Apnoea–hypopnoea index of 5 events·h^{-1} as a metabolomic threshold in patients with sleep complaints

Ott Kiens[1,2], Egon Taalberg[3,4], Viktoria Ivanova[2], Ketlin Veeväli[5], Triin Laurits[5], Ragne Tamm[5], Aigar Ottas[3,4], Kalle Kilk[3,4], Ursel Soomets[3,4] and Alan Altraja[1,2]

[1]Department of Pulmonary Medicine, University of Tartu, Tartu, Estonia. [2]Lung Clinic, Tartu University Hospital, Tartu, Estonia. [3]Institute of Biomedicine and Translational Medicine, University of Tartu, Tartu, Estonia. [4]Centre of Excellence for Genomics and Translational Medicine, University of Tartu, Tartu, Estonia. [5]Psychiatry Clinic, Tartu University Hospital, Tartu, Estonia.

Corresponding author: Alan Altraja (alan.altraja@ut.ee)

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The metabolomic breakpoint coincides with the apnoea–hypopnoea index threshold of ⩾5 events·h^{-1} required to confirm the diagnosis of obstructive sleep apnoea in patients with sleep complaints https://bit.ly/3SIswFC

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Abstract

Background The apnoea–hypopnoea index (AHI) forms the basis for severity of obstructive sleep apnoea (OSA), a condition expected to reprogramme metabolic pathways in humans. We aimed to identify the AHI breakpoint from which the majority of significant changes in the systemic metabolome of patients with sleep complaints occur.

Methods In a prospective observational study on symptomatic individuals, who underwent polysomnography for the diagnosis of OSA, profiles of 187 metabolites including amino acids, biogenic amines, acylcarnitines, lysophosphatidylcholines, phosphatidylcholines and sphingomyelins were analysed with liquid chromatography mass spectrometry in peripheral blood drawn at three different time points overnight. Comparisons of rank-transformed data using a general linear model for repeated measures after dichotomising the study group at different AHI levels were applied to define the best cut-off based on Cohen's f.

Results 65 subjects were recruited with a median AHI of 15.6 events·h^{-1}. The mean Cohen's f over the metabolites was highest (0.161) at an AHI level of 5 events·h^{-1} representing the metabolomic threshold. Of the particular between-group differences, eight phosphatidylcholines, nine acylcarnitines and one amino acid (threonine) had significantly lower concentrations in the individuals with an AHI level equal to or above the metabolomic threshold. The metabolomic changes at AHI levels defining moderate and severe OSA were smaller than at an AHI of 5 events·h^{-1}.

Conclusions The metabolomic threshold for patients with sleep complaints described in this report for the first time coincides with the AHI threshold required to confirm the diagnosis of OSA.

Introduction

Obstructive sleep apnoea (OSA) is diagnosed in the presence of symptoms (sleepiness, snoring, *etc*.) together with the occurrence of obstructed breathing events, hypopnoeas and apnoeas, during sleep [1]. OSA is diagnosed firstly in the presence of symptoms and/or concomitant diseases together with apnoea–hypopnoea index (AHI) ⩾5 events·h^{-1} during a night-time sleep study or secondly in the presence of AHI ⩾15 events·h^{-1} with or without the presence of symptoms/concomitant diseases [1, 2]. Untreated OSA increases the risk of cardiovascular disease [3, 4], motor vehicle accidents [5] and exacerbations and mortality of chronic airways disease [6].

OSA is expected to reprogramme metabolomic pathways due to mechanisms that include intermittent hypoxia, oral microbiome and inflammatory processes [7]. In metabolomic studies on individuals with OSA, the samples are taken often in the morning [8] and studies targeting dynamic changes in the

metabolome between multiple samples or sleep-time sampling, when OSA related changes should be at their peak, have been scarce. Some studies have used untargeted metabolomic analysis [8] that cannot be reliably replicated in separate laboratories [9]. However, targeted analyses have shown better interlaboratory reproducibility [10].

In-hospital polysomnography (PSG) measures the amount of apnoeas and hypopnoeas per hour of sleep, termed the AHI, whereas at-home polygraphy counts the breathing events (apnoeas and hypopnoeas with oxyhaemoglobin desaturation of ⩾4%) for the entire recording time, known as respiratory-event index [4]. In 1999, OSA was first classified into mild (AHI ⩾5 and <15 events·h^{-1}), moderate (AHI ⩾15 and <30 events·h^{-1}) and severe (AHI ⩾30 events·h^{-1}), based on AHI [11]. In recent years, the solely AHI-based classification of severity of OSA has received heavy criticism [12], because different definitions are used for the diagnosis of hypopnoea and the resulting AHI thresholds are not calibrated according to the definition used [13]. Also, there are many relevant variables to consider in assessing the clinical severity of OSA, *e.g.* degree of daytime sleepiness, cognitive function, occupation, presence of concomitant diseases, etc. [13]. The minimal AHI threshold for diagnosing OSA (5 events·h^{-1}) has the largest evidence to show that the deleterious health effects of OSA start from that level [5, 11, 14]. Therefore, a minimal diagnostic AHI value of ⩾5 events·h^{-1} would still be useful to diagnose OSA, whereas using AHI to classify the severity of OSA could be invalid [12].

AHI thresholds have been studied previously to find where AHI starts to affect different outcomes, such as cardiometabolic risk [15]. To our knowledge, there have been no studies investigating the AHI level, from where the majority of significant changes in the metabolome take place. The main aim of this study was to identify the level of AHI, where the largest change in the metabolome is observed in patients with sleep complaints, *i.e.* the metabolomic threshold.

Material and methods

Study subjects

Individuals aged ⩾18 years were recruited randomly from the Department of Psychiatry of the Tartu University Hospital (Tartu, Estonia), a reference centre for patients with sleep-related complaints, from April 2018 until January 2020. The participants were required to have at least one of the following sleep-related complaints: sleepiness, fatigue, insomnia symptoms, waking up gasping, snoring or night-time breathing interruptions. The main indications for PSG referral were the confirmation or exclusion of OSA or restless legs syndrome. The following exclusion criteria were used: treatment with continuous positive airway pressure during the past 6 months, any acute illness, defined as the presence of symptoms of acute infection, concomitant chronic illness such as heart failure in New York Heart Association class III–IV, autoimmune disease, type I and type II diabetes, degenerative cerebrovascular disease, chronic kidney disease stage IV–V, chronic liver disease, pulmonary disease with oxygen saturation levels permanently <93%, chronic neurological disease, active malignancy and treatment with drugs known to affect the metabolome (systemic corticosteroids, antirheumatic drugs and hormonal contraceptives). Efforts were made to minimise the effect of factors that can influence the body metabolome [16]: the patients were video-monitored, they stayed overnight in the same room under similar circumstances and did not eat during the study.

Study design

A single-centre prospective observational study was conducted to assess the relationship between the peripheral blood metabolomic profiles and AHI in a population of patients referred for PSG. The study was performed in accordance with the Declaration of Helsinki and the study protocol was approved by the Tallinn medical research ethics committee (decision number 2270). Written informed consent was obtained from each participant.

Polysomnography

A standardised PSG recording [2] was performed in an inpatient ward from 22:00 to 07:30, which included video monitoring, chin and leg electromyography, electrooculography, electroencephalography, nasal cannulas, thoracoabdominal bands, body position and snoring sensors, electrocardiography, heart rate and oxygen saturation sensors. We used either a NOX A1 (Nox Medical, Reykjavik, Iceland) or an Embletta MPR (Natus Medical, San Carlos, CA, USA) PSG recording devices. PSG data were scored manually: the AHI values, based on sleep-time obstructive respiratory events [17], were obtained according to the American Academy of Sleep Medicine guidelines [2].

Blood sampling

Blood for the analysis of metabolome was collected at the same time as PSG recording using peripheral venepuncture on three different occasions: 21:00, 05:00 and 07:00. These time points were selected to best characterise the metabolomic changes overnight on one hand, and to have minimal interference with sleep on the other, allowing the individuals to sleep for ⩾7 h before the second blood sample was taken.

BD Vacutainer silica-coated (ref 367614; Beckton Dickinson, Franklin Lakes, NJ, USA) extraction tubes were used for serum sample collection *via* venepuncture. Obtained samples were allowed to clot for 30 min at room temperature and were subsequently centrifuged at 1500 ×*g* for 15 min at 4°C. Sera were then frozen at −80°C until required for further analysis. The process was completed within 60 min of each venepuncture.

General biochemistry analyses were done as per laboratory standard protocol, details of which are presented in the supplementary material. Metabolites were measured in sera using liquid chromatography mass spectrometry. A targeted approach for determining the levels of different metabolites by AbsoluteIDQ p180 kit (Biocrates Life Sciences, Innsbruck, Austria) was used. Concentrations of a total of 187 metabolites were measured: amino acids, biogenic amines, acylcarnitines, lysophosphatidylcholines (lysoPC), phosphatidylcholines (PC) and sphingomyelins (the full list of compounds is presented in supplementary table S1). The kit is estimated to have interlaboratory coefficients of variation <10% for most metabolites used for the current study [10]. Sera were thawed at room temperature and analysed on a QTRAP 4500 mass spectrometer (Sciex, Framingham, MA, USA) connected to a high-performance liquid chromatography (Agilent 1260 series; Agilent Technologies, Waldbronn, Germany). Sample preparations and measurements were done as per manufacturer protocol in the test kit manual UM-P180, the details of which have been described previously [18]. Different metabolite concentrations were calculated automatically by the MetIDQ software (Biocrates Life Sciences). Deviations from quality control samples were evaluated and necessary corrections applied in the MetIDQ software before the data analysis. Invalid analyte values were excluded from the statistical analysis. Overall, the metabolomic analysis was conducted identically to our previous work [19].

Data analysis

To determine the sample size for this study, an *a priori* power analysis was made determining that 60 individuals were needed to be included to detect at least moderate effect size (Cohen's f ⩾0.25) [20] at 5% two-sided significance level and 90% power. A ranked general linear model for repeated measures was used to detect significant differences in the serum contents of metabolites between populations that remained below and equal to or higher than consecutively selected AHI cut-off values. To accomplish this, each variable was rank transformed before the statistical analysis, as were the outcome variables (metabolites) assigned standardised ranks at every time point. Backward elimination of explanatory clinical and demographic variables achieved the best fit. In the final model, the outcomes were adjusted to the following covariates: age, current smoking status, body mass index (BMI), gender, the number of episodes per hour of sleep with oxyhaemoglobin desaturation extending <90%, the number of episodes per hour of sleep with ⩾5% drop in oxyhaemoglobin saturation and serum contents of alanine aminotransferase (ALAT), aspartate aminotransferase, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, triglycerides, urea, potassium and sodium. Fisher's least significant difference method was used for correction for multiple comparisons.

The metabolomic threshold was determined by the maximum effect size assessed by the average Cohen's f over all metabolites out of the ranked general linear modelling for repeated measures performed with different AHI cut-offs. Mann–Whitney U-test and Pearson's Chi squared test were used for comparing characteristics of the participants, as well as the participants below and at/above the metabolomic threshold. In addition, the levels of specific metabolites were correlated with clinical parameters using Spearman correlation analysis. All data are presented as median (interquartile range) or n (%). The statistical analyses were performed using SPSS software (version 20.0; IBM, NY, USA).

Results

A total of 65 individuals (33 females and 32 males) were recruited with a median age of 54 (44–59) years. Overall, the median BMI was 29.4 (26.0–30.0) $kg \cdot m^{-2}$ and AHI was 15.6 (5.8–30.7) $events \cdot h^{-1}$ of sleep (table 1).

For our study population, the metabolites' average Cohen's f value was highest at AHI of 5 $events \cdot h^{-1}$ (0.161) (figure 1). The exact Cohen's f values together with the number of participants equal to or above and below a given AHI threshold are presented in supplementary table S2. The individuals with an AHI

TABLE 1 Baseline characteristics of the overall study population of symptomatic individuals: those with apnoea–hypopnoea index (AHI) below the metabolomic threshold (AHI <5 events·h^{-1}, MT^{-}) and those with AHI above the metabolomic threshold (AHI ⩾5 events·h^{-1}, MT^{+})

	Overall population	MT^{-}	MT^{+}	p-value$^{\#}$
Patients, n	65	14	51	
Age, years	54.0 (44.0–59.0)	44.5 (25.8–50.0)	57.0 (46.5–60.0)	0.014
BMI, kg·m^{-2}	29.4 (26.0–30.0)	24.7 (22.1–29.7)	29.7 (27.4–34.5)	0.008
Male, n (%)	32 (49.2)	5 (35.7)	27 (52.9)	0.40
Active smokers, n (%)	18 (27.7)	2 (14.3)	16 (31.4)	0.35
Neck circumference, cm	40.5 (38.0–43.0)	37.3 (35.0–41.1)	41.0 (39.0–43.0)	0.002
STOP-BANG score	5.0 (3.0–6.0)	2.5 (2.0–4.8)	5.0 (4.0–6.0)	0.003
ESS score	9.0 (5.0–12.0)	10.5 (6.0–11.0)	8.0 (4.5–12.0)	0.89
AHI, events·h^{-1}	15.6 (5.8–30.7)	1.9 (0.8–2.3)	19.5 (13.0–36.3)	<0.001
ALAT, U·L^{-1}	22.0 (18.0–29.0)	17.5 (13.3–21.8)	23.0 (19.5–29.5)	0.005
ASAT, U·L^{-1}	22.0 (19.0–26.0)	20.0 (17.0–23.8)	22.0 (20.0–26.5)	0.08
FBG, mmol·L^{-1}	5.8 (5.3–6.2)	5.4 (5.1–5.9)	5.8 (5.4–6.5)	0.017
LDL cholesterol, mmol·L^{-1}	3.17 (2.65–3.96)	2.97 (2.77–3.82)	3.40 (2.63–3.97)	0.62
HDL cholesterol, mmol·L^{-1}	1.21 (0.94–1.41)	1.37 (1.02–1.69)	1.10 (0.94–1.40)	0.067
Triglycerides, mmol·L^{-1}	1.50 (0.93–2.14)	0.95 (0.73–1.30)	1.61 (1.11–2.34)	0.003
β-blocker users, n (%)	26 (40)	2 (14.3)	24 (47.1)	0.056

Data are presented as n, median (interquartile range) or n (%), unless otherwise specified. BMI: body mass index; ESS: Epworth Sleepiness Scale; ALAT: alanine aminotransferase; ASAT: aspartate aminotransferase; FBG: fasting blood glucose; LDL: low-density lipoprotein; HDL: high-density lipoprotein. $^{\#}$: comparisons were done using either Mann–Whitney U-test (for continuous variables) or Pearson's Chi-squared test (for categorical variables) and p-values are reported for differences between the MT^{-} and MT^{+} groups.

<5 events·h^{-1} and those with AHI ⩾5 events·h^{-1} were deemed to be below (MT^{-}) and above (MT^{+}) the metabolomic threshold, respectively. This metabolomic threshold by AHI of 5 events·h^{-1} divided the study population into 14 (21.5%) MT^{-} individuals and 51 (78.5%) MT^{+} individuals (table 1).

Participants in the MT^{+} group were significantly older (p=0.014) and more obese (p=0.008) and had greater neck circumference (p=0.002) (table 1). Patients in the MT^{+} group had significantly higher STOP-BANG scores (p=0.003), but there were no differences for Epworth Sleepiness Scale scores (p=0.89) (table 1).

The concentrations of triglycerides correlated significantly with those of ALAT (ρ=0.33, p=0.020) and BMI (ρ=0.44, p=0.001) in the MT^{+} group. The values of ALAT also showed significant correlation with that of BMI (ρ=0.36, p=0.009) in the MT^{+} group.

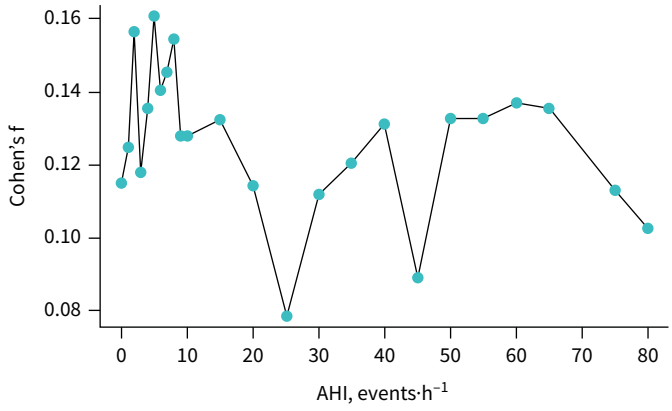

FIGURE 1 Metabolites' average Cohen's f values computed from ranked general linear modelling with repeated measures and plotted against different apnoea–hypopnea index (AHI) thresholds for the entire study population of individuals, who had symptoms characteristic of sleep disordered breathing (n=65). The metabolites' average Cohen's f value was highest at AHI of 5 events·h^{-1} (0.161).

Out of the different classes of metabolites, the number of significantly changed metabolites among the lysoPC (supplementary figure S1a), PC with diacyl residues (figure 2a) and acylcarnitines (figure 2b) peaked at the lower end of the AHI spectrum: at 8 events·h^{-1}, at 5 events·h^{-1} and at 2 events·h^{-1}, respectively. In contrast, the number of significantly changed metabolites among the PC with acyl-alkyl residues (supplementary figure S1b), sphingolipids (supplementary figure S1c) and amino acids (figure 2c) peaked at the higher end of the AHI spectrum: at 50 events·h^{-1}, at 65 events·h^{-1} and at 35 events·h^{-1}, respectively.

18 metabolites had significantly different concentrations between the MT^{-} and MT^{+} groups: eight PCs, nine acylcarnitines and one amino acid, threonine, all having lower concentration in the MT^{+} group than in the MT^{-} group (table 2).

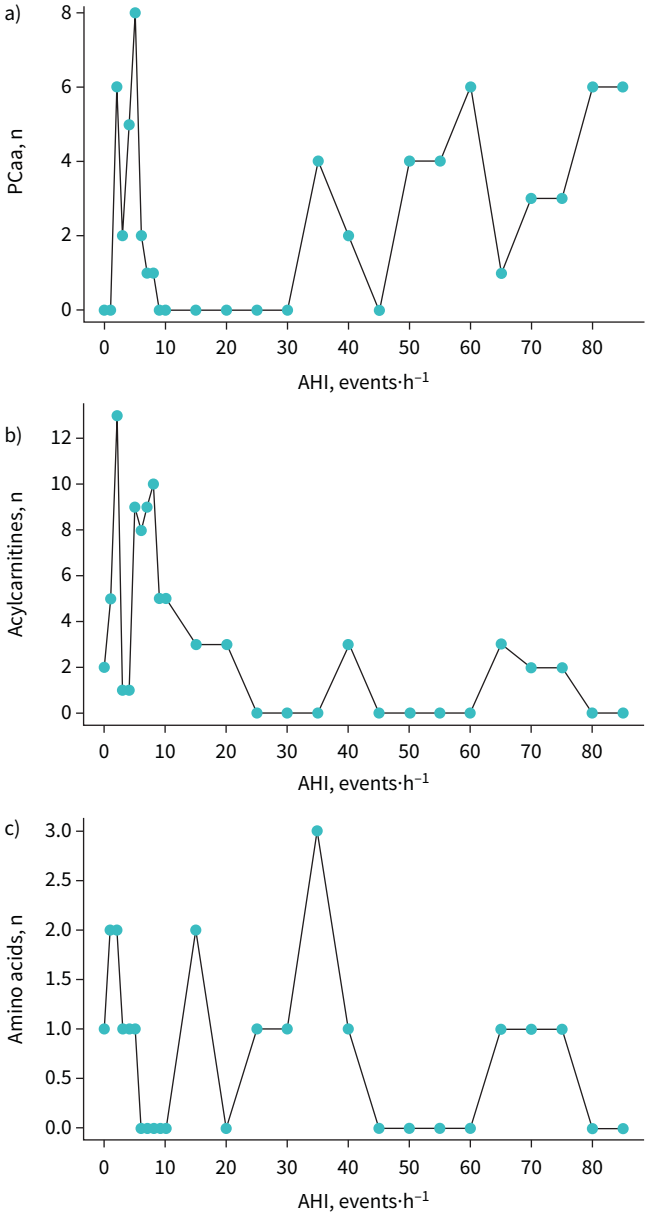

FIGURE 2 Numbers of metabolites with significantly different concentrations between symptomatic individuals with apnoea–hypopnoea index (AHI) below the metabolomic threshold (AHI <5 events·h^{-1}; MT^{-}) (n=14) and those with AHI above the metabolomic threshold (AHI ⩾5 events·h^{-1}; MT^{+}) (n=51) for different classes of metabolites. Comparisons between the groups were done using a ranked general linear model with repeated measures. a) Phosphatidylcholines with diacyl residue (PCaa); b) acylcarnitines; c) amino acids.

TABLE 2 Metabolites with significantly different concentrations between the sera of symptomatic individuals with apnoea–hypopnoea index (AHI) below the metabolomic threshold (AHI <5 events·h^{-1}; MT^{-}) (n=14) and those with AHI above the metabolomic threshold (AHI ⩾5 events·h^{-1}; MT^{+}) (n=51) at three time points: 21:00, 05:00 and 07:00.

	Group	Concentration, µmol·L^{-1}			p-value[#]
		21:00	05:00	07:00	
Phosphatidylcholines, µmol·L^{-1}					
PC aa C32:1	MT^{-}	13.25 (9.95–16.18)	11.25 (8.6–14.9)	11.8 (8.67–13.38)	0.030
	MT^{+}	12.10 (8.62–18.2)	10.6 (7.0–15.25)	10.3 (7.62–13.75)	
PC aa C32:2	MT^{-}	3.28 (2.69–3.80)	2.96 (2.25–3.20)	2.54 (2.10–3.15)	0.041
	MT^{+}	2.43 (1.75–3.62)	1.99 (1.17–2.77)	2.03 (1.25–2.64)	
PC aa C32:3	MT^{-}	0.49 (0.38–0.55)	0.45 (0.37–0.51)	0.36 (0.34–0.49)	0.041
	MT^{+}	0.40 (0.29–0.49)	0.32 (0.19–0.41)	0.31 (0.20–0.42)	
PC aa C34:4	MT^{-}	1.47 (0.91–1.60)	1.24 (0.85–1.51)	1.07 (0.76–1.43)	0.010
	MT^{+}	1.09 (0.81–1.51)	0.90 (0.53–1.25)	0.82 (0.55–1.17)	
PC aa C36:4	MT^{-}	169.0 (131.2–193.8)	151.5 (106.5–174.2)	143.5 (103.0–168.8)	0.010
	MT^{+}	137.0 (118.0–172.5)	120.0 (93.9–148.0)	123.0 (93.7–140.0)	
PC aa C36:6	MT^{-}	1.00 (0.61–1.14)	0.81 (0.59–0.89)	0.74 (0.60–0.90)	0.047
	MT^{+}	0.79 (0.54–0.96)	0.64 (0.34–0.84)	0.55 (0.38–0.87)	
PC aa C40:4	MT^{-}	2.16 (1.94–2.62)	2.12 (1.68–2.30)	2.07 (1.47–2.41)	0.033
	MT^{+}	1.95 (1–74–2.41)	1.90 (1.39–2.33)	1.88 (1.45–2.20)	
PC aa C42:4	MT^{-}	0.16 (0.15–0.18)	0.13 (0.11–0.15)	0.15 (0.10–0.16)	0.025
	MT^{+}	0.12 (0.10–0.16)	0.11 (0.10–0.14)	0.11 (0.09–0.15)	
Acylcarnitines, µmol·L^{-1}					
C4	MT^{-}	0.32 (0.30–0.39)	0.28 (0.25–0.31)	0.29 (0.26–0.31)	0.040
	MT^{+}	0.31 (0.28–0.35)	0.28 (0.23–0.33)	0.28 (0.23–0.34)	
C5:1-DC	MT^{-}	0.069 (0.054–0.076)	0.058 (0.035–0.080)	0.058 (0.041–0.090)	0.020
	MT^{+}	0.038 (0.031–0.056)	0.035 (0.023–0.048)	0.032 (0.024–0.044)	
C10:2	MT^{-}	0.18 (0.15–0.26)	0.16 (0.12–0.19)	0.17 (0.14–0.22)	0.011
	MT^{+}	0.08 (0.07–0.14)	0.08 (0.07–0.13)	0.08 (0.07–0.13)	
C12-DC	MT^{-}	0.20 (0.18–0.22)	0.19 (0.18–0.20)	0.20 (0.19–0.21)	0.022
	MT^{+}	0.18 (0.15–0.20)	0.18 (0.15–0.21)	0.18 (0.15–0.20)	
C14:2	MT^{-}	0.053 (0.043–0.088)	0.043 (0.028–0.057)	0.045 (0.031–0.066)	0.040
	MT^{+}	0.022 (0.015–0.037)	0.018 (0.013–0.039)	0.018 (0.014–0.034)	
C16:1	MT^{-}	0.054 (0.047–0.104)	0.052 (0.037–0.068)	0.047 (0.036–0.074)	0.020
	MT^{+}	0.036 (0.028–0.054)	0.032 (0.026–0.052)	0.032 (0.026–0.046)	
C16:2	MT^{-}	0.044 (0.028–0.083)	0.035 (0.017–0.049)	0.034 (0.023–0.061)	0.029
	MT^{+}	0.017 (0.013–0.026)	0.015 (0.012–0.028)	0.016 (0.013–0.026)	
C16:2-OH	MT^{-}	0.029 (0.025–0.044)	0.025 (0.020–0.034)	0.026 (0.021–0.038)	0.042
	MT^{+}	0.023 (0.018–0.024)	0.021 (0.018–0.027)	0.021 (0.019–0.027)	
C18:2	MT^{-}	0.041 (0.036–0.046)	0.038 (0.035–0.042)	0.041 (0.028–0.047)	0.026
	MT^{+}	0.032 (0.027–0.037)	0.032 (0.027–0.036)	0.030 (0.025–0.034)	
Amino acids, µmol·L^{-1}					
Threonine	MT^{-}	145.5 (124.8–158.5)	118.5 (100.4–162.0)	121.5 (106.5–154.8)	0.025
	MT^{+}	117.0 (98.6–127.0)	110.0 (93.3–124.0)	113.0 (89.4–11.5)	

Data are presented as median (interquartile range), unless otherwise stated. PC aa C32:1: phosphatidylcholine with diacyl residue sum C32:1; PC aa C32:2: phosphatidylcholine with diacyl residue sum C32:2; PC aa C32:3: phosphatidylcholine with diacyl residue sum C32:2; PC aa C34:4: phosphatidylcholine with diacyl residue sum C34:4; PC aa C36:4: phosphatidylcholine with diacyl residue sum C36:4; PC aa C36:6: phosphatidylcholine with diacyl residue sum C36:6; PC aa C40:4: phosphatidylcholine with diacyl residue sum C40:4; PC aa C42:4: phosphatidylcholine with diacyl residue sum C42:4; C4: butyrylcarnitine/isobutyrylcarnitine; C5:1-DC: glutaconylcarnitine/mesaconylcarnitine; C10:2: decadienoylcarnitine; C12-DC: dodecanedioylcarnitine; C14:2: tetradecadienoylcarnitine; C16:1: hexadecenoylcarnitine (=palmitoleylcarnitine); C16:2: hexadecadienoylcarnitine; C16:2-OH: hydroxyhexadecadienoylcarnitine; C18:2: octadecadienoylcarnitine (=linoleylcarnitine). [#]: comparisons were done using a ranked general linear model with repeated measures; p-values are reported for differences between the MT^{-} and MT^{+} groups.

Significant time-dependent effects were revealed by within-subject tests with significant time-by-group interactions for certain metabolites, which refers to significantly different dynamics of these metabolites. In particular, the values of PCs with acyl-alkyl residue sum C38:1 (p=0.002; supplementary figure S2) and hexenoylcarnitine (p=0.038; supplementary figure S3) were reduced overnight in the MT^{-} group, but remained unchanged in the MT^{+} group.

Discussion

Our study revealed that the average Cohen's f value was highest at AHI of 5 events·h^{-1}, thus denoting it as the metabolomic threshold. Furthermore, three of the highest Cohen's f values were at AHI levels

2 events·h^{-1}, 5 events·h^{-1} and 8 events·h^{-1} showing that the largest changes in the metabolome truly take place at relatively low AHI values. The fact that there were fewer significant changes in metabolite concentrations at the levels of moderate and severe OSA (supplementary table S2) may indicate the challenge of proper differentiation of these patients based on AHI alone. Assessment of different clinical variables can lead to better classification of these patients, as proposed previously [12], whereas our current study supports this modified approach metabolomically. In particular, the overall AHI metabolomic threshold of ⩾5 events·h^{-1} is mainly determined by certain lipids: PCs with diacyl residues, as well as acylcarnitines (figure 2). The serum levels of nine acylcarnitiness and eight PCs were significantly lower in the MT^{+} group than in the MT^{-} group (table 2).

Differences in acylcarnitine metabolism reflect the disturbances in fatty acid oxidation, as has been shown in obesity and type 2 diabetes [21]. MT^{+} individuals were more obese and had higher levels of fasting blood glucose. These conditions have been associated with disturbances in fatty acid oxidation resulting in the accumulation of acylcarnitines [21, 22]. Conversely, in our study, acylcarnitine concentrations in the peripheral blood were significantly lower in the MT^{+} group. In mice, β-adrenoceptor blockade with propranolol has been shown to abolish intermittent hypoxia-induced free fatty acid elevation in plasma [23]. In line with this, more participants in the MT^{+} group used β-blockers in the current study (47.1% *versus* 14.3%; table 1), although this difference did not quite reach statistical significance. Future focused studies will determine whether the administration of β-blockers influences intermittent hypoxia-induced free fatty acid elevations in humans.

PCs are synthesised in all mammalian nucleated cells *via* the Kennedy pathway [24]; an additional pathway is operative in the liver, where PCs are synthesised from phosphatidylethanolamines (PEs) [24, 25]. Changes in the absolute concentrations of PCs and PEs, but even more in the molar ratio of PC and PE, serve as key determinants of liver health [24]. Patients with nonalcoholic fatty liver disease (NAFLD) have decreased PC/PE ratios [26], mainly due to the decreased liver PC content [27]. In our study, ALAT concentrations were significantly elevated in the MT^{+} group in blood samples taken at 07:00 (table 1); however, a liver biopsy needed to confirm NAFLD [28] was not performed. Higher BMI values [29, 30] and increased ALAT concentrations have been shown previously in NAFLD patients [30]. Since OSA is associated with the development of NAFLD [31], there might be a connection between a possible NAFLD in the MT^{+} group supported by the increased ALAT levels and decreased PC content in the MT^{+} individuals. This urges a need for further studies on OSA patients with definite diagnostic workup regarding NAFLD and simultaneous measurement of both PC and PE. Besides being a substrate for energy, PC may also be used to rebuild damaged cell membranes or to compose surfactant, as lipids of a PC-type are important in determining surface tension of mucus and counteracting airway collapse in OSA [32].

The lipid species undergoing most significant changes at AHI 5 events·h^{-1} encompassed polyunsaturated fatty acids (table 2). There are supportive data in the literature indicating the synthesis of polyunsaturated fatty acid-derived eicosanoids or isoprostanes in patients with OSA [33].

In contrast, PCs with acyl-alkyl residues and sphingolipids with significantly different concentrations in the MT^{+} *versus* the MT^{-} individuals peaked in their numbers at AHI values that were above the threshold values for severe OSA (30 events·h^{-1}) (supplementary figure S1). Few significant differences among the metabolites were detected in moderate OSA (at AHI 15–30 events·h^{-1}) (figure 2, supplementary figure S1). Amino acids did not have a clear cut-off point in the current study, which is not surprising, as amino acids have more heterogenous biofunctions than lipids and different amino acids are used metabolically for different purposes. If the general protein turnover is not affected, amino acids may not outline a common AHI threshold. Taken together, the current results indicate that the increase in the severity of OSA affects different metabolic pathways. Certain pathways retain their activity at AHI 5 events·h^{-1}, where the metabolome as a whole shows the widest change, but will either wane or become upregulated as the AHI increases.

The strengths of our study include assessment of the metabolome at three different time points covering the night's sleep, when the effect of OSA on the metabolome is presumably the highest; the use of strict exclusion criteria; and obtaining samples for analysis using venepuncture. With regard to the possible role of the time of the day, in the current study, as well as in our previous work [19], relatively few time-dependent changes have occurred to the metabolome from the evening through the next morning. This may imply that there is little need for repeated assessments and even one night-time blood sample is enough to characterise the OSA-related metabolomic changes. The use of an indwelling catheter for blood sampling is associated with several metabolome changing factors, *e.g.* use of heparin to flush the catheter

and local inflammation caused by catheter placement [34]. The blood sampling during sleep maximised the characterisation of sleep-time changes. Finally, the PSG recording and blood sampling were done simultaneously to enable precise assessment of molecular phenotype and sleep quality.

One of the limitations of this study was the small sample size of 65 participants, although this was based on proper sample size calculations and its effect was counterbalanced by multiple sampling overnight. The participants in the MT^+ group were significantly older, had higher BMI, larger neck circumference and higher STOP-BANG scores. These differences mainly reflect the diagnosis of OSA [4]. The inequalities in the numbers of participants above or below a certain AHI value might have interfered with comparing between the subgroups, as there were fewer patients in the MT^- group than in the MT^+ group. Although the overall effect size found in our study is small, it still enables us to highlight the metabolic threshold, based on the classical interpretation of Cohen's f values [20]. Hence, it cannot be interpreted that the cumulative change in the metabolome in OSA is small, or more importantly, that the shifts are biologically irrelevant. What is more, due to our inclusion criteria, our current findings are limited to symptomatic individuals.

Conclusion

According to the current results, the metabolomic threshold of patients with sleep complaints is located at the AHI level of $\geqslant 5$ events·h^{-1}. Notably, this metabolomic threshold, described for the first time, coincides with the AHI threshold required to confirm the diagnosis of OSA [1]. Thus, our study further supports the diagnosis of OSA at AHI $\geqslant 5$ events·h^{-1}, reinforcing the idea of the inclusion of more variables to better classify patients with OSA, since in reality, the detrimental health effects may start at this AHI threshold.

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