ERJ Express. Published on July 2, 2009 as doi: 10.1183/09031936.00017909

Transcutaneous Carbon Dioxide Profile during Sleep Reveals Metabolic Risk Factors in Postmenopausal Women

Jenni Aittokallio[1], MD; Tarja Saaresranta[1,2] MD, PhD; Arho Virkki[1,3], PhD; Nina Karppinen[1] MB; Olli J Heinonen[4], MD, PhD; Tero Aittokallio[1,3,] PhD; Olli Polo[1,5] MD, PhD

[1]Sleep Research Unit, Department of Physiology, University of Turku, Finland; [2]Department of Pulmonary Diseases, Turku University Central Hospital, Turku, Finland; [3]Biomathematics Research Group, Department of Mathematics, University of Turku, Finland; [4]Paavo Nurmi Centre, Department of Physiology, University of Turku, Finland; [5]Department of Pulmonary Diseases, Tampere University Hospital, Tampere, Finland

Correspondence to Jenni Aittokallio, MD

Sleep Research Unit, University of Turku

Lemminkäisenkatu 14-18 A, FI-20014

Turku, Finland.

Tel +358 2 3337599

Fax +358 2 2410057

E-mail jemato@utu.fi

Keywords : transcutaneous carbon dioxide, sleep, postmenopause, metabolic syndrome

Abstract:

The risks of metabolic syndrome and sleep-disordered breathing increase around the time of menopause. We have previously shown that features of the nocturnal transcutaneous carbon dioxide ($TcCO_2$) profile are associated with metabolic variables such as cholesterol, glycosylated hemoglobin A1C (GHbA1C) and blood pressure in patients with sleep apnea. In the present study, we investigate whether these metabolic variables can be predicted using non-invasive $TcCO_2$ measurements during sleep in generally healthy postmenopausal women.

Twenty-two postmenopausal women went through an overnight polygraphic sleep study that involved the continuous monitoring of arterial oxyhemoglobin saturation (SaO_2) and $TcCO_2$. Body composition, GHbA1C, plasma cholesterol and blood pressure were measured prior to the sleep study.

Nocturnal $TcCO_2$ features were the most important predictors of lipoprotein cholesterols, triglycerides and blood pressure levels. A longer sleep period and higher $TcCO_2$ levels were linked with lower GHbA1C, and fragmented sleep with lower high-density lipoprotein cholesterol. Neither nocturnal SaO_2 indices nor the apnea-hypopnea index had a predictive power.

The results suggest that nocturnal $TcCO_2$ events reveal metabolic risk factors already in healthy postmenopausal women.

Introduction

There is increasing evidence to suggest that sleep disorders and cardiovascular diseases are linked. Aging and menopause increase the risk of sleep-disordered breathing (SDB) and poor sleep quality, and each of these are also associated with metabolic disorders [1-3]. Although the mechanisms of interaction between sleep disorders and cardiovascular diseases are not fully understood, an autonomic nervous system imbalance (increased sympathetic and decreased parasympathetic activity) and endothelial inflammation are likely to be involved.

The transcutaneous carbon dioxide ($TcCO_2$) sensor has been developed for noninvasive estimation of the partial pressure of arterial carbon dioxide. The method has not gained wide acceptance because the correlations between $TcCO_2$ and arterial carbon dioxide tension are affected by hemodynamic events such as vasoconstriction and vasodilatation [4,5]. On the other hand, measurement of local carbon dioxide (CO_2) is of special interest, since it is an important regulator of vascular nitric oxide production [6]. Using our algorithms to analyze the nocturnal $TcCO_2$ plateaus and sudden $TcCO_2$ descents we have previously been able to predict nitric oxide mediated vasodilatation in premenopausal women [7] and metabolic status in patients with suspected sleep apnea [8].

In women, the risk of metabolic syndrome (defined as insulin resistance, abdominal obesity, dyslipidemia and elevated blood pressure), increases during the menopausal transition [9,10]. The early detection of developing metabolic abnormalities in this population would be key to preventing or reducing the effects of metabolic syndrome and its complications. Therefore we performed sleep studies including all-night $TcCO_2$ recordings in a so far healthy population of postmenopausal women. We had two specific aims. First, we wanted to evaluate further the

performance of our $TcCO_2$ analysis in predicting metabolic variables such as glycosylated hemoglobin A1C (GHbA1C), blood pressure (BP), and plasma lipoprotein cholesterols and triglycerides in a group of individuals whose pre-test probability of metabolic syndrome was lower than that of the patients with suspected sleep apnea in our previous study. Our second aim was to screen a number of nocturnal $TcCO_2$ features among with other sleep parameters for their potential of predicting metabolic abnormalities and increased cardiovascular risk already at the preclinical stage.

Methods

Subjects

Twenty-two healthy postmenopausal women were recruited via a newspaper announcement advertising a sleep study. Subjects with a history of alcohol abuse, malignancies, diabetes, coronary heart disease, respiratory insufficiency or known SDB were excluded, as were subjects with medication for hypercholesterolemia or hypertension. Five women with estrogen therapy were allowed to continue with their medication, three used a transdermal gel, one a transdermal patch and one an oral preparation.

The study was approved by the Commission on Ethics of Turku University Central Hospital. A written informed consent was obtained from all subjects.

Subject Characteristics, Blood Tests and Questionnaires

The neck, waist and hip circumferences, body mass index (BMI), evening resting blood pressure and forced expiratory volume in one second (FEV_1) were measured as described earlier [11]. Venous blood samples for the assessment of plasma total cholesterol, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol and triglycerides (Modular Analytics P-analyzer®, Roche/Hitachi, Tokyo, Japan) as well as glycosylated hemoglobin A1C (GHbA1C; Variant II®, Bio-RAD Laboratories Diagnostics Group, Hercules, CA) and follicle stimulating hormone (FSH; AutoDelfia®, Wallac, Turku, Finland) were collected on the morning prior to the sleep study, following an overnight fast. LDL cholesterol was calculated using Friedewald's formula. A personal medical history, including smoking habits and medications, was collected using structured questionnaires in the presence of an investigator or study nurse.

Overnight Measurements

Sleep recordings involved the overnight monitoring of the electroencephalogram (C3/A2, C4/A1, O1/A2, O2/A1), electrooculogram and the mandibular electromyogram (Embla®, Medcare Flaga hf. Medical Devices, Reykjavik, Iceland). Nasal air flow was measured with nasal prongs attached to a pressure sensor of the Embla®/Somnologica system. The arterial oxyhemoglobin saturation (SaO_2) was measured by two finger probe pulse oximeters (Nonin® oximeter build into the Embla®/Somnologica system, Medcare Flaga hf, Reykjavik, Iceland and Ohmeda Biox 3700 Pulse Oximeter®, Biomed Technologies Inc. US, recorded using the Uniplot® software, Unesta, Turku, Finland). $TcCO_2$ was measured using a TCM3 device (Radiometer®; Copenhagen, Denmark) [12]. After cleansing the skin with alcohol the skin sensor was placed on the upper part of the chest parasternally and heated to 43˚C, at which temperature the sensor remained attached during the

night for approximately eight hours [13]. Before each recording, the $TcCO_2$ signal was calibrated by flushing the sensor with a calibration gas containing a 5 % concentration of CO_2.

The time that the subjects spent in bed was not strictly limited. Subjects went to bed at around their usual bedtime and were woken up at around 7.30 am if they had not already woken up earlier. Sleep stages including stage 1, stage 2, combined slow-wave sleep (SWS, stages 3 and 4) and rapid eye movement (REM) sleep, were visually scored in 30-second epochs according to the Rechtschaffen and Kales criteria [14]. Sleep onset was determined by the appearance of the first 30-seconds of sleep. The time before sleep onset was defined as evening wakefulness. The end of the sleep period was determined by the final arousal leading to wakefulness. Sleep latency was defined as the period from the beginning of the recording to sleep onset. Sleep efficiency was expressed as the percentage of total sleep time in the sleep period. Arousals were defined by using the American Sleep Disorders Association Definition [15].

The SaO_2 signal was recorded with a sampling frequency of 1 Hz and $TcCO_2$ with a sampling frequency of 100 Hz throughout the night using the Embla® system. The episodes of arterial oxyhemoglobin desaturation of 3% and 4% units or more per hour (ODI_3 and ODI_4) were calculated using the Embla-Somnologica® or the Uniplot® software. The apnea-hypopnea index (AHI) was visually determined using the Embla-Somnologica ® software and the AASM criteria [16]. An episode of apnea was defined as a cessation of airflow for at least 10 seconds. Hypopnea was defined as a marked reduction in the nasal flow signal lasting at least 10 seconds that was associated with a reduction in the oxyhemoglobin saturation from the pre-event baseline of at least 4%.

Processing of the $TcCO_2$ Signal

Details of the $TcCO_2$ signal processing are provided in the Supplementary Data, and only briefly described here. First, obvious artifacts at the beginning or at the end of the $TcCO_2$ recordings were manually removed. The artifacts in the middle of the recordings were replaced with constant line segments. The median overnight $TcCO_2$ levels were calculated for each sleep stage (S1, S2, SWS, REM sleep and wakefulness) as well as the percentage of time the signal stayed above 7 kPa. Special attention was paid to abrupt $TcCO_2$ descents, referred to as pit patterns. Each pit pattern was characterized by its descent (amplitude, duration and slope). The highest $TcCO_2$ plateau (maximal plateau) was defined visually from each curve. An example of a $TcCO_2$ curve with a pit pattern and maximal plateau is shown in Figure 1.

Regression Analyses

Standard multiple linear regression analyses with stepwise feature selection were carried out individually for each metabolic variable (GHbA1C, HDL and LDL cholesterol and triglycerides, and blood pressure). Predictors included the overnight $TcCO_2$ and SaO_2 features as well as the sleep architecture measures. The neck and waist circumferences, waist to hip ratio, BMI and FSH were used as confounding factors in the prediction models. At each step, the most significant feature not yet in the regression model was entered, provided that its individual significance was sufficient ($p < 0.05$), and the insignificant ($p > 0.10$) features were removed. The standard *F*-test was used to assess the significance of each feature in terms if its contribution to the R^2-change. The feature selection algorithm was terminated as soon as no more features could be included or removed. Multicollinearity was tested by computing the variance inflation factor (VIF) for each model variable. VIF factors for the model variables were below 2 (1.023 and 1.011), which

indicates that possible collinearity of the model variables was not a concern. The difference in the frequency of pit patterns (pit index) between non-REM (NREM) and REM sleep was assessed with paired *t*-tests. The statistical analyses were performed with the default values in the SPSS 12 software (SPSS Inc., Chicago, IL).

Results

Each of the 22 women participated in the overnight measurements (Figure 1). The demographic data of the study group is presented in Table 1. The women were mildly overweight (mean BMI 25.3). Two women had a BMI greater than 30 kg/m^2. Seven women had a systolic blood pressure (SBP) greater than 140 mmHg and six women had a diastolic blood pressure (DBP) greater than 90 mmHg. One subject had a GHbA1C greater than 6 % (6.1 %) and 17 subjects had total cholesterol greater than 5 mmol/L. The inter-relationships between the metabolic variables used in the linear regression models are presented in Table 2. The FEV_1 ranged from 77 % to 146 % of predicted values and the median FEV_1 was 95 %. In all of the women, FSH was in postmenopausal levels ranging from 31 to 140 IU/L. The subjects included four habitual smokers, two occasional smokers, and four habitual snorers (snoring during at least three nights a week). None of the subjects had chronic obstructive pulmonary disease or asthma. Two women regularly used acetylsalicylic acid. One woman used cetirizine for allergic symptoms and gastric mucoprotective drugs. One woman was on citalopram.

The mean and S.D. values of the sleep architecture measures from the sleep study are shown in Table 3. The SaO_2 and $TcCO_2$ measurements are presented in Table 4. None of the subjects had an ODI_4 of over 5 events per hour. Two subjects had an AHI of greater than 5 events per hour (7 and

8 events per hour). The nocturnal frequency of pit patterns was computed individually for both REM and NREM sleep states, with the pit index being considerably higher in REM sleep (paired *t*-test, $p < 0.001$). Age was not taken as a predictor into the final multivariate results because it did not show a significant correlation with any of the metabolic variables (correlations ranged from 0.233 to 0.303, and the p-values from 0.171 to 0.947).

Nocturnal Measurements as Predictors of Metabolic Variables

The features selected using the stepwise multiple linear regression analyses supported the importance of the novel $TcCO_2$ features in predicting metabolic variables (Table 5). In contrast, none of the SaO_2 or demographic features were selected as predictors of the metabolic variables. Also AHI, ODI_3 and ODI_4 were insignificant predictors. In addition to the nocturnal $TcCO_2$ features, a longer sleep period was found to be an important predictor of lower GHbA1C, and increased sleep fragmentation of lower HDL cholesterol. High levels of $TcCO_2$ (% of time over 7 kPa) were linked with lower GHbA1C and triglycerides. The maximal plateau of the $TcCO_2$ curve associated positively with the HDL/total cholesterol ratio. In addition, high evening levels of $TcCO_2$ were linked with a lower evening systolic BP. Further, a high nocturnal frequency of pit patterns predicted lower HDL cholesterol. The slope of the pit patterns was related both with low total cholesterol and low LDL cholesterol. Moreover, a higher amplitude of the pit pattern was associated with lower diastolic blood pressure.

Discussion

In our study population of generally healthy 55-year-old postmenopausal women, nocturnal $TcCO_2$ features were the most important predictors of GHbA1C, blood pressure and cholesterol levels (Table 5). A longer sleep period was linked with a lower GHbA1C, and fragmented sleep with lower HDL cholesterol, as suggested by previous studies [17,18]. Mean and nadir SaO_2, ODI_4, AHI, BMI values and waist circumference were worse predictors of metabolic variables in this population. Subjects were all generally healthy, although marginally overweight and with BP and waist circumference slightly exceeding the International Diabetes Federation reference values [19] (Table 1). Despite this, the overnight $TcCO_2$ features were systematically associated with the metabolic variables. Our results provide further support to our earlier findings that, irrespective of the study population, the nocturnal $TcCO_2$ profile contains risk factor information on metabolic diseases [8]. It is possible that increasing $TcCO_2$ levels during sleep indicate decent vasodilatation capacity in the silence of sympathetic drive whereas sudden $TcCO_2$ decreases (pit patterns) indicate surges of sympathetic activity that cause exaggerated vasoconstrictive responses when endothelial dysfunction is present. The feasibility of interaction between the local CO_2 events and endothelial dysfunction is further strengthened by the observation that the CO_2-mediated autoregulatory vasodilatation is mediated through nitric oxide [6]. The nocturnal $TcCO_2$ features have a high predictive power on daytime endothelial function tests [7], suggesting that local CO_2 is a major controller of local nitric oxide production. Further studies are needed to establish to which extent nocturnal $TcCO_2$ events display local nitric oxide production.

Menopause increases the risk of SDB [20,21], which in turn is also known to be a risk factor for metabolic syndrome and cardiovascular disorders [2,22]. Our study population was a group of clinically healthy women with age and postmenopausal status as risk factors, which gave us a

unique setting to search for signs of emerging metabolic syndrome. Carbon dioxide, which is the final metabolic end product, turned out to play a major role in our results. A $TcCO_2$ sensor measures the CO_2 that diffuses through the skin, and its measurements are affected by central respiratory drive, peripheral vascular perfusion and local tissue metabolism [12, 23]. Conventional severity indexes of sleep apnea (AHI, ODI_3, ODI_4 or SaO_2) were not found to be important in this population with relatively little sleep-disordered breathing. This suggests that the $TcCO_2$ features predict metabolic variables independently of hypoxemia or SDB.

The high overnight levels of $TcCO_2$ was one of the new key predictors for protective metabolic variables in our earlier study in patients with sleep apnea [8]. This association was also found in the present study, despite essentially different study population (healthy postmenopausal women). The longer the subjects maintained their $TcCO_2$ over 7 kPa, the lower their levels of GHbA1C and triglycerides were. Furthermore, the visually detected maximal plateau of the $TcCO_2$ curve associated positively with the HDL/total cholesterol ratio. These results are encouraging, yet they should be interpreted with caution, since we have no explicit data about "the normal" $TcCO_2$ ranges during sleep. It is probable that nocturnal $TcCO_2$ variables differ between genders and change with increasing age, after menopause [11] or during estrogen therapy [24]. However, the findings are in line with the earlier $TcCO_2$ profile results, and in particular with the result in our previous work that the proportion of high $TcCO_2$ levels measured during sleep was one of the most important features for classifying insulin resistance [8].

Obstructive sleep apnea (OSA) and snoring are associated with insulin resistance and an impaired lipid profile [3,25,26]. OSA may decrease the arterial CO_2 tension due to repetitive arousals and hyperventilation following each apnea. It is therefore possible that people with even mild sleep disturbances cannot achieve as high $TcCO_2$ levels as coeval healthy people, because episodes of

apnea, snoring or frequent arousals interrupt sleep. This is in line with the finding that awakening is usually followed by a notable descent in the $TcCO_2$ tension (Figure 1), while falling asleep is typically related to a rise in $TcCO_2$ [11].

In the present study, we classified the $TcCO_2$ features according to the sleep stages and found that $TcCO_2$ pit patterns occur significantly more often in REM sleep and wakefulness than in other sleep stages (Figure 1). Normally sympathetic activity dominates during wakefulness and appears as bursts during REM sleep. NREM sleep is characterized by parasympathetic dominance. Sympathovagal imbalance is common in metabolic syndrome, but its definitive causative role has not been demonstrated [27]. Pit patterns may result from sudden bursts of sympathetic activity that produce peripheral vasoconstriction. These bursts may appear more consistently during sympathetic dominance. Subjects with a high pit index had a lower HDL cholesterol concentration, confirming our previous findings [8]. Low HDL cholesterol has previously been linked with a greater frequency of arousals [17]. Likewise, in our study, sleep fragmentation was the other predictor of HDL cholesterol. As arousals and awakenings increase sympathetic activation during sleep, it is possible that they also produce the pit patterns. Spiegel et al have shown that glucose metabolism is impaired with increased sympathetic tone which has been induced by partial short-term sleep deprivation [28]. If $TcCO_2$ reflects sympathovagal balance, then during parasympathetic dominance when periferic blood vessels are dilated, $TcCO_2$ levels are high.

Another feature of the $TcCO_2$ patterns is the amplitude and sharpness of the pits. The fast and deep descents appear to be associated with low LDL and total cholesterol levels. In addition, a high amplitude of pit pattern was the only predictor of low diastolic blood pressure. This further supports the idea of $TcCO_2$ as a reflector of sympathovagal balance. By monitoring the transient

$TcCO_2$ events against the prevailing parasympathetic tone, the bursts of sympathetic nervous activity can be distinguished more clearly. The dominance of sympathetic activity may diminish the amblitudes of the pit patterns.

The only predictor for systolic blood pressure was the evening wakefulness level of $TcCO_2$. As CO_2 is a known vasodilatator [29], this may explain why subjects with a high $TcCO_2$ had a lower systolic blood pressure. The possible protective effect of higher $TcCO_2$ is also in line with the findings in women with SDB. Those with a predominantly partial upper airway obstruction during sleep (flow limitation) combined with increased $TcCO_2$ levels, had less hypertension than in patients with OSA [30]. However, high $TcCO_2$ did not associate with diastolic blood pressure. Andersson et al. have previously demonstrated that high end tidal CO_2 ($EtCO_2$) predicts high systolic blood pressure in women [31]. $EtCO_2$ measures CO_2 concentration in the alveoli and is strongly affected by changes in ventilation, whereas $TcCO_2$ is also affected by tissue metabolism and local vasodynamics. Hence, $EtCO_2$ and $TcCO_2$ are likely to measure different phenomena. However, the results of Anderson et al. show that breathing is an important contributor in the development of hypertension. The blood pressure of OSA patients is higher than in healthy controls [32] and nocturnal hypoxia elevates blood pressure [33]. Generally, poor sleep seems to be an important risk factor for hypertension, because sleep fragmentation, arousals and short self-reported sleep duration associate with high blood pressure [17,34,35]. These variables did not predict blood pressure in our study. This may be due to the relatively small and healthy study population.

The risk of metabolic syndrome increases around the time of menopause [9,10,36]. Therefore, we expected to find some variation in our study population, even if all of the women were generally healthy. Central obesity is a known risk factor for insulin resistance [37]. However, in our study, it

did not turn out to be an important contributor, probably because most of our subjects were rather lean (Table 1). Neither the duration nor the proportion of SWS turned out to be important predictors in our study population, even though van Cauter et al recently showed that a short SWS duration associates with an increased risk of diabetes [1]. This may be because of our healthy sample, as only one woman had a GHbA1C over 6%. In addition to the SWS duration, self-reported short sleep has been linked with obesity and diabetes [18,38]. These studies are in line with our results, that a long sleep period (assuming that the length of a subject's sleep period in a sleep laboratory reflects their normal sleep period) was the most important predictor of lower GHbA1C. In addition, sleep fragmentation was a predictor of decreased HDL cholesterol. Recently, Ekstedt et al. showed that the number of arousals predicted lower HDL cholesterol [17]. These results together suggest the importance of adequate length and quality of sleep in the prevention of metabolic disorders. Short and fragmented sleep may disturb the $TcCO_2$ signal, which seems to be sensitive to subtle nocturnal changes. This may explain why in our results the $TcCO_2$ features play such an important role. However, more studies are still needed to confirm the results.

Our study has some potential confounders and limitations. The cross-sectional study design does not allow us to confirm whether CO_2 is playing a causative role, or whether the $TcCO_2$ profile is a marker of some other underlying pathophysiological process. Moreover, the number of subjects was relatively small and heterogeneous in terms of hormone replacement therapy. Although the subjects were all of around the same age, the time from menopause varied (Table 1). The time from menopause was not included in linear regression models, for only 13 women (59 %) remembered their exact time of menopause. This together with the cross-sectional study design makes it impossible to separate the influence of age and menopause. In addition, estrogen deficiency affects metabolism [10]. Estrogen usage was not selected as an exclusion criterion,

because our main goal was not to study the effects of estrogen, but to find the predictors for wide range of metabolic variables. Moreover, grouping the subjects based on the estrogen usage did not reveal differences in any of the measurements (unpaired t-test, $p>0.1$). Some potential pitfalls in the subject selection could be criticized as well. The subjects were recruited through newspaper announcements, calling healthy postmenopausal women for a sleep and cardiovascular study. Some members of this group of "healthy" subjects, may have been compelled to participate in such a study due to subclinical hidden sleep problems or cardiovascular family risk factors. However, none of the subjects regularly used hypnotic drugs.

Conclusions

Nocturnal $TcCO_2$ features can predict metabolic variables including GHbA1C, HDL and LDL cholesterol, triglycerides and blood pressure in healthy postmenopausal women. Conventional measures such as waist circumference and nocturnal hypoxia were not important predictors in our study population. Monitoring $TcCO_2$ events (the pit patterns) against the prevailing parasympathetic tone ($TcCO_2$ plateaus) during sleep may reveal abnormal endothelium responses to the activation of the sympathetic nervous system, which may result from abnormal metabolic processes. These results may have important medical implications, ranging from an understanding of the potential mechanisms underlying the disease pathogenesis to improved diagnostic methods for assessing the risk of developing metabolic syndrome.

References

1. Tasali E, Leproult R, Ehrmann DA, Van Cauter E. Slow-wave sleep and the risk of type 2 diabetes in humans. *Proc Natl Acad Sci U S A* 2008;105:1044-9.
2. Vgontzas AN, Bixler EO, Chrousos GP. Sleep apnea is a manifestation of the metabolic syndrome. *Sleep Med Rev* 2005; 9:211-24.
3. Williams CJ, Hu FB, Patel SR, Mantzoros CS. Sleep duration and snoring in relation to biomarkers of cardiovascular disease risk among women with type 2 diabetes. *Diabetes Care* 2007;30:1233-40.
4. Clark JS, Votteri B, Ariagno RL, Cheung P, Eichhorn JH, Fallat RJ, et al. Noninvasive assessment of blood gases. *Am Rev Respir Dis* 1992;145:220-32.
5. Healey CJ, Fedullo AJ, Swinburne AJ, Wahl GW. Comparison of noninvasive measurements of carbon dioxide tension during withdrawal from mechanical ventilation. *Crit Care Med* 1987;15:764-8.
6. Lavi S, Gaitini D, Milloul V, Jacob G. Impaired cerebral CO_2 vasoreactivity: Association with endothelial dysfunction. *Am J Physiol Heart Circ Physiol* 2006;291:H1856-61.
7. Aittokallio J, Polo O, Hiissa J, Virkki A, Toikka J, Raitakari O, et al. Overnight variability in transcutaneous carbon dioxide predicts vascular impairment in women. *Exp Physiol* 2008;93: 880-91.
8. Virkki A, Polo O, Saaresranta T, Laapotti-Salo A, Gyllenberg M, Aittokallio T. Overnight features of transcutaneous carbon dioxide measurement as predictors of metabolic status. *Artif Intell Med* 2008;42:55-65.
9. Davidson MH, Maki KC, Karp SK, Ingram KA. Management of hypercholesterolaemia in postmenopausal women. *Drugs Aging* 2002;19:169-78.

10. Salpeter SR, Walsh JM, Ormiston TM, Greyber E, Buckley NS, Salpeter EE. Meta-analysis: Effect of hormone-replacement therapy on components of the metabolic syndrome in postmenopausal women. *Diabetes Obes Metab* 2006;8:538-54.

11. Aittokallio J, Virkki A, Aittokallio T, Saaresranta T, Polo-Kantola P, Polo O. Non-invasive respiratory monitoring during wakefulness and sleep in pre- and postmenopausal women. *Respir Physiol Neurobiol* 2006;150:66-74.

12. Baumbach P. Understanding transcutaneous pO_2 and pCO_2 measurements. Radiometer Medical A/S, Denmark, 1997.

13. Janssens JP, Perrin E, Bennani I, de Muralt B, Titelion V, Picaud C. Is continuous transcutaneous monitoring of PCO2 (TcPCO2) over 8 h reliable in adults? *Respir Med* 2001;95:331-5.

14. Rechtschaffen A, Kales A. A manual of standardized terminology, techniques and scoring systems for sleep stages of human subjects. National Institutes of Health, Washington, DC, 1968.

15. A preliminary report from the sleep disorders atlas task force of the American sleep disorders association. EEG arousals: Scoring rules and examples. *Sleep* 1992;15:173-84.

16. Iber C, Ancoli-Israel S, Chesson A, Quan S. The AASM manual for the scoring of sleep and associated events: Rules, terminology and technical specification, 1st ed. American Academy of Sleep Medicine, Westchester, Illinois, 2007.

17. Ekstedt M, Åkerstedt T, Söderström M. Microarousals during sleep are associated with increased levels of lipids, cortisol, and blood pressure. *Psychosom Med* 2004;66:925-31.

18. Gangwisch JE, Heymsfield SB, Boden-Albala B, Buijs RM, Kreier F, Pickering TG, et al. Sleep duration as a risk factor for diabetes incidence in a large U.S. sample. *Sleep* 2007; 30:1667-73.

19. Alberti KG, Zimmet P, Shaw J, IDF Epidemiology Task Force Consensus Group. The metabolic syndrome--a new worldwide definition. *Lancet* 2005;366:1059-62.

20. Bixler EO, Vgontzas AN, Lin HM, Ten Have T, Rein J, Vela-Bueno A, et al. Prevalence of sleep-disordered breathing in women: Effects of gender. *Am J Respir Crit Care Med* 2001;163:608-13.

21. Young T, Finn L, Austin D, Peterson A. Menopausal status and sleep-disordered breathing in the Wisconsin Sleep Cohort Study. *Am J Respir Crit Care Med* 2003;167:1181-5.

22. Hamilton GS, Solin P, Naughton MT. Obstructive sleep apnoea and cardiovascular disease. *Intern Med J* 2004;34:420-6.

23. Lubbers DW. Theoretical basis of the transcutaneous blood gas measurements. *Crit Care Med* 1981;9:721-33.

24. Aittokallio J, Hiissa J, Saaresranta T, Polo-Kantola P, Aittokallio T, Polo O. Nocturnal transcutaneous carbon dioxide tension in postmenopausal estrogen-users and non-users. Menopause Int (in press).

25. Borgel J, Sanner BM, Bittlinsky A, Keskin F, Bartels NK, Buechner N, et al. Obstructive sleep apnoea and its therapy influence high-density lipoprotein cholesterol serum levels. *Eur Respir J* 2006;27:121-7.

26. Ip MS, Lam B, Ng MM, Lam WK, Tsang KW, Lam KS. Obstructive sleep apnea is independently associated with insulin resistance. *Am J Respir Crit Care Med* 2002;165:670-6.

27. Frontoni S, Bracaglia D, Gigli F. Relationship between autonomic dysfunction, insulin resistance and hypertension, in diabetes. *Nutr Metab Cardiovasc Dis* 2005;15:441-9.

28. Spiegel K, Leproult R, Van Cauter E. Impact of sleep dept on metabolic and endocrine function. *Lancet* 1999;354:1435-9.

29. Atkinson JL, Anderson RE, Sundt TM,Jr. The effect of carbon dioxide on the diameter of brain capillaries. *Brain Res* 1990;517:333-40.

30. Anttalainen U, Polo O, Vahlberg T, Saaresranta T. Reimbursed drugs in patients with sleep-disordered breathing - a static-charge-sensitive bed study. *Sleep Med* (in press).

31. Anderson DE, Parsons DJ, Scuteri A. End tidal CO2 is an independent determinant of systolic blood pressure in women. *J Hypertens* 1999;17:1073-80.

32. Hla KM, Young TB, Bidwell T, Palta M, Skatrud JB, Dempsey J. Sleep apnea and hypertension. A population-based study. *Ann Intern Med* 1994;120:382-8.

33. Arabi Y, Morgan BJ, Goodman B, Puleo DS, Xie A, Skatrud JB. Daytime blood pressure elevation after nocturnal hypoxia. *J Appl Physiol* 1999;87:689-98.

34. Gangwisch JE, Heymsfield SB, Boden-Albala B, Buijs RM, Kreier F, Pickering TG, et al. Short sleep duration as a risk factor for hypertension: Analyses of the first national health and nutrition examination survey. *Hypertension* 2006;47:833-9.

35. Morrell MJ, Finn L, Kim H, Peppard PE, Badr MS, Young T. Sleep fragmentation, awake blood pressure, and sleep-disordered breathing in a population-based study. *Am J Respir Crit Care Med* 2000;162:2091-6.

36. Reckelhoff JF, Fortepiani LA. Novel mechanisms responsible for postmenopausal hypertension. *Hypertension* 2004;43:918-23.

37. Greenfield JR, Campbell LV. Insulin resistance and obesity. *Clin Dermatol* 2004;22:289-95.

38. Gangwisch JE, Malaspina D, Boden-Albala B, Heymsfield SB. Inadequate sleep as a risk factor for obesity: Analyses of the NHANES I. *Sleep* 2005;28:1289-96.

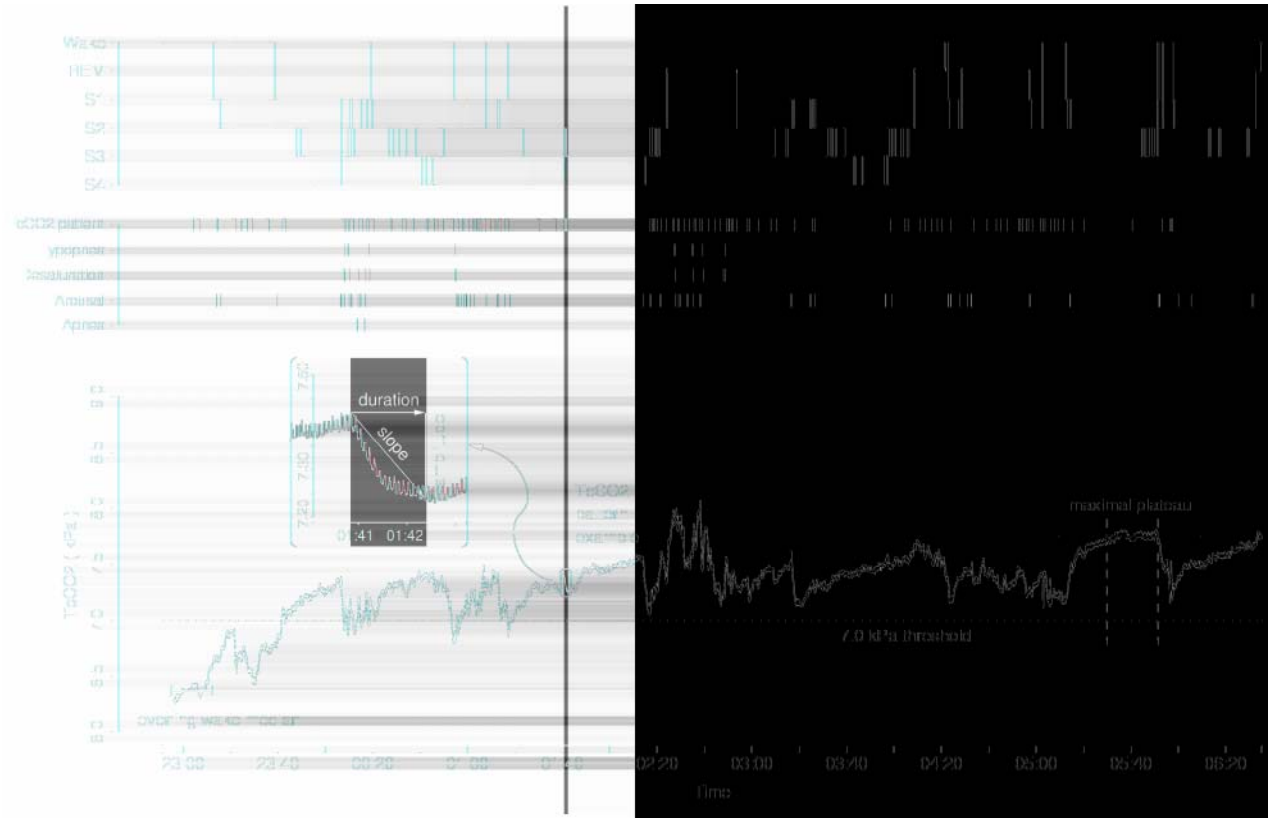
Acknowledgements

This work was supported by grants from the Finnish Anti-Tuberculosis Association Foundation, Tampere Tuberculosis Foundation, Aarne and Aili Turunen Foundation, Väinö and Laina Kivi Foundation, Academy of Finland, Paulo Foundation, The Jalmari and Rauha Ahokas Foundation, Orion-Farmos Research Foundation, The Research Foundation for Respiratory Diseases and the governmental EVO-grant of the Turku University Hospital.

Legends to the Figures

Figure 1. Representative overnight recording of the transcutaneous carbon dioxide ($TcCO_2$) as function of sleep stages and events.

Episodes of $TcCO_2$ pit patterns, apnea, hypopnea, desaturation, and arousals per hour are illustrated below the hypnogram. The enlarged insert in the $TcCO_2$ signal corresponds to a single pit pattern. The pit patterns were analyzed for amplitude, duration and slope. The first horizontal line indicates the $TcCO_2$ level during evening wakefulness and the second horizontal line indicates the maximal $TcCO_2$ plateau. The dotted line indicates the 7 kPa threshold. Other features extracted from the $TcCO_2$ signal include median $TcCO_2$ levels in the various sleep stages (Table 4).



Tables

Table 1. Subject Characteristics and Metabolic Measurements

n = 22	Mean	SD	Range
Age, years	55.5	1.2	[53, 57]
Age of menopause (n = 13)*, years	51.1	2.5	[47, 54]
FSH, IU/L	78.9	30.8	[31, 140]
FEV_1, % of reference	97.9	15.7	[77, 146]
Body mass index, kg/m^2	25.3	2.6	[21.9, 31.2]
Waist circumference, cm	84.3	8.4	[71.6, 97.0]
Neck circumference, cm	34.8	1.6	[33.0, 38.3]
Waist-to-hip ratio, %	82.4	4.5	[73.5, 90.5]

SBP, mmHg	130.8	15.9	[102, 163]
DBP, mmHg	83.1	10.4	[64, 106]
Total cholesterol, mmol/L	5.8	0.9	[4.0, 7.9]
LDL cholesterol, mmol/L	3.3	0.8	[2.2, 5.4]
HDL cholesterol, mmol/L	2.0	0.5	[1.3, 3.0]
HDL/cholesterol, %	35.4	8.1	[16, 52]
Triglycerides, mmol/L	1.0	0.5	[0.4, 2.7]
GHbA1C, %	5.6	0.3	[5.2, 6.1]

The values above correspond to the mean ± SD [range]. Definition of abbreviations: FSH = follicle stimulating hormone; SBP = systolic blood pressure; DBP = diastolic blood pressure; FEV_1 = forced expiratory volume in one second presented as % of the predicted values; LDL = low-density lipoprotein; HDL = high-density lipoprotein; GHbA1C = glycosylated hemoglobin A1C. *Only 13 subjects remembered the exact time of their menopause.

Table 2. Pairwise inter-relationships between Metabolic Variables.

	Total Cholesterol	Trigly	HDL	HDL /Cholesterol	LDL	Systolic BP	Diastolic BP
GHbA1C	r= 0.181 p= 0.419	**r= 0.546** **p= 0.009**	r= -0.329 p= 0.135	**r= -0.444** **p= 0.039**	r= 0.253 p= 0.257	r= 0.223 p= 0.319	r= 0.016 p= 0.942
Total Cholesterol		**r= 0.424** **p= 0.049**	r= 0.229 p= 0.305	r= -0.378 p= 0.082	**r= 0.903** **p= 0.000**	r= -0.061 p= 0.786	r= 0.096 p= 0.670
Trigly			**r= -0.572** **p= 0.005**	**r= -0.760** **p= 0.000**	**r= 0.563** **p= 0.006**	r= -0.019 p= 0.932	r= -0.005 p= 0.981
HDL				**r= 0.802** **p= 0.000**	r= -0.181 p= 0.420	r= 0.106 p= 0.637	r= 0.219 p= 0.327
HDL/Cholesterol					**r= -0.713** **p= 0.000**	r= 0.085 p= 0.705	r= 0.141 p= 0.530
LDL						r= -0.135 p= 0.548	r= -0.027 p= 0.904
Systolic BP							**r= 0.738** **p= 0.000**

GHbA1C, glycosylated hemoglobin A1C; Trigly, triglycerides; LDL, low-density lipoprotein; HDL, high-density lipoprotein; BP, blood pressure. GHbA1c, cholesterol and triglyceride levels are correlated, as are the systolic and diastolic blood pressure. *p*-values < 0.05 are bolded.

Table 3.
Sleep Architecture Measures

Stage 1*, %	9.0 (4.6)
Stage 2*, %	35.8 (10.3)
Stage 3 + 4*, %	32.6 (10.8)
REM sleep*, %	22.6 (6.2)
Stage 1, min	34.3 (18.3)
Stage 2, min	137.3 (42.4)
Stage 3 + 4, min	123.3 (39.7)
REM sleep, min	87.8 (29.3)
Arousals, events/hour	12.7 (2.9)
Sleep latency, min	32.9 (23.1)
Total sleep time, min	382.6 (47.0)
Sleep period**, min	433.3 (34.1)
Sleep efficiency***, %	88.4 (8.0)
Sleep fragmentation****, events/hour	1.9 (0.9)

The values above correspond to the mean (S.D.). * Percentage of total sleep time, ** Time from the sleep onset to the final awakening, *** the percentage of sleep time in the sleep period, **** shifts to wakefulness during the sleep period. REM, rapid eye movement sleep.

Table 4.

Overnight $TcCO_2$ and SaO_2 Measurements

Evening wakefulness median $TcCO_2$, kPa	6.46 (0.61)
Total sleep time median $TcCO_2$, kPa	6.56 (0.70)
SWS median $TcCO_2$, kPa	6.88 (0.72)
REM sleep median $TcCO_2$, kPa	6.88 (0.74)
Maximal plateau of $TcCO_2$, kPa	7.27 (0.75)
Pit index, events/hour	11.7 (3.8)
Pit index in REM, events/hour	19.7 (3.8)
Pit index in NREM, events/hour	6.4 (3.9)
Amplitude (pit pattern), kPa	0.19 (0.04)
Duration (pit pattern), s	57.0 (8.4)
Slope (pit pattern), kPa/min	0.20 (0.06)
Percentage $TcCO_2$ > 7 kPa, %	38.0 (44.2)
ODI_4, events/hour	3.2 (3.0)
ODI3, events/hour	7.9 (4.9)
AHI, events/hour	2.1 (2.0)
Nadir SaO_2, %	86.7 (4.2)
Mean SaO_2, %	95.2 (0.8)

The values above correspond to the mean (S.D.). $TcCO_2$, transcutaneous carbon dioxide; SWS, slow wave sleep (sleep stages 3 and 4); REM, rapid eye movement sleep; NREM, non-REM sleep; ODI_x, arterial oxyhemoglobin desaturation of x% units or more per hour; SaO_2, arterial oxyhemoglobin saturation; AHI, apnea-hypopnea index. $TcCO_2$ features are illustrated in Figure 1.

Table 5.
Stepwise Linear Regression Analysis with Overnight Measurements as Predictors of Metabolic Variables.

Metabolic variable	Predictors	Beta-value	P-value
GHbA1C	Sleep period	-0.511	0.009
	Percentage $TcCO_2$ > 7 kPa	-0.484	0.013
Total cholesterol	Slope ($TcCO_2$ pit pattern)	-0.500	0.018
LDL cholesterol	Slope ($TcCO_2$ pit pattern)	-0.439	0.041
HDL cholesterol	Pit index ($TcCO_2$)	-0.487	0.012
	Sleep fragmentation	-0.483	0.013
HDL/Total cholesterol ratio	Maximal plateau ($TcCO_2$)	0.428	0.047
Triglycerides	Percentage > 7 kPa ($TcCO_2$)	-0.472	0.027
Diastolic BP	Amplitude ($TcCO_2$ pit pattern)	-0.425	0.049
Systolic BP	Evening wakefulness ($TcCO_2$)	-0.550	0.008

Only the predictors with a p-value under 0.05 were accepted into the final linear regression models. GHbA1C, glycosylated hemoglobin A1C; $TcCO_2$, transcutaneous carbon dioxide; LDL, low-density lipoprotein; HDL, high-density lipoprotein; BP, blood pressure. $TcCO_2$ pit pattern, percentage >7 and maximal plateau are demonstrated in Figure 1.