UPPER AIRWAY FLOW LIMITATION AND TRANSCUTANEOUS CARBON DIOXIDE DURING SLEEP IN NORMAL PREGNANCY

Ville Rimpilä [a,e], Riina Jernman [b], Katariina Lassila [a], Jukka Uotila [b], Heini Huhtala [c], Johanna Mäenpää [a,b], Olli Polo [d,e]

[a] Faculty of Medicine and Life Sciences, PL100, 33014, University of Tampere, Tampere, Finland

[b] Department of Obstetrics and Gynaecology, PL2000, 33521, Tampere University Hospital, Tampere, Finland

[c] Faculty of Social Sciences, PL100, 33014, University of Tampere, Tampere, Finland

[d] Department of Pulmonary Diseases, PL2000, 33521, Tampere University Hospital, Tampere, Finland

[e] Unesta Research Center, Näsilinnankatu 48 D, 4th floor, 33520, Tampere, Finland

Correspondence: Ville Rimpilä, Unesta Research Center, Näsilinnankatu 48 D, 4th floor, 33520 Tampere, Finland. Email: rimpila.ville.p@student.uta.fi

ABSTRACT

Background: Sleep during pregnancy involves a physiological challenge to provide sufficient gas exchange to the fetus. Enhanced ventilatory responses to hypercapnia and hypoxia may protect from deficient gas exchange, but sleep-disordered breathing (SDB) may predispose to adverse events. Aim of this study was to analyze sleep and breathing in healthy pregnant women compared to non-pregnant controls, with a focus on CO_2 changes and upper airway flow limitation.

Methods: Healthy women on their third trimester and healthy non-pregnant women with normal BMI were recruited for polysomnography. Conventional analysis of sleep and breathing was performed. Transcutaneous carbon dioxide ($TcCO_2$) was determined for each sleep stage. Flow-limitation was analyzed using the flattening index and $TcCO_2$ values were recorded for every inspiration.

Results: Eighteen pregnant women and 12 controls were studied. Pregnancy was associated with shorter sleep duration and more superficial sleep. Apnea-hypopnea index, arterial oxyhemoglobin desaturation, flow-limitation, snoring or periodic leg movements were similar in the two groups. Mean SaO_2 and minimum SaO_2 were lower and average heart rate was higher in pregnant group. $TcCO_2$ levels did not differ between groups but variance of $TcCO_2$ was smaller in pregnant women during NREM. $TcCO_2$ profiles showed transient $TcCO_2$ peaks, which seem specific to pregnancy.

Conclusions: Healthy pregnancy does not predispose to SDB. Enhanced ventilatory control manifests as narrowing threshold of $TcCO_2$ between wakefulness and sleep. Pregnant women have a tendency for rapid CO_2 increases during sleep which might have harmful consequences if not properly compensated.

KEYWORDS: Pregnancy, transcutaneous carbon dioxide, sleep-disordered breathing, inspiratory flow-limitation, hypopnea, control of breathing

1. INTRODUCTION

Pregnancy is a challenge for the cardiorespiratory system, particularly during sleep when the body needs to rest without compromising fetal oxygen supply and carbon dioxide removal. In normal pregnancy, breathing during sleep is well preserved [1]. Plasma concentrations of progesterone are elevated during pregnancy and contribute to the increased ventilatory responses to hypoxia [2] and hypercapnia [3]. Factors compromising breathing during pregnancy are the growing uterus that elevates the diaphragm, resulting in decreased functional residual capacity of the lung. The decreased tracheal traction in turn predisposes to upper airway narrowing, and hormonal changes increase the upper airway edema. Obesity during pregnancy is an additional factor predisposing to obstructive sleep apnea or snoring [4]. Breathing abnormalities are purported to be common during pregnancy, with partial upper airway obstruction rather than obstructive sleep apnea (OSA) usually observed [5]. SDB in pregnant women is associated with intrauterine growth retardation [[6], [7]].

In sleep studies CO_2 is rarely measured, and little is known about CO_2 control during SDB, but we have previously shown the effect of progressively developing flow-limitation as well as steady flow-limitation on transcutaneous CO_2 increase [[8], [9], [10]]. CO_2 has been suggested to play a role in hypertension in preeclampsia [11]. Central chemoreceptor sensitivity to CO_2 is also increased during pregnancy [3], which could destabilize the ventilation when operated near threshold values.

The purpose of this study was to evaluate the differences in SDB and transcutaneous carbon dioxide ($TcCO_2$) parameters during normal pregnancy compared to non-gravid controls. It was hypothesized that pregnant women may have more flow-limitation and snoring compared to non-pregnant women (1). In addition, flow-limitation in pregnant women may cause greater $TcCO_2$ increase (2), and consequently, $TcCO_2$ values during sleep should differ between pregnant and non-pregnant women (3).

2. METHODS

2.1 Participants

We recruited 18 pregnant women from maternity clinics in Tampere and its nearby regions and from the antenatal outpatient clinic and antenatal ward of Tampere University Hospital. Inclusion criteria were 18-45 years of age, singleton pregnancy without fetal demise, and gestational age 33±1 weeks. Women with pre-eclampsia or other complications warranting constant monitoring and/or induction of labour were excluded.

Twelve non-pregnant women with body mass index similar to that of pregnant women in the beginning of pregnancy were chosen as controls. These women were recruited from Tampere University of Applied Sciences, Medical School of University of Tampere, and Tampere University Hospital, Department of Obstetrics and Gynecology, using recruitment posters. The study was approved by the local Ethics Committee (Identification Number R12102), and all women received oral and written information on the trial and signed a consent form before attending.

2.2 Obstetrical examination

The patients were first seen by an obstetrician (R.J.) at the antenatal outpatient clinic or ward. From the maternity card the following baseline information (standard recordings of the first maternity clinic visit in early pregnancy) was obtained: initial weight, height, body mass index (BMI), initial blood pressure, and the results of the oral glucose tolerance test if performed. Fetal ultrasound was done using Voluson ultrasound equipment (Voluson S6 ultrasound, GE Healthcare, CT, USA) to record a fetal weight estimate, amniotic fluid index (AFI), fetal movements, and to assess the flow of the umbilical artery (uA). After a minimum of 15 minute rest in a supine position in the examination room, blood pressure was measured from the right arm using a validated oscillometric technique (Omron automated manometer, M4-I Intellisense, Omron Corporation, Japan) with medium cuff-size. Weight was measured on a regular weighing scale. Urine dip stick test was analyzed for protein and glucose (Combur3 Test, Roche Diagnostics, Germany).

2.3 Sleep recordings

An overnight polysomnography was performed at Unesta Research Centre within a week after the obstetrical examination in the pregnant group. Controls visited the sleep laboratory once and all the information needed was then gathered. Recording montage contained electroencephalogram (EEG) with 8 –channels (A1,A2,O1,O2,F3,F4,C3,C4), electro-oculogram (EOG), submental electromyography (EMG), anterior tibial EMG, nasal flow (prongs/cannula), body position, and inductance plethysmography (RIP) belts, which reflect the respiratory effort of the abdomen and thorax (Somnologica, Medcare Flaga hf, Reykjavik, Iceland). The sleep investigations included also nocturnal measurement of transcutaneous partial pressure of carbon dioxide ($TcCO_2$) and transcutaneous partial pressure of oxygen (TcO_2). A parasternally fixed dual sensor ($TcCO_2$ and $TcCO_2$) warmed up to 43.0°C was used (TCM4, Radiometer, Copenhagen, Denmark).

2.4 Data analysis

Sleep was scored according to AASM (American Academy of Sleep Medicine) criteria [12], and former stages S3-S4 were used in the breath-by-breath analysis. Proprietary scoring function of Somnologica was used to score flow limitation and snoring (default flattening index of 0.13 was used) and reported as percentage of total sleep time. Episodes of apnea were scored according to AASM rules [13], hypopnea was scored when a 30% reduction of flow was observed for a minimum of 10 seconds and apnea-hypopnea-index (AHI) was calculated. Oxyhemoglobin desaturations of 3% (ODI3) or more were tabulated. Transcutaneous CO_2 values were sampled with the frequency of 1 Hz and the data values during each sleep stage were pooled to calculate the statistics, including the median and quartiles for each sleep stage. This means that each epoch produced 30 data points to corresponding sleep stage data pool. $TcCO_2$ values were also determined separately during inspirations with and without flow-limitation in each sleep stage. For this respiratory analysis the $TcCO_2$ data was advanced 30 seconds in order to correct the physiological delay between breathing and the CO_2 reading on the skin. In addition, to avoid the disproportionately marked effect of individuals with low levels of flow-limited breathing on overall data, the $TcCO_2$ data was excluded from this analysis if less than 75 data points were available in a given sleep stage. One to two data points were available from each inspiration. Accordingly, short episodes (less than 3-5 min, depending on respiratory rate) of flow-limitation were not included. Poor quality $TcCO_2$ data (missing, unphysiological behavior and drift more than 1 kPa between evening and morning wakefulness for sleep stage analysis) as well as data during calibrations were omitted. $TcCO_2$ drift corrections were not done. Technical drift occurs both upwards and downwards, which results a reduced effect of signal drift on a group level, but at the same time increases the variance of the sample which affects statistical analysis.

2.4.1 Peak analysis

The overnight profile revealed $TcCO_2$ fluctuations, which have not been reported or identified earlier in any other patient population or healthy controls. Based on initial visual observation a set of rules to identify and numerically characterize these events were established. A $TcCO_2$ peak was scored when a sudden $TcCO_2$ increase followed by decrease to baseline was observed. Since no guidelines exist for scoring this type of event, $TcCO_2$ increase of more than 0.1 kPa was chosen as a loose criterion to score a $TcCO_2$ peak. For exclusion of clear episodes of apnea and hypopnea, minimum event duration was set to 1 minute and 30 seconds. Peaks during rapid eye movement (REM) sleep were excluded due to normal ventilatory instability and fluctuating $TcCO_2$ that is commonly seen. Sleep state was required for the peak to be scored and peak events containing two or more consecutive epochs of wakefulness were excluded. Association to arousal or respiratory arousal was also scored. Respiratory arousal was scored when inspiratory flow shape improved simultaneously with arousal. The start of the peak was marked when $TcCO_2$ slope started to increase. The end of the event was marked when $TcCO_2$ returned to starting value or slope returned near zero (applied when the peak ended at higher $TcCO_2$). Signal drift was considered insignificant during these shorter events.

2.5 Statistics

The data were analyzed using SPSS (IBM SPSS Statistics for Windows, Version 24.0. Armonk, NY: IBM Corp.). The differences between pregnant and controls were analyzed with independent samples Mann-Whitney U test. Non-parametric tests were used due to small sample size and skewed distributions. Results are expressed as medians with interquartile range (IQR) except for age and BMI where full range is shown. Pearson's chi-square test was used to test the prevalence of peaks between groups. Non-parametric Levene's test was used to compare the variance of $TcCO_2$ between groups in different sleep stages. A p-value of <0.05 was considered statistically significant.

3. RESULTS

3.1 Obstetrical characteristics

Study enrollment ran from 1.1.2013 to 31.12.2015. Findings on general physical and obstetrical examination were normal. All subjects were normotensive without proteinuria. All fetuses presented with normal bioprofiles in ultrasound examination. The outcomes of the pregnancies were analyzed (possible complications during pregnancy, time and mode of delivery, birthweight, Apgar scores at 1 and 5 minutes, umbilical artery pH and base excess). Fourteen patients delivered vaginally and four via caesarean section. Gestation at birth ranged from 37+3 to 42+0 weeks. Obstetrical characteristics are presented in Table 1.

3.2 Sleep and demographics

The control population was younger than the pregnant population (26, range: 24-31, vs. 30, range: 26 -36, $p < 0.001$). There was no difference in BMI in the beginning of pregnancy compared to controls (22.0, range 19.0 – 29.4, vs. 21.7, range: 18.8 – 25.4, $p = 0.245$). Sleep quality of the pregnant women was worse compared to controls. Pregnant women had more light sleep (N1) and less deep sleep (N3) and REM sleep. Also, total sleep time (TST) was shorter and wake after sleep onset (WASO) was longer in pregnant women. Clear difference between groups was seen on oxyhemoglobin parameters (SaO_2), which were lower in pregnant women. Considerable increase in average heart rate was observed in pregnant women also. Common sleep-disordered breathing parameters AHI and ODI3 showed no difference. Additional measures

that were used (FL-index (%) and snore time (%)) did not show statistical difference. Two pregnant subjects and one control subject were identified as significant snorers with episodes of prolonged flow limitation. Detailed values are presented in Table 2.

Table 1. Obstetrical characteristics of 18 healthy pregnant women participating in the sleep study.

Subject	Age	P	US	RR	BMI	Del	Mode	Sex	BW	Apgar	uApH
1	36	2	34+6	130/75	25.7	40+3	V	Boy	3740	9/9	7.28
2	29	1	32+1	108/69	27.3	39+4	V	Boy	3605	9/10	7.26
3	29	0	31+4	112/72	21.0	42+0	V	Girl	3300	9/9	7.28
4	36	2	33+2	118/82	NA	39+1	CS	Boy	3470	9/9	7.37
5	31	0	33+2	102/67	21.6	40+6	CS	Girl	3740	9/9	7.34
6	36	0	33+0	122/71	25.8	40+2	V	Boy	3650	8/9	7.21
7	36	2	32+6	124/79	27.7	39+0	V	Boy	3120	9/9	7.19
8	29	0	32+6	121/72	29.4	40+1	CS	Girl	3080	9/8	7.34
9	29	0	32+3	99/51	25.0	41+3	V	Girl	3260	7/8	7.17
10	29	3	32+4	104/58	19.2	39+0	V	Girl	3195	9/10	7.24
11	31	1	32+4	120/76	33.0	38+3	V	Boy	3800	9/9	7.17
12	30	1	31+4	112/84	22.0	39+3	V	Boy	3920	8/9	7.30
13	29	0	34+0	108/72	28.0	41+1	CS	Girl	4480	8/8	7.36
14	30	0	31+2	118/74	21.6	39+6	V	Boy	3255	7/9	7.13
15	35	0	32+4	112/70	19.2	37+3	V	Girl	2385	8/8	7.17
16	34	0	31+5	113/77	19.0	41+1	V	Girl	3340	8/9	7.28
17	30	0	33+4	110/80	23.0	39+4	V	Girl	3250	6/9	7.20
18	26	0	34+0	124/74	22.0	42+0	V	Girl	4190	9/9	7.20

P=parity; US=ultrasound scan; RR=blood pressure at ultrasound visit; BMI=body mass index in the beginning of pregnancy; Del=gestation at delivery, weeks+days; Mode=mode of delivery; V=vaginal; CS=ceasarean section; BW=birth weight (grams); Apgar=Apgar score 1 min/5 min; uApH=umbilical artery pH at birth; NA=not available

Table 2. Polysomnography data

	Pregnant median (Q1-Q3)	Controls median (Q1-Q3)	P
TST (min)	361.0 (330.5 – 418.9)	415.5 (391.5 – 429.4)	0.007
Sleep onset (min)	17.5 (11.5 – 27.6)	21.5 (15.1 – 27.0)	0.518
WASO (min)	110.5 (58.5 – 151.1)	39.7 (21.8 – 77.0)	0.003
Sleep Efficiency (%)	76.7 (68.3 – 88.6)	91 (85.0 – 95.0)	0.002
N1 sleep (min)	30.3 (25.5 – 45.3)	21.0 (16.3 – 30.8)	0.004
N2 sleep (min)	141.8 (96.0 – 168.0)	140.5 (109.9 – 174.0)	0.884
N3 sleep (min)	118.5 (97.5 – 143.6)	156.5 (133.3 – 190.8)	0.009
REM sleep (min)	53.0 (40.3 – 69.5)	91.5 (73.5 – 99.6)	0.001
AHI (#/h)	1.55 (0.73 – 3.25)	0.75 (0.23 – 1.45)	0.124
ODI3 (%)	0.4 (0 – 2.1)	0.2 (0 – 0.38)	0.200
SaO_2 mean (%)	95.9 (95.2 – 96.3)	97.5 (96.1 – 97.9)	<0.001
SaO_2 min (%)	93.0 (91.5 – 94.0)	94.5 (93.3 – 95.8)	0.008
FL-index (%)	11.3 (6.1 – 23.6)	10.10 (6.3 – 18.4)	0.573
Snore time (%)	0 (0 – 0.33)	0 (0 – 0.08)	0.914
PLM (#/h)	2.4 (0 – 5.2)	0.5 (0 – 1.4)	0.296
Avg. HR (bpm)	71.7 (64.3 – 79.0)	59.6 (55.7 – 66.9)	0.002

Data presented with medians and IQR. TST = total sleep time, WASO = wake after sleep onset, N1,N2,N3 = Non rapid eye movement (NREM) sleep, REM sleep = rapid eye movement sleep, AHI = apnea-hypopnea-index, ODI3 = oxyhemoglobin desaturation for 3% or more, FL-index = percentage of flow limited breaths during sleep, PLM = periodic leg movements, Avg. HR = average heart rate.

3.3 $TcCO_2$

In terms of $TcCO_2$ levels (absolute or relative) there were no differences in $TcCO_2$ between the pregnant and control group in any of the sleep stages (Table 3). In general, the pregnant women had a wider range for $TcCO_2$ when absolute values were used. However, we discovered that in pregnant women the variance of $TcCO_2$ across sleep stages became smaller for NREM when $TcCO_2$ during wakefulness was used as the reference value (Table 3 and Figure 1). Sleep stage and $TcCO_2$ analysis was possible only for 15 pregnant and 8 non-pregnant women due to $TcCO_2$ signal drift and poor data in three pregnant woman and four controls. Inspiratory flow-limitation did not cause greater $TcCO_2$ increase in pregnant women compared to controls in any of the sleep stages (data not shown).

Table 3. Absolute and relative transcutaneous CO_2 data for sleep stages

	Pregnant (N=15) median (Q1-Q3)	Controls (N=8) median (Q1-Q3)	p	p (variance)
$TcCO_2$, absolute (kPa)				
Wake	6.19 (5.41 – 6.65)	6.16 (5.96 – 6.51)	0.776	
N1 sleep	6.28 (5.35 – 6.75)	6.49 (6.04 – 6.74)	0.428	
N2 sleep	6.28 (5.43 – 6.75)	6.57 (5.97 – 6.89)	0.392	
N3 sleep	6.32 (5.92 – 6.65)	6.52 (5.97 – 7.08)	0.506	
REM sleep	6.41 (5.28 – 6.95)	6.39 (5.72 – 6.78)	0.825	
NREM sleep	6.29 (5.48 – 6.67)	6.56 (5.98 – 7.01)	0.357	
$\Delta TcCO_2$ (kPa)				
Wake (reference)	0	0	-	
N1 sleep	0.08 (-0.03 – 0.18)	0.20 (0.03 – 0.61)	0.115	0.323
N2 sleep	0.12 (0.05 – 0.29)	0.24 (-0.04 – 0.65)	0.506	0.213
N3 sleep	0.15 (0.08 – 0.38)	0.21 (0.04 – 0.53)	0.825	0.075
REM sleep	0.15 (0.08 – 0.43)	0.16 (- 0.15 – 0.49)	0.925	0.067
NREM sleep	0.11 (0.07 – 0.22)	0.24 (0.03 – 0.62)	0.506	0.038

Data presented with medians and IQR. $TcCO_2$ = transcutaneous carbon dioxide, NREM = non-rapid eye movement, REM = rapid eye movement, p (variance) denotes Levene's test.

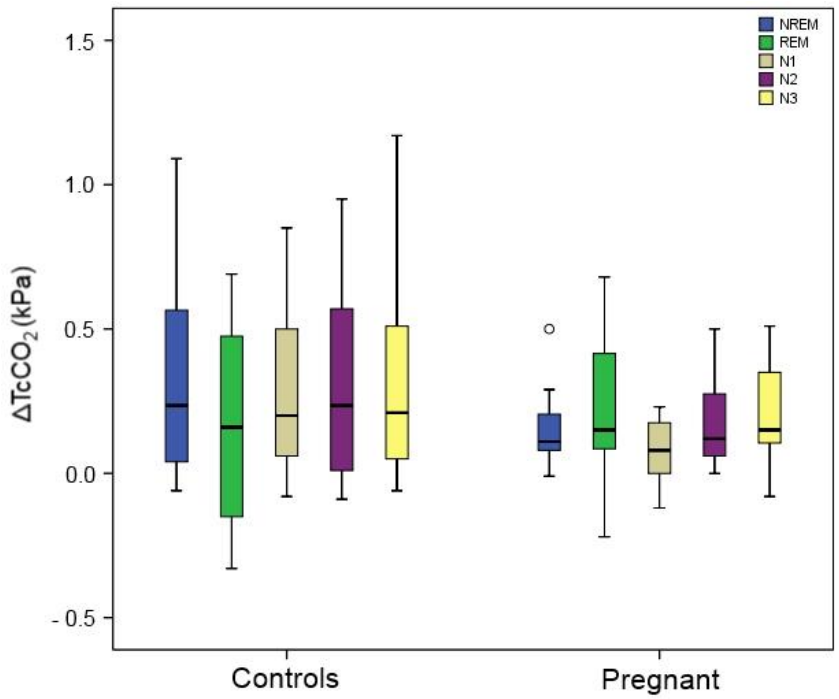

Figure 1. Relative $TcCO_2$ in pregnant and controls during different sleep stages. Wakefulness was assigned as 0 kPa and sleep stages are relative to that. $TcCO_2$ levels did not differ between groups, but the variance

(Levene´s test) was different for pooled NREM. Boxplots show median values with inter-quartile range and min-max. Circle denotes outlier.

3.3.1 Transcutaneous carbon dioxide peaks

A further look into $TcCO_2$ overnight profiles of the pregnant group showed a number of distinctive peaks (Figures 2 and 3), which are not commonly seen in overnight profiles of non-pregnant subjects. Eleven of 18 pregnant women had these peaks, whereas 4 of 12 non-pregnant women had similar $TcCO_2$ peaks ($p = 0.136$). Altogether, only 5 peaks were observed in the control population versus 78 peaks in the pregnant population (0.42 peaks/person in controls vs. 4.33 peaks/person in pregnant, $p= 0.028$) rendering the peaks approximately 10-times more frequent in the pregnant population. Mean amplitude of the peak was 0.20 kPa with duration of 241 seconds (range 92 – 430 seconds). Mean slopes were 0.11 kPa/min upwards and 0.09 kPa/min downwards. When present, the number of peaks per person ranged from 2 to 22 in pregnant women. Fifty-two $TcCO_2$ peaks (62.7 %) were associated with clear inspiratory flow-limitation (Figure 2). Respiratory arousal was associated with 14 peaks and arousal with 37 peaks. Amplitude criterion of hypopnea was reached in 24 peaks and apnea in one peak. Oxyhemoglobin desaturation of 3 to 4 % was associated with 8 events.

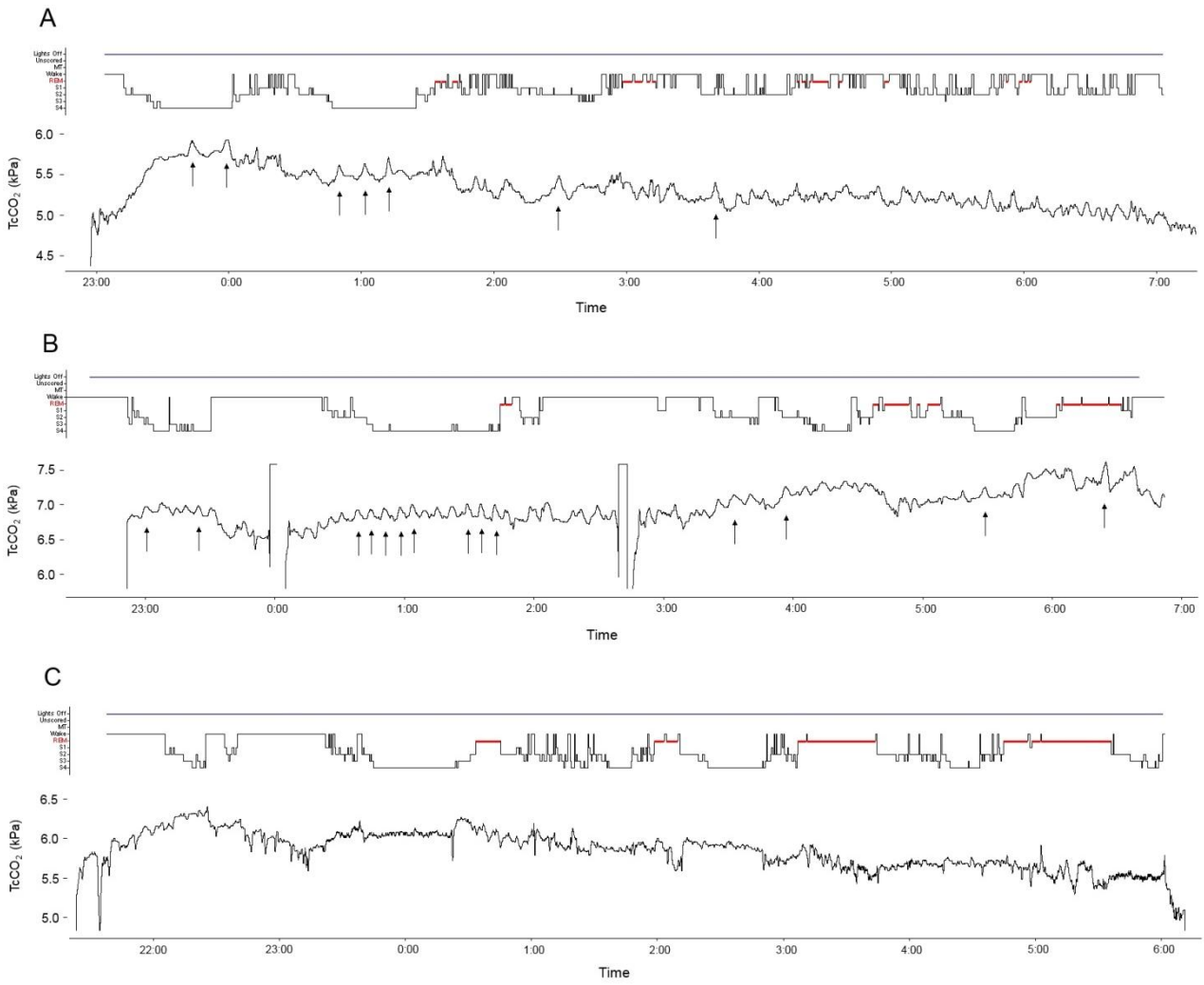

Figure 2. Three examples of overnight $TcCO_2$ profile with hypnogram. For drift assessment, we expect that the wakefulness $tcCO_2$ should be the same in the evening and in the morning. Also, the plateau levels during SWS should repeat at about same levels. A) A pregnant woman with $TcCO_2$ peaks (arrows) associated with short episodes of flow-limitation. Overnight flow-limitation index was 4.3 %. Downward trend of the signal is considered physiological, related to respiratory stimuli from circadian process and/or sleep fragmentation. B) A pregnant woman with $TcCO_2$ peaks (arrows) without flow-limitation episodes. Overnight flow-limitation index was 4.7 %. A minor upwards drift is probable, since the wakefulness levels are higher in the morning. The lower fluctuating $tcCO_2$ level during the early part of the recording seems to hinder $tcCO_2$ increase to the so called plateau level during SWS, so drift may seem more accentuated than what it really is. C) A control woman without any $TcCO_2$ peaks. Overnight flow-limitation index was 3.1 %. Decreasing trend of the plateau levels and evening/morning wakefulness levels suggest a technical drift.

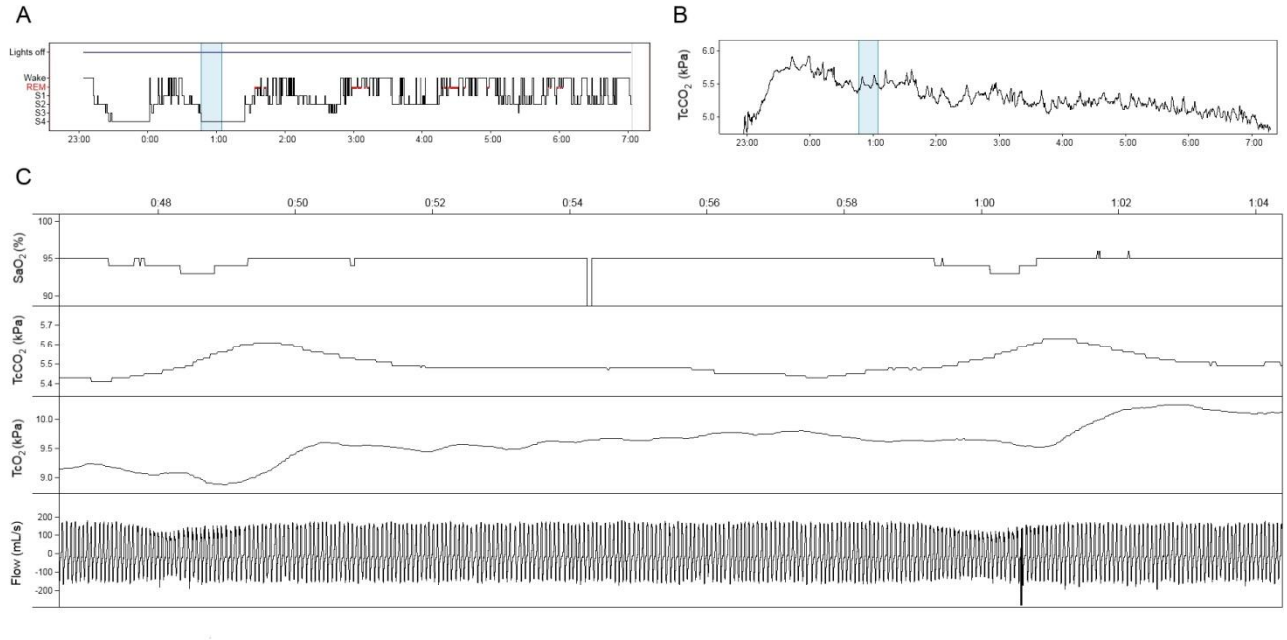

Figure 3. A pregnant woman with two transcutaneous CO_2 peaks associated with flow-limitation episodes. Upper left panel shows the hypnogram (A). Upper right panel shows the overnight $TcCO_2$ (B). Bottom panel (C) shows a detailed 18-minute time window, from darkened area shown in upper panels. Traces: SaO_2 (%) = arterial oxyhemoglobin saturation; TcO_2 (kPa) = transcutaneous partial pressure of oxygen; $TcCO_2$ (kPa) = transcutaneous partial pressure of carbon dioxide; Flow (ml/s) = flow at nasal prongs.

4. DISCUSSION

We studied sleep and breathing parameters in 18 healthy women during pregnancy weeks 31-34, and in 12 healthy non-gravid controls. Pregnant women slept less and stayed less time in slow wave sleep and REM sleep. No difference was observed between groups in terms of flow-limitation or snoring, AHI or PLM. However, pregnant women had lower average and minimum SaO_2 levels and increased heart rate. Unexpectedly, the $TcCO_2$ levels showed no difference between groups during different sleep stages, only the variance of NREM $TcCO_2$ was smaller in the pregnant group. These findings suggest that the $TcCO_2$, which represents the peripheral pCO_2 at the tissue level, remains unchanged and is carefully controlled

during pregnancy. The most intriguing finding of our study was that more than half of the pregnant women presented with transient $TcCO_2$ increases in a pattern that to our knowledge has not been previously described in any other control or patient population. Post-hoc analysis showed that most of these $TcCO_2$ increases were associated with transient episodes of inspiratory flow-limitation, although in some pregnant women flow limitation was absent. The transient $TcCO_2$ increases in pregnant women are in contrast with otherwise strictly controlled $TcCO_2$ and may reveal episodes when the maintenance of homeostasis is challenged.

Our study corroborates previous findings that sleep quality is diminished during pregnancy [14]. Total sleep time was shorter with 40 minute reductions in N3 and REM sleep and increase in N1 sleep. We showed that breathing is only minimally disturbed in late pregnancy, a finding that has been reported several times before. A recent study performed by Sarberg et al. [15] had a similar, albeit larger, healthy study population with comparable results of very low levels of AHI, ODI and nadir SaO_2 from the respiratory recordings. We observed lower levels of snoring than have been previously reported, but this difference may result from methodological differences.

The average SaO_2 during sleep was lower in pregnant women compared to the controls. This is in line with earlier findings showing progressively decreasing PaO_2 levels [16] from second to third trimester of pregnancy seen in supine position. The fact that PaO_2 does not decrease during pregnancy in sitting position [16] suggests that also the lower SaO_2 in our study during sleep is associated with body position and the progressive impact of elevating diaphragm on functional residual capacity (FRC) and oxygenation [17]. The cardiac output increases during pregnancy [18] but also becomes sensitive to body position suggesting compression of inferior vena cava by uterus [19]. Decreased venous return to the heart could contribute to the observed increasing heart rate in the third trimester since stroke volume decreases from the second trimester [18].

The role of CO_2 in SDB during pregnancy has been speculated [[11], [20]], as well as in preeclampsia [11]. CO_2 measurements are not routinely measured during adult sleep studies [21], mainly due to lack of simple, robust and noninvasive methods of CO_2 monitoring. The reference measurement is partial pressure of the arterial CO_2 ($PaCO_2$), which is a reliable measure of ventilation but requires blood samples and is not suitable for sleep studies. End-tidal CO_2 ($EtCO_2$) is noninvasive and allows for continuous monitoring of the mixed venous CO_2 provided that the tidal volumes are sufficient to produce readable end-tidal CO_2-plateau: this may be a challenge in subjects with mouth breathing, sleep-disordered-breathing, nocturnal hypoventilation or usage of CPAP mask, which is often the case in subject whom this type of measurement is indicated [13].

Transcutaneous carbon dioxide measurement is probably best adapted for sleep recordings. It reflects both ventilation and peripheral blood perfusion [[22], [23]]. The main issues with $TcCO_2$ are slow response time and potential signal drift during prolonged recording [[24], [25]]. The overnight $tcCO_2$ profiles can display either technical drift or physiological trends. The technical drifts can be related to changes in the contact between the sensor and the skin after changes in body position but may also occur within the sensor. The physiological trends arise from changes in control of breathing and circulation at the system level or changes in metabolism and blood perfusion locally. The $tcCO_2$ signal is a combination of ventilation at system level and perfusion at local level, which are both under control of the sympathetic nervous system. The sympathetic tone from hypothalamic origin displays a circadian rhythm with high tone during wakefulness and a nadir tone around 03-04 am. During the morning hours this sympathetic tone starts to

increase and is potentially reflected as a decreasing trend in $tcCO_2$, as superficial skin perfusion starts to increase. A physiological trend may also occur, if the proportion of SWS decreases and the proportion of sleep stages N1-N2, REM or wake increase as they often do during the latter part of the night. This is the likely explanation of decreasing trend in Figure 2 panel A, since the wakefulness levels are the same before sleep onset and after sleep. Frequent arousals from sleep decrease the $tcCO_2$ (hyperventilation) whereas periods of upper airway flow-limitation increase it (hypoventilation). Long or repeated periods of flow-limitation may also cause a physiological $tcCO_2$ increase over time. In the populations of the current study, prolonged flow-limitation was rare and its contribution to $tcCO_2$ trends was unlikely. We conclude that at present, it is not possible to distinguish between technical and physiological trends. Therefore, we preferred only to exclude cases with extreme trend/drifts but include signals with limited drift.

We are not aware of previous reports of $TcCO_2$ during sleep in pregnancy. Therefore, we can only compare our results with studies using other methods, including also measurement while awake. It is well established that the minute ventilation increases and $PaCO_2$ decreases during pregnancy [26]. This effect is considered as an exaggeration of ventilation to ensure sufficient gas exchange to the fetus and is at least partly driven by increasing levels of progesterone during pregnancy [3]. Against our expectations, we failed to show decreased $TcCO_2$-levels in pregnant women. There are several possible explanations for this observation. First, the sensitivity or accuracy of the $TcCO_2$ method is not sufficient to demonstrate difference. This interpretation is not supported by the observed lower variance in our larger study group (15 pregnant women vs. eight controls), which may reflect increased chemoreceptor sensitivity [3]. The $PaCO_2$ increases during sleep [27] but decreases during pregnancy while awake [26]. When using $tcCO_2$, we see sleep-related increase but not pregnancy-related decrease while awake (Table 3), which should be of about similar magnitude. This suggests that the effects of sleep and pregnancy on the $TcCO_2$ are mediated through different mechanisms. One possible explanation for similar $TcCO_2$ values could be that the $TcCO_2$ measures the combination of the increased metabolic rate with increased CO_2 production and decreased $PaCO_2$, resulting in no change. However, the lower partial pressure of CO_2 in the arteries and higher heart rate during pregnancy could represent adaptive measures to maintain constant pCO_2 at the tissue level.

Discovering the $TcCO_2$ peaks in the healthy pregnant women (but not in controls) is new and seems contradictory in relation to the mechanisms mentioned above. One would assume that changes in $TcCO_2$ should be minimal. Yet, visually detectable transient $TcCO_2$ increases are common pregnancy specific findings. The transient $TcCO_2$ increases with upper airway flow limitation can be explained as obstructive events. Partial upper airway collapse decreases minute ventilation, which results in transient CO_2 increase followed by corrective response. We have previously shown that upper airway flow limitation during sleep is associated with increasing $TcCO_2$ [[8], [9]]. There are several reasons for upper airway narrowing during pregnancy, including upper airway edema and decreased tracheal traction due to pregnancy induced elevation of the diaphragm. On the other hand, tight respiratory control during pregnancy ensures that the minor upper airway obstructive event is promptly corrected and does not develop into an episode of obstructive sleep apnea. Examples of transient $TcCO_2$ increases with flow limitation are presented in Figure 2A and Figure 3. These episodes may play a role in pre-eclampsia [11].

In contrast to $TcCO_2$ peaks associating with flow-limitation, in some individuals the phenomenon is more likely to be associated with unstable respiratory control. Different type of $TcCO_2$ pattern is seen in Figure 2B. This fluctuation was counted as peaks, but since flow-limitation is not observed it can be speculated that ventilatory plant gain is increased (small changes in ventilation cause augmented CO_2/O_2 response). Slight fluctuation of ventilation is observed during these peaks in slow-wave sleep which indicates an

overall increase in loop gain, as ventilation during slow-wave sleep is usually very stable. Respiratory stimulation during pregnancy probably prohibits the development of (central) apneic behavior because reaching the apneic threshold would require further increase in ventilation [27]. In fact a recent study showed that central sleep apnea is almost non-existent during late pregnancy [20]. This could also explain why the observed large fluctuations in $TcCO_2$ are accompanied by very little waxing and waning behavior. In our previous studies we have used the concept of $TcCO_2$ plateau to describe optimal $TcCO_2$ during sleep and normal breathing [9]. If this level is exceeded, respiratory efforts increase. Figure 2A represents this idea well during the first sleep cycle; flow-limitation causes upward deviation of the $TcCO_2$ from the target level, but compensatory mechanisms quickly restore the optimal level. On the other hand, if a disturbance such as an arousal decreases CO_2 from the target level, the ventilation is repressed in order to restore that optimal level. Extreme case is the momentary repression to zero when apneic threshold is reached. Pregnant woman in figure 2B presumably fluctuates around the plateau level since there is neither flow-limitation nor hypopneic breathing present. Our results show the dynamic behavior of CO_2 during sleep in pregnancy, which could benefit the understanding of SDB during this vulnerable state. It remains to be investigated whether the CO_2 events observed here are prolonged or exaggerated in risk populations and how they may contribute to pathophysiological processes.

The strength of this study is the use of overnight $TcCO_2$ measurement and a healthy population. Changes that are seen in $TcCO_2$ profile should be considered as normal findings, which is useful when $TcCO_2$ is measured in high-risk populations or in pre-eclamptic patients. In addition, a full polysomnographic recording allowed detailed determination of sleep. Determination of $PaCO_2$ during sleep would have been informative, but being invasive and uncomfortable procedure it would have disturbed sleep unduly. $EtCO_2$ measurement would have also been useful, but was not available for this study. The number of subjects in this exploratory study is quite small and thus decreases the statistical power. However, recruitment of women for an overnight sleep recording in late pregnancy is challenging, particularly when there exists no previous measurement standards for all the events. Hence, a novel method for scoring $TcCO_2$ peaks was developed and used in this study.

Our approach for respiratory analysis has limitations, which should be considered when evaluating the data. First, the use of automated scoring function for breathing may not be optimal, but subject-to-subject variation is minimal and systematic error is similar between subjects. Second, the $TcCO_2$ signal is known to drift and this will have an effect if normal breathing and flow-limited breathing occur at different times during the night. In order to counter this effect, data with large differences between the evening and morning were discarded. Also, large fluctuations in $TcCO_2$ signal without any discernible change in ventilation were considered erroneous. Some individuals had virtually no flow-limitation and in order to avoid overrepresentation in the data, data from sleep stages with small amounts of flow-limited breathing were excluded.

For the analysis of sleep stage $tcCO_2$ the data was pooled from the whole night to give a sleep stage specific $tcCO_2$ value. In the case of signal drift this approach is problematic since it introduces bias. Signal drift downwards will give too low values and upward drift will give too high $tcCO_2$ values. Technical drift however, occurs in both directions and when the data is analyzed on a group level the effect of drift is diminished to some extent. Unfortunately the variance is increased at the same time, which makes it more difficult to see statistical differences between groups. This may explain why we failed to see differences between sleep stages. Despite of these limitations we see that our data gives a detailed view of overnight CO_2 dynamics that should be further studied.

Our results open new perspectives to cardiorespiratory monitoring during sleep and pregnancy. In healthy individuals, even subtle changes in ventilation may lead to notable changes in $TcCO_2$. Our subjects were healthy and had a relatively low level of sleep-disordered breathing, but in individuals with complicated pregnancies upper airway flow-limitation could result in marked $(Tc)CO_2$ changes affecting heart and endothelial function. CO_2 increase related to flow-limitation may contribute to overnight increase in blood pressure [11] that is reversible with treatment. Identification of clinically significant $TcCO_2$ patterns could in the future guide to treatment decisions in symptomatic patients with low AHI. Flow limitation can be effectively controlled with CPAP (continuous positive airway pressure), a mode of therapy which helps to stabilize CO_2 levels and sleep.

5. CONCLUSIONS

In conclusion, during normal pregnancy sleep-disordered breathing is very mild and comparable to non-pregnant controls. In contrast to the earlier findings of decreased $PaCO_2$ during pregnancy, the absolute levels of $TcCO_2$ showed no difference between the groups. However, the variance of $TcCO_2$ is smaller in the pregnant group when relative values are used. We suggest that the decreased $PaCO_2$ during pregnancy is an adaptive measure to maintain constant pCO_2 at the tissue level. Transient changes are seen in overnight $TcCO_2$ profile, which reflects altered respiratory control. Further studies with a larger population are evidently needed in the field of sleep disorders during pregnancy and different pregnancy complications.

6. FUNDING

V.R. was supported by grants from Tampere Tuberculosis Foundation, Foundation of the Finnish Anti-Tuberculosis Association, Finnish Research Foundation of Pulmonary Diseases and Finnish Sleep Research Society. K.L. was supported by Finnish Research Foundation of Pulmonary Diseases. O.P. was supported by Tampere Tuberculosis Foundation. Funding bodies were not involved in any aspect of the study.

7. REFERENCES

[1] Nikkola E, Ekblad U, Ekholm E, Mikola H, Polo O. Sleep in multiple pregnancy: Breathing patterns, oxygenation, and periodic leg movements. Am J Obstet Gynecol. 1996 May;174(5):1622-5.

[2] Moore LG, McCullough RE, Weil JV. Increased HVR in pregnancy: Relationship to hormonal and metabolic changes. J Appl Physiol (1985). 1987 Jan;62(1):158-63.

[3] Jensen D, Wolfe LA, Slatkovska L, Webb KA, Davies GA, O'Donnell DE. Effects of human pregnancy on the ventilatory chemoreflex response to carbon dioxide. Am J Physiol Regul Integr Comp Physiol. 2005 May;288(5):R1369-75.

[4] Maasilta P, Bachour A, Teramo K, Polo O, Laitinen LA. Sleep-related disordered breathing during pregnancy in obese women. Chest. 2001 Nov;120(5):1448-54.

[5] Connolly G, Razak AR, Hayanga A, Russell A, McKenna P, McNicholas WT. Inspiratory flow limitation during sleep in pre-eclampsia: Comparison with normal pregnant and nonpregnant women. Eur Respir J. 2001 Oct;18(4):672-6.

[6] Franklin KA, Holmgren PA, Jonsson F, Poromaa N, Stenlund H, Svanborg E. Snoring, pregnancy-induced hypertension, and growth retardation of the fetus. Chest. 2000 Jan;117(1):137-41.

[7] Pamidi S, Marc I, Simoneau G, Lavigne L, Olha A, Benedetti A, et al. Maternal sleep-disordered breathing and the risk of delivering small for gestational age infants: A prospective cohort study. Thorax. 2016 Aug;71(8):719-25.

[8] Rauhala E, Himanen SL, Saastamoinen A, Polo O. Prolonged spiking in the emfit sensor in patients with sleep-disordered breathing is characterized by increase in transcutaneous carbon dioxide. Physiol Meas. 2007 Oct;28(10):1163-73.

[9] Rimpilä V, Saaresranta T, Huhtala H, Virkki A, Salminen AV, Polo O. Transcutaneous CO(2) plateau as set-point for respiratory drive during upper airway flow-limitation. Respir Physiol Neurobiol. 2014 Jan 15;191:44-51.

[10] Rimpila V, Hosokawa K, Huhtala H, Saaresranta T, Salminen AV, Polo O. Transcutaneous carbon dioxide during sleep-disordered breathing. Respir Physiol Neurobiol. 2015 Dec;219:95-102.

[11] Edwards N, Blyton DM, Kirjavainen T, Kesby GJ, Sullivan CE. Nasal continuous positive airway pressure reduces sleep-induced blood pressure increments in preeclampsia. Am J Respir Crit Care Med. 2000 Jul;162(1):252-7.

[12] Iber C, Ancoli-Israel S, Chesson A, Quan SF. The AASM manual for the scoring of sleep and associated events: Rules, terminology and technical specifications. 1st. ed. ed. Iber C, Ancoli-Israel S, Chesson A,L.., Quan S,F., editors. Westchester, Illinois: American Academy of Sleep Medicine; 2007.

[13] Berry RB, Budhiraja R, Gottlieb DJ, Gozal D, Iber C, Kapur VK, et al. Rules for scoring respiratory events in sleep: Update of the 2007 AASM manual for the scoring of sleep and associated events. deliberations of the sleep apnea definitions task force of the american academy of sleep medicine. J Clin Sleep Med. 2012 Oct 15;8(5):597-619.

[14] Bourjeily G, Fung JY, Sharkey KM, Walia P, Kao M, Moore R, et al. Airflow limitations in pregnant women suspected of sleep-disordered breathing. Sleep Med. 2014 May;15(5):550-5.

[15] Sarberg M, Bladh M, Josefsson A, Svanborg E. Sleepiness and sleep-disordered breathing during pregnancy. Sleep Breath. 2016 Apr 16.

[16] Spiropoulos K, Prodromaki E, Tsapanos V. Effect of body position on PaO2 and PaCO2 during pregnancy. Gynecol Obstet Invest. 2004;58(1):22-5.

[17] Prodromakis E, Trakada G, Tsapanos V, Spiropoulos K. Arterial oxygen tension during sleep in the third trimester of pregnancy. Acta Obstet Gynecol Scand. 2004 Feb;83(2):159-64.

[18] Mahendru AA, Everett TR, Wilkinson IB, Lees CC, McEniery CM. A longitudinal study of maternal cardiovascular function from preconception to the postpartum period. J Hypertens. 2014 Apr;32(4):849-56.

[19] Trakada G, Tsapanos V, Spiropoulos K. Normal pregnancy and oxygenation during sleep. Eur J Obstet Gynecol Reprod Biol. 2003 Aug 15;109(2):128-32.

[20] Bourjeily G, Sharkey KM, Mazer J, Moore R, Martin S, Millman R. Central sleep apnea in pregnant women with sleep disordered breathing. Sleep Breath. 2015 Sep;19(3):835-40.

[21] Gerdung CA, Adeleye A, Kirk VG. Noninvasive monitoring of CO2 during polysomnography: A review of the recent literature. Curr Opin Pulm Med. 2016 Nov;22(6):527-34.

[22] Stock MC. Noninvasive carbon dioxide monitoring. Crit Care Clin. 1988 Jul;4(3):511-26.

[23] Clark JS, Votteri B, Ariagno RL, Cheung P, Eichhorn JH, Fallat RJ, et al. Noninvasive assessment of blood gases. Am Rev Respir Dis. 1992 Jan;145(1):220-32.

[24] 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 May;95(5):331-5.

[25] Berlowitz DJ, Spong J, O'Donoghue FJ, Pierce RJ, Brown DJ, Campbell DA, et al. Transcutaneous measurement of carbon dioxide tension during extended monitoring: Evaluation of accuracy and stability, and an algorithm for correcting calibration drift. Respir Care. 2011 Apr;56(4):442-8.

[26] Machida H. Influence of progesterone on arterial blood and CSF acid-base balance in women. J Appl Physiol Respir Environ Exerc Physiol. 1981 Dec;51(6):1433-6.

[27] Dempsey JA. Crossing the apnoeic threshold: Causes and consequences. Exp Physiol. 2005 Jan;90(1):13-24.