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Transcutaneous carbon dioxide profile during sleep reveals metabolic risk factors in post-menopausal females

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ABSTRACT: The risks of metabolic syndrome and sleep-disordered breathing increase around the time of the 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 haemoglobin A1C (GHbA1C) and blood pressure in patients with sleep apnoea. In the present study, we investigated whether these metabolic variables can be predicted using noninvasive $TcCO_2$ measurements during sleep in generally healthy post-menopausal females.

22 post-menopausal females underwent an overnight polygraphic sleep study that involved the continuous monitoring of arterial oxygen saturation (Sa,O_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 Sa,O_2 indices nor the apnoea/hypopnoea index had a predictive power.

The results suggest that nocturnal $TcCO_2$ events revealed metabolic risk factors already present in healthy post-menopausal females.

KEYWORDS: Metabolic syndrome, post menopause, sleep, transcutaneous carbon dioxide

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 haemodynamic events, such as vasoconstriction and vasodilatation [4, 5]. However, measurement of local carbon dioxide is of special interest, since it is an important regulator of vascular nitric oxide production [6]. Using our algorithms to analyse the nocturnal $TcCO_2$ plateaus and sudden $TcCO_2$ descents, we have previously been able to predict nitric oxide mediated vasodilatation in pre-menopausal females [7] and metabolic status in patients with suspected sleep apnoea [8].

In females, 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 post-menopausal females with two specific aims. First, we wanted to evaluate further the

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performance of our $TcCO_2$ analysis in predicting metabolic variables such as glycosylated haemoglobin 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 apnoea in our previous study. Our second aim was to screen a number of nocturnal $TcCO_2$ features, along with other sleep parameters, for their potential to predict metabolic abnormalities and increased cardiovascular risk in these subjects.

METHODS

Subjects

22 healthy post-menopausal females 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 taking medication for hypercholesterolemia or hypertension. Five females with oestrogen 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 (Turku, Finland). 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 BP and forced expiratory volume in 1 s (FEV_1) were measured as described previously [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), GHbA1C (Variant II®; Bio-RAD Laboratories Diagnostics Group, Hercules, CA, USA) and follicle stimulating hormone (FSH) (AutoDelfia®; Wallac, Turku) 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), electro-oculogram 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 (Medcare Flaga hf. Medical Devices). The arterial oxygen saturation ($S_{a,O2}$) was measured by two finger-probe pulse oximeters (Nonin® oximeter built into the Embla®/Somnologica system and the Ohmeda Biox 3700 Pulse Oximeter® (Biomed Technologies Inc., Stoughton, MA, USA; recorded using the Uniplot® software, Unesta, Turku)). $TcCO_2$ 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 ~8 h [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 around their usual bedtime and were woken up at around 07.30 h 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-s epochs according to the criteria of RECHTSCHAFFEN and KALES [14]. Sleep onset was determined by the appearance of the first 30 s 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 using the American Sleep Disorders Association definition [15].

The S_{a,O_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. Episodes of arterial oxyhaemoglobin 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 apnoea/hypopnoea index (AHI) was visually determined using the Embla/Somnologica® software and the American Academy of Sleep Medicine criteria [16]. An episode of apnoea was defined as a cessation of airflow for $\geqslant$10 s. Hypopnoea was defined as a marked reduction in the nasal flow signal lasting for $\geqslant$10 s that was associated with a reduction in the oxyhaemoglobin 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 are only described briefly herein. First, obvious artefacts at the beginning or at the end of the $TcCO_2$ recordings were manually removed. The artefacts 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 characterised 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 $S_{a,O2}$ 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

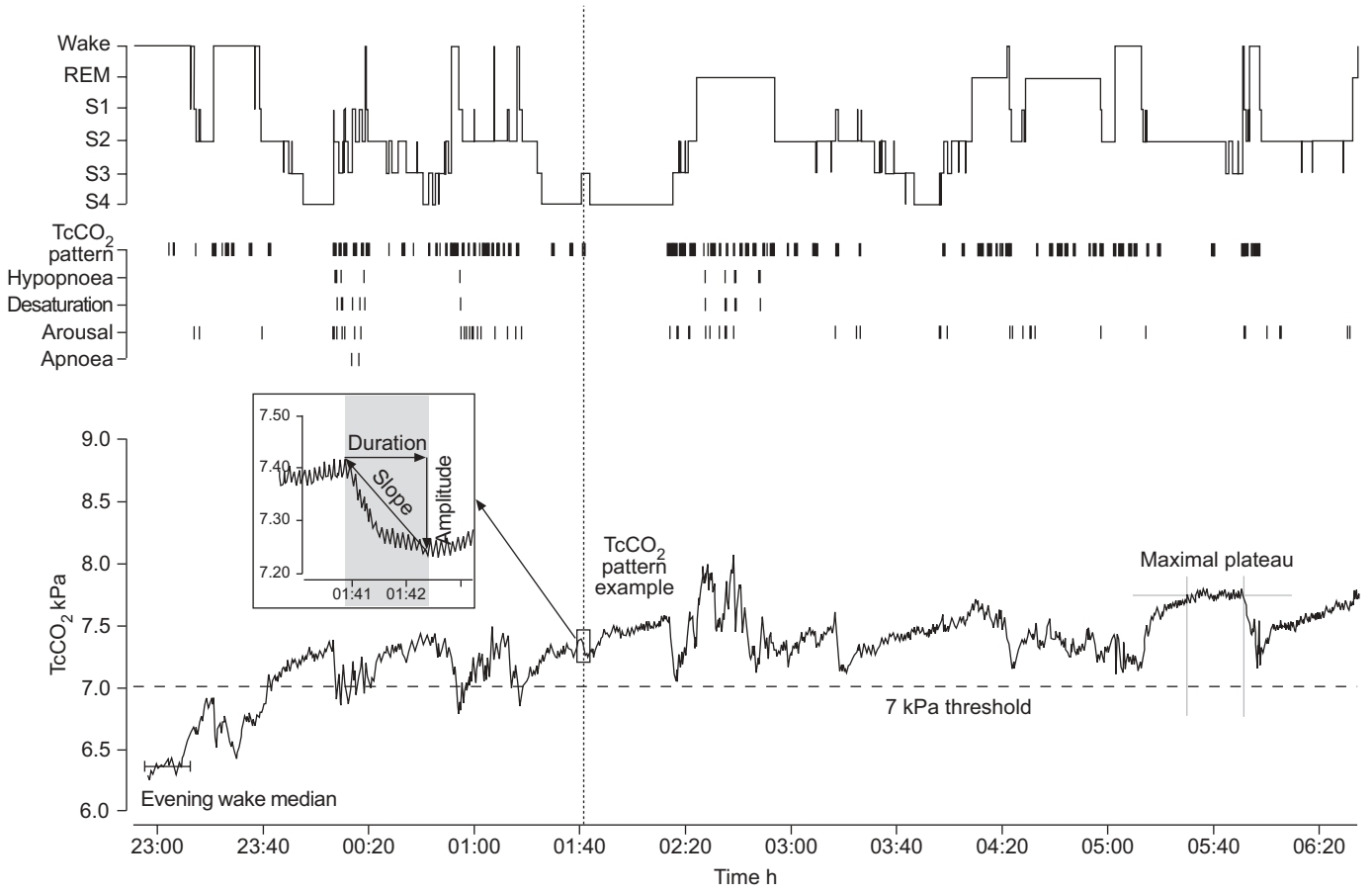

FIGURE 1. Representative overnight recording of the transcutaneous carbon dioxide ($TcCO_2$) as function of sleep stages and events. Episodes of $TcCO_2$ pit patterns, apnoea, hypopnoea, desaturation and arousals per hour are illustrated below the hypnogram. The $TcCO_2$ signal insert corresponds to a single pit pattern. The pit patterns were analysed for amplitude, duration and slope. The $TcCO_2$ level during evening wakefulness and the maximal $TcCO_2$ plateau are indicated. - - - -: the 7 kPa threshold. Other features extracted from the $TcCO_2$ signal include median $TcCO_2$ levels in the various sleep stages. REM: rapid eye movement.

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 <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, USA).

RESULTS

Each of the 22 females participated in the overnight measurements (fig. 1). The demographic data of the study group is presented in table 1. The females were mildly overweight (mean BMI 25.3 $kg \cdot m^{-2}$). Two of the females had a BMI >30 $kg \cdot m^{-2}$. Seven females had a systolic BP (SBP) >140 mmHg and six females had a diastolic BP (DBP) >90 mmHg. One subject had a GHbA1C of 6.1 %, and 17 subjects had total cholesterol >5 $mmol \cdot L^{-1}$. The inter-relationships between the metabolic variables used in the linear regression models are presented in table 2. The FEV1 ranged from 77% to 146% of predicted values and the median FEV1 was 95%. In all of the females, FSH was in the post-menopausal levels ranging from 31 to 140 $IU \cdot L^{-1}$. The subjects included four habitual smokers, two occasional smokers and four habitual snorers (snored at least three nights per week). None of the subjects had chronic obstructive pulmonary disease or asthma. Two females regularly used acetylsalicylic acid, one used cetirizine for allergic symptoms and gastric mucoprotective drugs and one female was on citalopram.

The mean $\pm$SD values of the sleep architecture measures from the sleep study are shown in table 3. The Sa,O_2 and $TcCO_2$ measurements are presented in table 4. None of the subjects had an ODI_4 of >5 events$\cdot h^{-1}$. Two subjects had an AHI of >5 events$\cdot h^{-1}$ (7 and 8 events$\cdot h^{-1}$, respectively). 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 ($p<0.001$). In the final multivariate results, age was not taken as a predictor 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).

TABLE 1 Subject characteristics and metabolic measurements

Subjects n	22
Age yrs	55.5±1.2 (53–57)
Age of menopause# yrs	51.1±2.5 (47–54)
FSH IU·L^{-1}	78.9±30.8 (31–140)
FEV$_1$ % pred	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^{-1}	5.8±0.9 (4.0–7.9)
LDL cholesterol mmol·L^{-1}	3.3±0.8 (2.2–5.4)
HDL cholesterol mmol·L^{-1}	2.0±0.5 (1.3–3.0)
HDL/cholesterol %	35.4±8.1 (16–52)
Triglycerides mmol·L^{-1}	1.0±0.5 (0.4–2.7)
GHbA1C %	5.6±0.3 (5.2–6.1)

Data are presented as mean±SD (range), unless otherwise stated. FSH: follicle stimulating hormone; FEV$_1$: forced expiratory volume in 1 s; % pred: % predicted; SBP: systolic blood pressure; DBP: diastolic blood pressure; LDL: low-density lipoprotein; HDL: high-density lipoprotein; GHbA1C: glycosylated haemoglobin A1C. #: only 13 subjects remembered the exact time of their menopause.

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·h^{-1}	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·h^{-1}	1.9±0.9

Data are presented as mean±SD. REM: rapid eye movement. #: 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.

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 S_{a,O_2} or demographic features were selected as predictors of the metabolic variables. In addition, AHI, ODI_3 and ODI_4 were insignificant predictors. As well as 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 $TcCO_2$ (percentage 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 SBP. Furthermore, 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, higher amplitude of the pit pattern was associated with lower DBP.

TABLE 2 Pairwise inter-relationships between metabolic variables

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

GHbA1C: glycosylated haemoglobin A1C; HDL: high-density lipoprotein; LDL: low-density lipoprotein; SBP: systolic blood pressure; DBP: diastolic blood pressure. GHbA1c, cholesterol and triglyceride levels are correlated, as are the SBP and DBP. Statistically significant correlations (r-values with $p<0.05$) are bolded.

TABLE 4 Overnight transcutaneous carbon dioxide ($TcCO_2$) and arterial oxygen saturation (Sa,O_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·h^{-1}	11.7±3.8
Pit index in REM events·h^{-1}	19.7±3.8
Pit index in NREM events·h^{-1}	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^{-1}	0.20±0.06
Percentage $TcCO_2$ >7 kPa %	38.0±44.2
ODI_4 events·h^{-1}	3.2±3.0
ODI_3 events·h^{-1}	7.9±4.9
AHI events·h^{-1}	2.1±2.0
Nadir Sa,O_2 %	86.7±4.2
Mean Sa,O_2 %	95.2±0.8

Data are presented as mean±SD. SWS: slow wave sleep (sleep stages 3 and 4); REM: rapid eye movement sleep; NREM: non-REM; ODI_x: arterial oxyhaemoglobin desaturation of x% units or more per hour; AHI: apnoea/hypopnoea index. The $TcCO_2$ features are shown in figure 1.

TABLE 5 Stepwise linear regression analysis with overnight measurements as predictors of metabolic variables

Metabolic variable	Predictors	β-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
DBP	Amplitude ($TcCO_2$ pit pattern)	-0.425	0.049
SBP	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 haemoglobin A1C; LDL: low-density lipoprotein; HDL: high-density lipoprotein; DBP: diastolic blood pressure; SBP: systolic blood pressure; $TcCO_2$: transcutaneous carbon dioxide. $TcCO_2$ pit pattern, the percentage of time $TcCO_2$ >7 kPa and maximal plateau are shown in figure 1.

DISCUSSION

In our study population of generally healthy 55-yr-old post-menopausal females, nocturnal $TcCO_2$ features were the most important predictors of GHbA1C, BP 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 Sa,O_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 what 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 females with age and post-menopausal status as risk factors, which gave us a unique setting to search for signs of emerging metabolic syndrome. CO_2, which is the final metabolic end product, played 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 apnoea (AHI, ODI_3, ODI_4 or Sa,O_2) were not found to be important in this population with relatively little SDB. This suggests that the $TcCO_2$ features predict metabolic variables independently of hypoxaemia or SDB.

The high overnight levels of $TcCO_2$ were one of the new key predictors for protective metabolic variables in our earlier study in patients with sleep apnoea [8]. This association was also found in the present study, despite an essentially different study population (healthy post-menopausal females). The longer the subjects maintained their $TcCO_2$ at >7 kPa, the lower their levels of GHbA1C and triglycerides. 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 sexes and change with increasing age, after the menopause [11] or during oestrogen therapy [24]. However, the findings are in line with the earlier $TcCO_2$ profile results and, in particular, with the results from our previous work that the proportion of high $TcCO_2$ levels measured during sleep were one of the most important features for classifying insulin resistance [8].

Obstructive sleep apnoea (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 apnoea. Therefore, it is possible that people with even mild sleep disturbances cannot achieve as high $TcCO_2$ levels as coeval healthy people because episodes of apnoea, 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 (fig. 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 (fig. 1). Normally sympathetic activity dominates during wakefulness and appears as bursts during REM sleep. NREM sleep is characterised 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.* [28] have shown that glucose metabolism is impaired with increased sympathetic tone, which has been induced by partial short-term sleep deprivation. 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, high amplitude of pit pattern was the only predictor of low DBP. 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 amplitudes of the pit patterns.

The only predictor for SBP 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 SBP. The possible protective effect of higher $TcCO_2$ is also in line with the findings in females 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 DBP. ANDERSON *et al.* [31] have previously demonstrated that high end-tidal CO_2 predicts high SBP in females. End-tidal CO_2 measures the 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, end-tidal CO_2 and $TcCO_2$ are likely to measure different phenomena. However, the results of ANDERSON *et al.* [31] show that breathing is an important contributor in the development of hypertension. The BP of OSA patients is higher than in healthy controls [32] and nocturnal hypoxia elevates BP [33]. Generally, poor sleep seems to be an important risk factor for hypertension because sleep fragmentation, arousals and short self-reported sleep duration are associated with high blood pressure [17, 34, 35]. These variables did not predict BP 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, one would expect to find some variation in our study population, even if all of the females 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 TASALI *et al.* [1] recently showed that a short SWS duration is associated with an increased risk of diabetes. This may be because of our healthy sample, as only one female had a GHbA1C >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.* [17] showed that the number of arousals predicted lower HDL cholesterol. Together, these results 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 plays 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 around the same age, the time from menopause varied (table 1). The time from menopause was not included in linear regression models as only 13 (59%) females 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, oestrogen deficiency affects metabolism [10]. Oestrogen usage was not selected as an exclusion criterion because our main goal was not to study the effects of oestrogen but to find the predictors for a wide range of metabolic variables. Moreover, grouping the subjects based on the oestrogen 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 criticised as well. The subjects were recruited through newspaper announcements, calling healthy post-menopausal females 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 BP in healthy post-menopausal females. 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.

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STATEMENT OF INTEREST

None declared.

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