18 (0.38)	0.001
Blood pressure, mmHg				
Sys_E	125.3 (13.1)#	134.2 (18.6)#	8.7 (15.0)	< 0.001
Dia_E	84.4 (8.9)#	86.7 (9.7)#	2.1 (9.7)	0.096
Sys_M**	108.7 (12.9)	122.11 (17.4)	13.1 (14.3)	< 0.001
Dia_M**	69.1 (9.1)	82.32 (10.3)	13.2 (8.3)	< 0.001

Values presented with means and SD, except for neck circumference shown with median and interquartile range. *, N = 55; **, N = 29; #, evening BP vs. morning BP p < 0.001; S-FSH, serum follicle-stimulating hormone; IU/L, international units per litre; WHR, waist-to-hip ratio; FEV1, forced expiratory volume in one second; Sys, systolic; Dia, diastolic; $_E$, evening; $_M$, morning.

Table 2. Sleep-disordered breathing characteristics at baseline and follow-up (N=64)

Variable	Baseline	10-year follow-up	Difference	p
AHI/h	*2.5 (3.6)*	*5.8 (12.2)*	*3.6 (9.3)*	*<0.001*
AI/h	*0.3 (1.1)*	*1.9 (4.0)*	*0.8 (3.9)*	*<0.001*
HI/h	*1.9 (3.2)*	*3.5 (7.67)*	*1.5 (6.1)*	*<0.001*
ODI_3/h	*2.2 (3.5)*	*5.2 (10.1)*	*2.9 (7.3)*	*<0.001*
IFL, % (TST)	*12.2 (20.24)*	*18.6 (22.7)*	*2.7 (20.4)*	*0.032*
N, (AHI > 5)	12 (18.8 %)	40 (62.5 %)		
NREM				
AHI/h	*1.4 (2.8)*	*3.7 (8.6)*	*1.8 (7.9)*	*<0.001*
AI/h	*0.2 (0.9)*	*0.7 (2.3)*	*0.4 (4.5)*	*<0.001*
HI/h	*0.9 (2.6)*	*2.5 (5.0)*	*1.3 (4.5)*	*<0.001*
ODI_3/h	*1.6 (2.7)*	*3.1 (9.2)*	*1.1 (7.1)*	*<0.001*
IFL, % (TST)	*13.9 (23.1)*	*20.7 (25.7)*	*3.6 (24.2)*	*0.014*
REM				
AHI/h	*5.6 (12.2)*	*14.7 (36.0)*	*8.3 (28.7)*	*<0.001*
AI/h	*1.1 (2.1)*	*4.8 (16.0)*	*3.2 (12.9)*	*<0.001*
HI/h	*4.0 (8.5)*	*6.7 (13.5)*	*2.0 (10.1)*	*0.013*
ODI_3/h	*3.2 (7.1)*	*13.9 (23.4)*	*7.9 (18.6)*	*<0.001*
IFL, % (TST)	*1.2 (10.81)*	*0.0 (8.5)*	*0.0 (8.1)*	*0.237*
N, (AHI > 5)	34 (53.1 %)	48 (75.0 %)		

Values presented with medians and (interquartile range) for full night and separately for NREM and REM sleep. AHI, apnea-hypopnea-index; AI, apnea-index; HI, hypopnea-index; IFL, inspiratory flow-limitation-index; ODI_3, oxygen desaturation index of 3%; TST, total sleep time.

Table 3. Correlations between the baseline and follow-up indices for sleep-disordered breathing

Variable	r_s	p
AI/h	0.259	0.039
HI/h	0.475	<0.001
AHI/h	0.443	<0.001
ODI_3/h	0.596	<0.001
IFL, % (TST)	0.504	<0.001
NREM		
AI/h	0.262	0.037
HI/h	0.491	<0.001
AHI/h	0.482	<0.001
ODI_3/h	0.489	<0.001
IFL, % (TST)	0.496	<0.001
REM		
AI/h	0.289	0.022
HI/h	0.453	<0.001
AHI/h	0.437	<0.001
ODI_3/h	0.557	<0.001
IFL, % (TST)	0.211	0.097

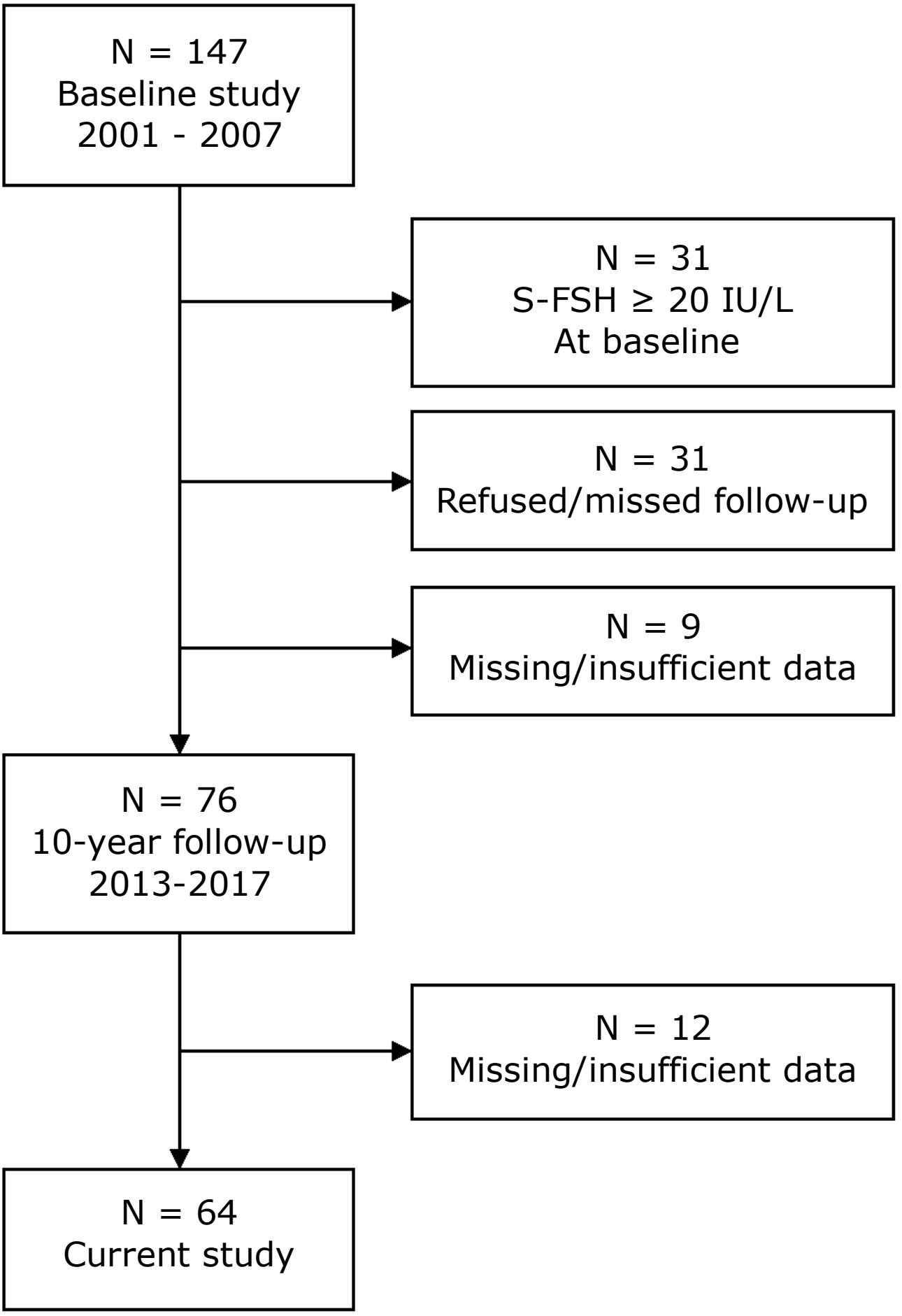
AHI, apnea-hypopnea-index; AI, apnea-index; HI, hypopnea-index; IFL, inspiratory flow-limitation-index; ODI_3, oxygen desaturation index of 3%; TST, total sleep time.

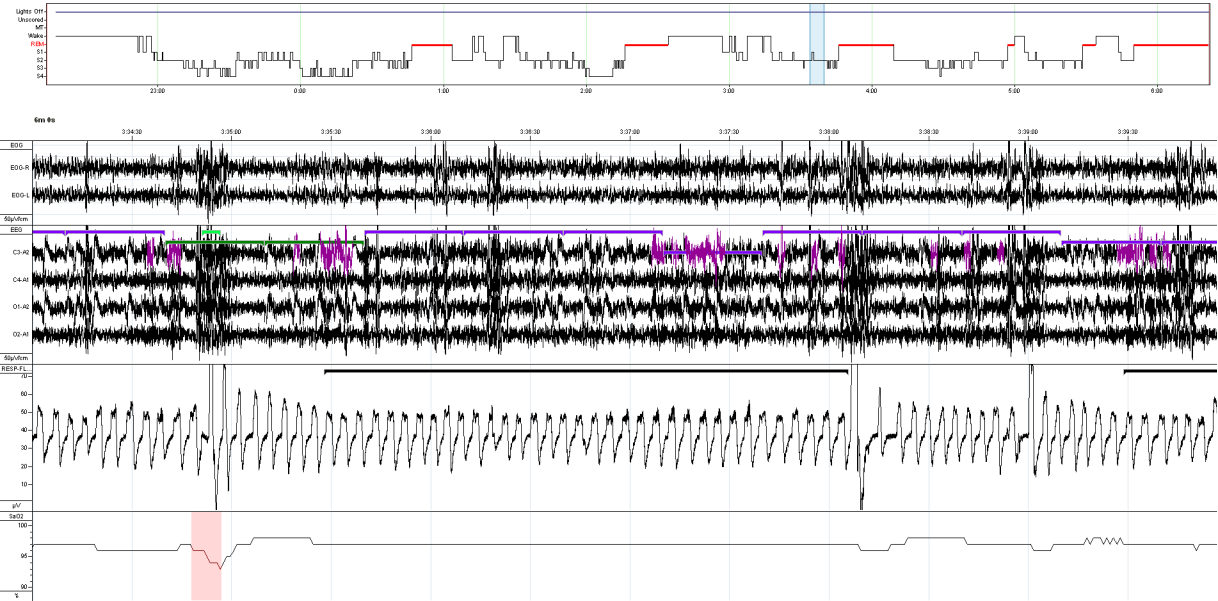
Table 4. The effect of sleep-disordered breathing on blood pressure. Stratified by sleep stage.

	ΔAHI				ΔIFL				Unadjusted		Adjusted	
	β	(95% CI)	p	adj. p	β	(95 % CI)	p	adj. p	p	adj. R^2	p	adj. R^2
Full night												
Evening BP												
ΔSys_E	- 0.043	(- 0.392; 0.306)	0.807	0.519	0.335	(0.086; 0.584)	0.009	0.043	0.023	0.089	0.036#	0.117
ΔDia_E	- 0.034	(- 0.251; 0.183)	0.754	0.703	0.282	(0.128; 0.437)	0.001	0.003	0.001	0.172	0.005	0.191
Morning BP**												
ΔSys_M	0.335	(- 0.198; 0.867)	0.208	0.783	0.140	(- 0.240; 0.521)	0.455	0.962	0.404	- 0.004	0.017#	0.321
ΔDia_M	0.184	(- 0.124; 0.491)	0.230	0.698	0.122	(- 0.098; 0.341)	0.264	0.597	0.338	0.009	0.098#	0.175
NREM												
Evening BP												
ΔSys_E	- 0.028	(- 0.413; 0.356)	0.883	0.665	0.317	(0.093; 0.541)	0.006	0.028	0.014	0.103	0.027	0.129
ΔDia_E	- 0.028	(- 0.265; 0.210)	0.817	0.824	0.266	(0.128; 0.404)	<0.001	0.002	0.001	0.193	0.003	0.211
Morning BP **												
ΔSys_M	0.321	(- 0.276; 0.917)	0.279	0.861	0.123	(- 0.224; 0.471)	0.473	0.984	0.506	- 0.022	0.017#	0.319
ΔDia_M	0.200	(- 0.143; 0.542)	0.242	0.649	0.110	(- 0.090; 0.309)	0.269	0.595	0.366	0.003	0.097#	0.176
REM												
Evening BP												
ΔSys_E	- 0.015	(- 0.218; 0.187)	0.881	0.513	0.027	(- 0.167; 0.222)	0.780	0.982	0.948	- 0.032	0.267#	0.026
ΔDia_E	- 0.007	(- 0.139; 0.124)	0.910	0.706	0.014	(- 0.113; 0.140)	0.831	0.825	0.970	- 0.033	0.270##	0.026
Morning BP **												
ΔSys_M	0.247	(- 0.033; 0.527)	0.082	0.321	0.073	(- 0.196; 0.342)	0.583	1.000	0.195	0.050	0.011#	0.349
ΔDia_M	0.123	(- 0.042; 0.288)	0.138	0.439	0.050	(- 0.109; 0.208)	0.526	0.808	0.283	0.023	0.087#	0.186

Columns for ΔAHI and ΔIFL show regression coefficients (β), 95% CI intervals and p-values from the unadjusted model and p-values from the adjusted model. Regression model p – values and adjusted R^2-values are shown on the right columns. Independent variables in the unadjusted model are: ΔAHI and ΔFLI. Independent variables in the adjusted model are: ΔAHI, ΔFLI, ΔBMI, ΔS-FSH and MHT. #, p(BMI) ≤ 0.05; ##, p(MHT use) ≤ 0.05; **, N = 29.
Abbreviations: AHI, Apnea-hypopnea-index; BMI, Body mass-index; BP, Blood pressure; Dia, diastolic blood pressure; IFL, inspiratory flow-limitation; MHT,

menopausal hormone therapy; NREM, non-rapid eye movement sleep; REM, rapid eye movement sleep; S-FSH, serum follicle-stimulating hormone; Sys, systolic blood pressure; $_{E}$, evening; $_{M}$, morning;


N = 147
Baseline study
2001 - 2007
N = 31
S-FSH ≥ 20 IU/L
At baseline
N = 31
Refused/missed follow-up
N = 9
Missing/insufficient data
N = 76
10-year follow-up
2013-2017
N = 12
Missing/insufficient data
N = 64
Current study


Lights Off
Uncscored
MT
Wake
REM
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S2
S3
S4
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0:00
1:00
2:00
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5:00
6:00
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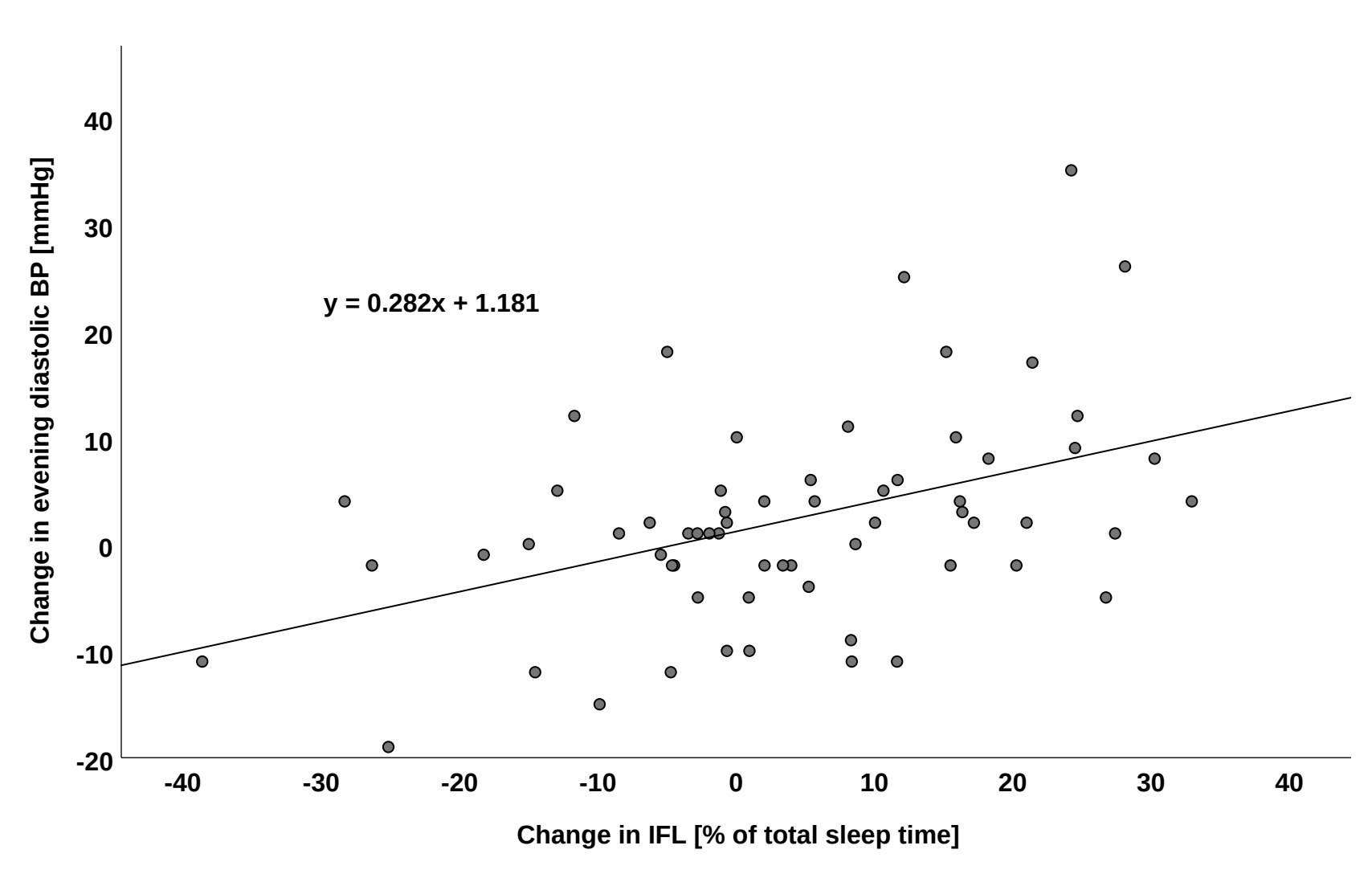
3:37:30
3:38:00
3:38:30
3:39:00
3:39:30
EOG
EOG-R
EOG-L
50µV/cm
EEG
C3-A2
C4-A1
O1-A2
O2-A1
50µV/cm
RESP-FL
70
60
50
40
30
20
10
µV
SaO2
100
95
90
%


y = 0.282x + 1.181
Change in evening diastolic BP [mmHg]
40
30
20
10
0
-10
-20
-40
-30
-20
-10
0
10
20
30
40
Change in IFL [% of total sleep time]