Early View

Original research article

Apnoea-hypopnoea index of 5 as a metabolomic threshold in patients with sleep complaints'

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Title: „Apnoea-hypopnoea index of 5 as a metabolomic threshold in patients with sleep complaints"

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Take home message: The metabolomic breakpoint coincides with the apnoea-hypopnoea index threshold of ≥5 required to confirm the diagnosis of obstructive sleep apnoea in patients with sleep complaints.

Abstract

Background: Apnoea-hypopnoea index (AHI) forms the basis for severity of obstructive sleep apnoea (OSA), a condition expected to reprogram metabolic pathways in humans. We aimed at identifying 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, lysophasphatidylcholines, phosphatidylcholines and sphingomyelins were analysed with liquid chromatography-mass-spectrometry in peripheral blood drawn at 3 different time points overnight. Comparisons of rank-transformed data with general linear model for repeated measures after dichotomizing the study group at different AHI levels were applied to define the best cut-off based on Cohen's f.

Results: Sixty-five subjects were recruited with their median AHI of 15.6 events per hour. The mean Cohen's f over the metabolites was highest (0.161) at an AHI level of 5/h representing the metabolomic threshold (MT). Of the particular between-group differences, 8 phosphatidylcholines, 9 acylcarnitines and one amino acid (threonine) had significantly lower concentrations in the individuals with an AHI level equal to or above the MT. The metabolomic changes at AHI levels defining moderate and severe OSA were smaller than at AHI of 5/h.

Conclusions: The MT 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.

Keywords: metabolome; metabolism; obstructive sleep apnoea; apnoea-hypopnoea index; metabolomic threshold

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) $\geqslant$5/h during the night-time sleep study or secondly in the presence of AHI $\geqslant$15/h 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 reprogram 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 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]. Targeted analyses, however, have shown better interlaboratory reproducibility [10].

In-hospital polysomnography (PSG) measures the amount of apnoeas and hypopnoeas per hour of sleep, termed as apnoea-hypopnoea index (AHI), whereas at-home polygraphy counts the breathing events (apnoeas and hypopnoeas with oxyhaemoglobin desaturation of $\geqslant$4%) for the entire recording time, known as respiratory-event index [4]. In 1999, OSA was first classified into mild (AHI $\geqslant$5 and <15/h), moderate (AHI $\geqslant$15 and <30/h) and severe (AHI $\geq$30/h), 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/h) 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/h would still be useful to diagnose OSA, whereas using AHI to classify the severity of OSA could be invalid [12].

AHI thresholds have been previously studied 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 (MT).

Material and methods

Study subjects

Individuals aged ≥18 years were randomly recruited from the Department of Psychiatry of the Tartu University Hospital, a reference centre for patients with sleep-related complaints from April of 2018 until January of 2020. The participants were required to have at least one of the following sleep-related complaint: 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 last 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 below 93%, chronic neurological disease, active malignancy and treatment with drugs known to affect metabolome (systemic corticosteroids, antirheumatic drugs and hormonal contraceptives). Efforts were made to minimize 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 standardized PSG recording [2] was performed in in-patient ward from 10 p.m. to 7:30 a.m. that 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 Inc., San Carlos, CA, USA) PSG recording devices. PSG data was 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 analyses of metabolome was collected at the same time as PSG recording using peripheral venepuncture on 3 different occasions: 9:00 p.m.; 5:00 a.m.; 7:00 a.m. These time points were selected to best characterize the metabolomic changes overnight on one hand and to have minimal interference with sleep on the other with allowing the individuals to sleep for at least 7 hours 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 minutes at room temperature and were subsequently centrifuged at 1,500 g for 15 min at 4°C. Sera were then frozen at -80°C until the conducting of 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 materials. 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 AG, Innsbruck, Austria) was used. Concentrations of a total of 187 metabolites were measured: amino acids, biogenic amines, acylcarnitines (AC), lysophasphatidylcholines (LysoPC), phosphatidylcholines (PC) and sphingomyelins (the full list of particular compounds is presented in supplementary table S1). The kit is estimated to have inter-laboratory coefficients of variation below 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 AG). Deviations from quality control samples were evaluated and necessary corrections applied in 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, a priori power analysis was made resulting in that 60 individuals were needed to be included to detect at least moderate effect size (Cohen's f $\geqslant$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, each variable was rank transformed before the statistical analysis, as were the outcome variables (metabolites) assigned standardized ranks at every time point. Backward elimination of explanatory clinical and demographic variables to achieve 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 below 90%, the number of episodes per hour of sleep with $\geqslant$5% drop in oxyhaemoglobin saturation and serum contents of ALAT, ASAT, high density lipoprotein cholesterol, low density lipoprotein cholesterol, TG, urea, potassium, and sodium. Fischer's least significant difference method was used for correction for multiple comparisons.

The MT 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 Square test were used for comparing characteristics of the participants, as well as the participants below and at/above the MT. In addition, the levels of specific metabolites were correlated with clinical parameters using Spearman correlation analysis. All data are presented as medians and interquartile ranges or numbers (%). The statistical analyses were performed using SPSS software, version 20.0 (IBM Co, 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) and AHI was 15.6 (5.8-30.7) events per hour of sleep (table 1).

For our study population, the metabolites' average Cohen's f value was highest at AHI of 5/h (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 <5/h and those with AHI ⩾5/h were deemed to be below (MT-) and above (MT+) the MT,

respectively. This MT by AHI of 5/h divided the study population into 14 MT- (21.5%) and 51 MT+

individuals (78.5%) (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 ($\rho=0.33$,

p=0.020) and BMI ($\rho=0.44$, p=0.001) in the MT+ group. The values of ALAT also showed significant

correlation with that of BMI ($\rho=0.36$, p=0.009) in the MT+ group.

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 AC (figure 2b)

peaked at the lower end of the AHI spectrum: at 8/h, at 5/h and at 2/h, respectively. 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), on the contrary, peaked at the

higher end of the AHI spectrum: at 50/h, at 65/h and at 35/h, respectively.

There were 18 metabolites that had significantly different concentrations between the MT- and

MT+ groups: 8 PC, 9 AC and one amino acid, threonine, all having lower concentration in the MT+

group than in the MT- group (table 2).

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 PC 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/h, thus denoting it as the MT. Furthermore, 3 of the highest Cohen's f values were at AHI levels 2/h, 5/h, and 8/h 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 differentiating 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 $\geqslant 5$ is mainly determined by certain lipids: PC with diacyl residues, as well as AC (figure 2). The serum levels of 9 ACs and 8 PCs were significantly lower in the MT+ group than in the MT- group (table 2).

Differences in the AC metabolism reflect the disturbances in fatty acid oxidation, as it 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 ACs [21, 22]. Contrary to this, in our study, AC 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 beta blockers in the current study (47.1 vs 14.3%, table 1), although this difference did not quite reach statistical significance. Future focused studies will determine whether administration of beta-blockers influences intermittent hypoxia-induced free fatty acid elevations in humans.

PC are synthesized in all mammalian nucleated cells via the Kennedy pathway [24], whereas an additional pathway is operative in the liver, where PCs are synthesized from phosphatidylethanolamines (PE) [24, 25]. Changes in the absolute concentrations of PC and PE, but even more in the molar ratio of PC and PE, serve as key determinants of liver health [24]. Patients

with non-alcoholic fatty liver disease (NAFLD) have decreased PC/PE ratios [26], mainly due to the decreased liver PC content [27]. In our study, the ALAT concentrations were significantly elevated in the MT+ group in blood samples taken at 7:00 a.m. (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 previously shown 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 contents in the MT+ individuals. This urges a need for further studies on OSA patients with definite diagnostic work-up 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 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 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].

PC with acyl-alkyl residues and sphingolipids with significantly different concentrations in the MT+ versus the MT- individuals peaked in their numbers, on the contrary, at AHI values that were above the threshold values for severe OSA (30/h) (supplementary figure S1). Few significant differences among the metabolites were detected in moderate OSA (at AHI 15-30/h) (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 metabolically used 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, 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 3 different time points with covering the night sleep, when the effect of OSA on the metabolome is presumably the highest, the use of strict exclusion criteria and obtaining the analyses using venepuncture. With regard to the possible role of the time of the day, in the current, as well as in our previous study [19], relatively little 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 characterize the OSA-related metabolomic changes. The use of an indwelling catheter for blood sampling is associated with several metabolome changing factors e.g. usage of heparin to flush the catheter and local inflammation caused by catheter placement [34]. The blood sampling during sleep maximized the characterization 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, albeit 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, based on the classical interpretation of Cohen's f values [20], to highlight the metabolic threshold. 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 per hour. Notably, this MT described for the first time coincides

with the AHI threshold required to confirm the diagnosis of OSA [1]. Our study thus further supports diagnosing OSA at AHI $\geqslant 5$/h with reinforcing the idea of inclusion of more variables to better classify patients with OSA, since the detrimental health effects may in reality start at this AHI threshold.

Author contributions

All authors listed were involved in elaborating the concept and design of the study. O. Kiens, V. Ivanova, K. Veeväli, T. Laurits and R. Tamm made substantial contribution to selecting the study individuals, sampling of blood and ensuring the quality of the sleep study data. E. Taalberg conducted the metabolomic analyses and obtained the concentration values for metabolites. A. Altraja and A. Ottas performed statistical analysis, whereas all authors were involved in interpretation of the results. O. Kiens wrote the first draft of the manuscript and K. Kilk and A. Altraja made considerable revisions thereafter. All authors revised the drafts of the manuscript, approved its final version submitted for publication and take responsibility for the accuracy and integrity of the whole work.

Table 1. Baseline characteristics of the overall study population of symptomatic individuals: those with apnoea-hypopnoea index (AHI) below the metabolomic threshold (AHI <5/h, MT-) and those with AHI above the metabolomic threshold (AHI ⩾5/h, MT+).

Variable	Overall population (n=65)	MT- (n=14)	MT+ (n=51)	p-value[#]
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 gender, 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	15.6 (5.8-30.7)	1.9 (0.8-2.3)	19.5 (13.0-36.3)	<0.001
ALAT, U/L	22.0 (18.0-29.0)	17.5 (13.3-21.8)	23.0 (19.5-29.5)	0.005
ASAT, U/L	22.0 (19.0-26.0)	20.0 (17.0-23.8)	22.0 (20.0-26.5)	0.08
FBG, mmol/L	5.8 (5.3-6.2)	5.4 (5.1-5.9)	5.8 (5.4-6.5)	0.017
LDL-Chol, mmol/L	3.17 (2.65-3.96)	2.97 (2.77-3.82)	3.40 (2.63-3.97)	0.62
HDL-Chol, mmol/L	1.21 (0.94-1.41)	1.37 (1.02-1.69)	1.10 (0.94-1.40)	0.067
TG, mmol/L	1.50 (0.93-2.14)	0.95 (0.73-1.30)	1.61 (1.11-2.34)	0.003
Beta blocker users, n (%)	26 (40)	2 (14.3)	24 (47.1)	0.056

Data are presented as median (interquartile range) unless otherwise specified. [#]: Comparisons were done using either Mann-Whitney U test (for continuous variables) or Pearson's Chi square test (for categorical variables) and p-values are reported for differences between the MT- and MT+ groups.

ALAT: alanine aminotransferase; ASAT: aspartate aminotransferase; BMI: body mass index; ESS: Epworth sleepiness scale; FBG: fasting blood glucose; HDL-Chol: high-density lipoprotein cholesterol; IQR: interquartile range; LDL-Chol: low-density lipoprotein cholesterol; OSA: obstructive sleep apnoea; TG: triglycerides.

Table 2. Metabolites with significantly different concentrations between the sera of symptomatic individuals with apnoea-hypopnoea index (AHI) below the metabolomic threshold (AHI <5/h, MT-) (n=14) and those with AHI above the metabolomic threshold (AHI ⩾5/h, MT+) (n=51) at three time points: 9:00 p.m., 5:00 a.m. and 7:00 a.m.

Variable	Group	Time point

		9:00 p.m.	5:00 a.m.	7:00 a.m.	p- value[#]
Phosphatidylcholines					
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					
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					
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)	

Expressed as μmol/L (interquartile range). [#]: Comparisons were done using ranked general linear model with repeated measures and p-values are reported for differences between the MT- and MT+ groups.

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).

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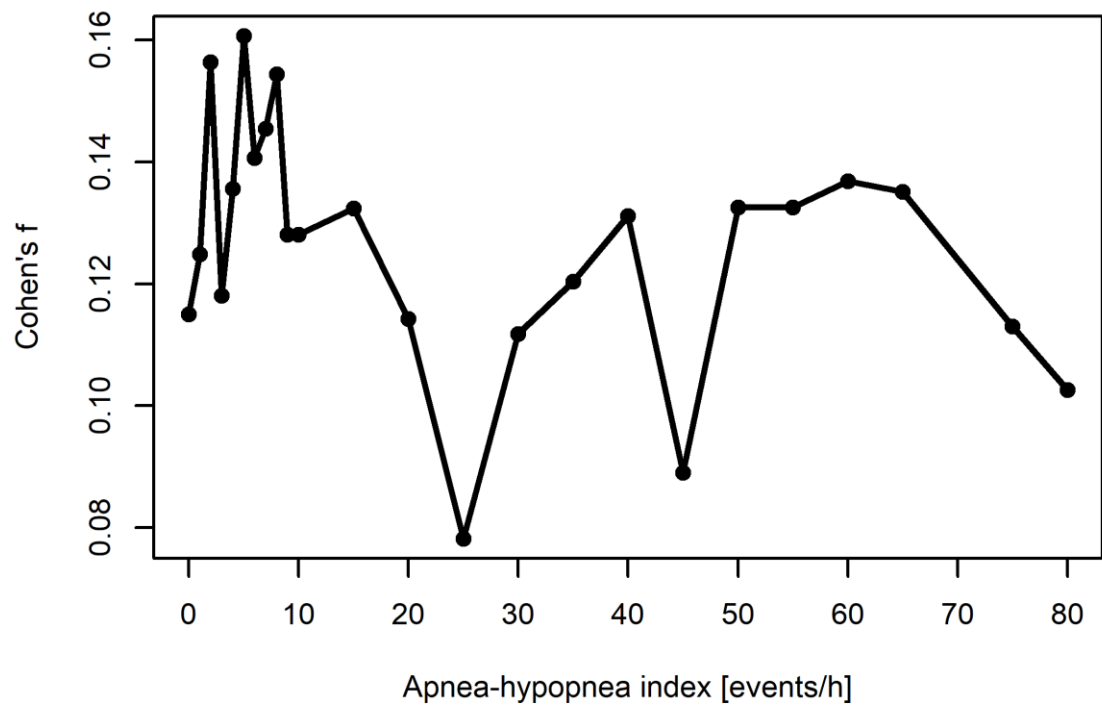
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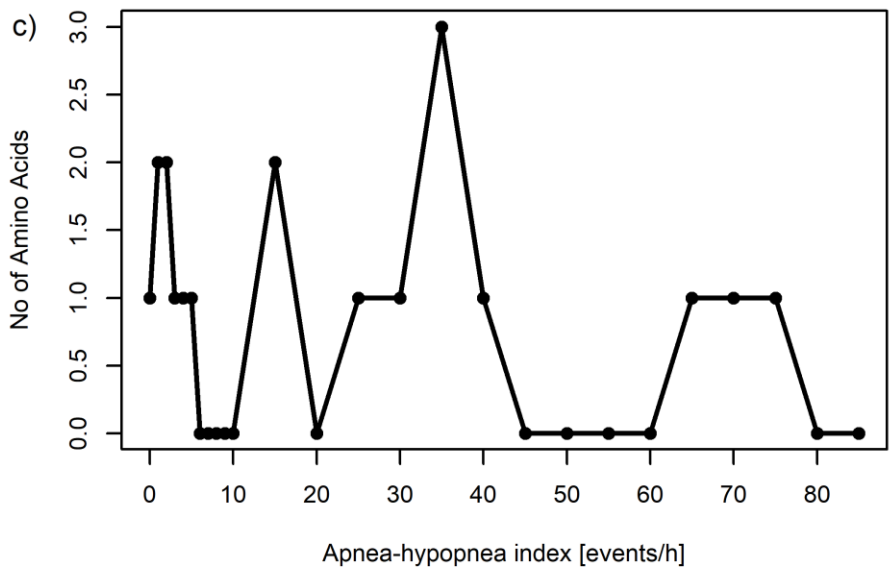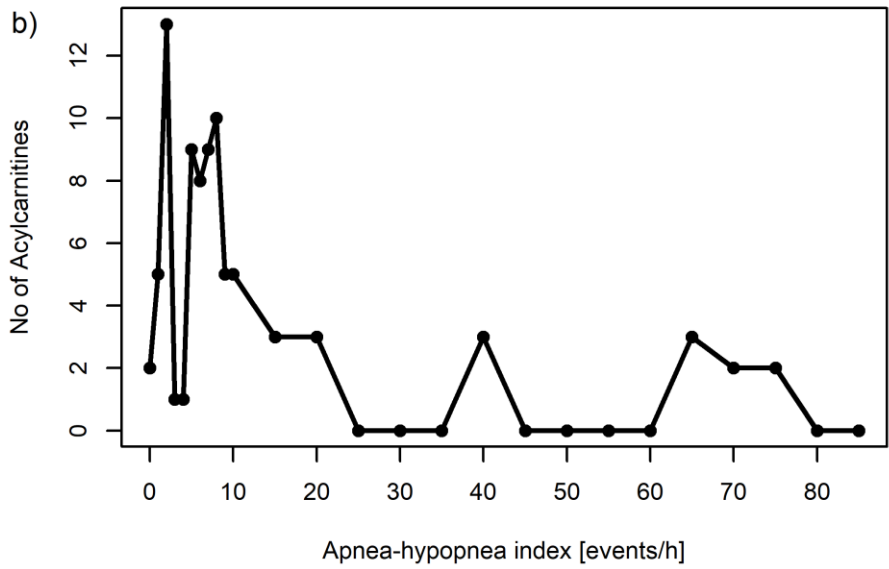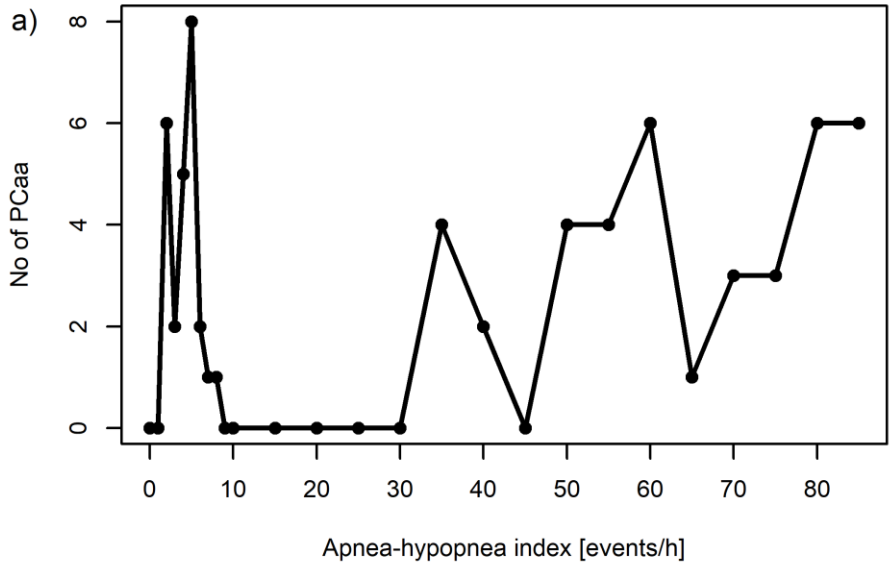
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Figure legends

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/h (0.161).

FIGURE 2 a-c Numbers of metabolites with significantly different concentrations between

symptomatic individuals with apnoea-hypopnoea index (AHI) below the metabolomic threshold (AHI

<5/h, MT-) (n=14) and those with AHI above the metabolomic threshold (AHI $\geqslant$5/h, MT+) (n=51) for

different classes of metabolites. Comparisons between the groups were done using ranked general

linear model with repeated measures. a) phosphatidylcholines with diacyl residue (PCaa); b)

acylcarnitines; c) amino acids.

a)

No of PCaa

Apnea-hypopnea index [events/h]

b)

No of Acylcarnitines

Apnea-hypopnea index [events/h]

c)

No of Amino Acids

Apnea-hypopnea index [events/h]

Supplementary material to „Apnoea-hypopnoea index of 5 as a metabolomic threshold in patients with sleep complaints"

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General serum biochemistry analyses

General analyses on serum biochemistry including measurements of potassium, sodium, creatinine, urea, alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT), triglycerides (TG), cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol were done once at 7:00 a.m., whereas high-sensitivity C-reactive protein, haptoglobin and ceruloplasmin levels were measured thrice at 9:00 p.m., 5:00 a.m. and 7:00 a.m. BD Vacutainer® Heparin (REF 368886, Beckton Dickinson, Franklin Lakes, NJ, USA) extraction tubes were used for the blood collection for the above-mentioned analyses. Fasting blood glucose was also analysed via finger-prick testing at 7:00 a.m.

Supplementary table S1. Full list of metabolites (n=187) analysed using the AbsoluteIDQ™ p180 kit (BIOCRATES Life Sciences AG, Innsbruck, Austria) with their abbreviations.

Abbreviation	Full name
Amino acids (n=22)	
Ala	Alanine
Arg	Arginine
Asn	Asparagine
Asp	Aspartate
Cit	Citrulline
Gln	Glutamine
Glu	Glutamate
Gly	Glycine
His	Histidine
Ile	Isoleucine
Leu	Leucine
Lys	Lysine
Met	Methionine
Orn	Ornithine
Phe	Phenylalanine
Pro	Proline
Ser	Serine
Thr	Threonine
Trp	Tryptophan
Try	Tyrosine
Val	Valine

Biogenic amines (n=20)

Ac-Orn	Acetylornithine
ADMA	Asymmetric dimethylarginine
SDMA	Symmetric dimethylarginine
alpha-AAA	alpha-Aminoadipic acid
Histamine	Histamine
Met-SO	Methionine-Sulfoxide
Kyn	Kynurenine
Putrescine	Putrescine
Spermidine	Spermidine
Spermine	Spermine
Serotonin	Serotonin
PEA	Phenylethylamine
Nitro-Tyr	Nitrotyrosine
c4-OH-Pro	cis-4-Hydroxyproline
t4-OH-Pro	trans-4-Hydroxyproline
Creatinine	Creatinine
Carnosine	Carnosine
Taurine	Taurine
DOPA	Dihydroxyphenylalanine
Dopamine	Dopamine

Acylcarnitines (n=40)

C0	Carnitine (free)
C2	Acetylcarnitine
C3	Propionylcarnitine

C3:1	Propenoylcarnitine
C3-OH	Hydroxypropionylcarnitine
C4	Butyrylcarnitine/Isobutyrylcarnitine
C4:1	Butenoylcarnitine
C4-OH (C3-DC)	Hydroxybutyrylcarnitine (Malonylcarnitine)
C5	Isovalerylcarnitine/2-Methylbutyrylcarnitine/Valerylcarnitine
C5:1	Tiglylcarnitine/3-Methyl-crotonylcarnitine
C5:1-DC	Glutaconylcarnitine/Mesaconylcarnitine
C5-DC (C6-OH)	Glutarylcarnitine (Hydroxyhexanoylcarnitine (= Hydroxycaproylcarnitine))
C5-M-DC	Methylglutarylcarnitine
C5-OH (C3-DC-M)	Hydroxyisovalerylcarnitine/Hydroxy-2-methylbutyryl/Hydroxyvalerylcarnitine (Methylmalonylcarnitine)
C6 (C4:1-DC)	Hexanoylcarnitine (= Caproylcarnitine) (Fumarylcarnitine)
C6:1	Hexenoylcarnitine
C7-DC	Pimelylcarnitine
C8	Octanoylcarnitine (= Caprylylcarnitine)
C9	Nonanoylcarnitine (= Pelargonylcarnitine)
C10	Decanoylcarnitine (= Caprylcarnitine)
C10:1	Decenoylcarnitine
C10:2	Decadienoylcarnitine
C12	Dodecanoylcarnitine (= Laurylcarnitine)
C12:1	Dodecenoylcarnitine
C12-DC	Dodecanedioylcarnitine
C14	Tetradecanoylcarnitine (= Myristylcarnitine)

C14:1	Tetradecenoylcarnitine (= Myristoleylcarnitine)
C14:1-OH	Hydroxytetradecenoylcarnitine (= Hydroxymyristoleylcarnitine)
C14:2	Tetradecadienoylcarnitine
C14:2-OH	Hydroxytetradecadienoylcarnitine
C16	Hexadecanoylcarnitine (= Palmitoylcarnitine)
C16:1	Hexadecenoylcarnitine (= Palmitoleylcarnitine)
C16:1-OH	Hydroxyhexadecenoylcarnitine (= Hydroxypalmitoleylcarnitine)
C16:2	Hexadecadienoylcarnitine
C16:2-OH	Hydroxyhexadecadienoylcarnitine
C16-OH	Hydroxyhexadecanolycarnitine (= Hydroxypalmitoylcarnitine)
C18	Octadecanoylcarnitine (= Stearylcarnitine)
C18:1	Octadecenoylcarnitine (= Oleylcarnitine)
C18:1-OH	Hydroxyoctadecenoylcarnitine (= Hydroxyoleylcarnitine)
C18:2	Octadecadienoylcarnitine (= Linoleylcarnitine)

Lysophosphatidylcholines (n=14)

lysoPC a C14:0	Lysophosphatidylcholine with acyl residue C14:0
lysoPC a C16:0	Lysophosphatidylcholine with acyl residue C16:0
lysoPC a C16:1	Lysophosphatidylcholine with acyl residue C16:1
lysoPC a C17:0	Lysophosphatidylcholine with acyl residue C17:0
lysoPC a C18:0	Lysophosphatidylcholine with acyl residue C18:0
lysoPC a C18:1	Lysophosphatidylcholine with acyl residue C18:1
lysoPC a C18:2	Lysophosphatidylcholine with acyl residue C18:2
lysoPC a C20:3	Lysophosphatidylcholine with acyl residue C20:3
lysoPC a C20:4	Lysophosphatidylcholine with acyl residue C20:4

lysoPC a C24:0   Lysophosphatidylcholine with acyl residue C24:0

lysoPC a C26:0   Lysophosphatidylcholine with acyl residue C26:0

lysoPC a C26:1   Lysophosphatidylcholine with acyl residue C26:1

lysoPC a C28:0   Lysophosphatidylcholine with acyl residue C28:0

lysoPC a C28:1   Lysophosphatidylcholine with acyl residue C28:1

Phosphatidylcholines (n=76)

PC aa C24:0   Phosphatidylcholine with diacyl residue sum C24:0

PC aa C26:0   Phosphatidylcholine with diacyl residue sum C26:0

PC aa C28:1   Phosphatidylcholine with diacyl residue sum C28:1

PC aa C30:0   Phosphatidylcholine with diacyl residue sum C30:0

PC aa C30:2   Phosphatidylcholine with diacyl residue sum C30:2

PC aa C32:0   Phosphatidylcholine with diacyl residue sum C32:0

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 C32:3

PC aa C34:1   Phosphatidylcholine with diacyl residue sum C34:1

PC aa C34:2   Phosphatidylcholine with diacyl residue sum C34:2

PC aa C34:3   Phosphatidylcholine with diacyl residue sum C34:3

PC aa C34:4   Phosphatidylcholine with diacyl residue sum C34:4

PC aa C36:0   Phosphatidylcholine with diacyl residue sum C36:0

PC aa C36:1   Phosphatidylcholine with diacyl residue sum C36:1

PC aa C36:2   Phosphatidylcholine with diacyl residue sum C36:2

PC aa C36:3   Phosphatidylcholine with diacyl residue sum C36:3

PC aa C36:4   Phosphatidylcholine with diacyl residue sum C36:4

PC aa C36:5   Phosphatidylcholine with diacyl residue sum C36:5

PC aa C36:6	Phosphatidylcholine with diacyl residue sum C36:6
PC aa C38:0	Phosphatidylcholine with diacyl residue sum C38:0
PC aa C38:1	Phosphatidylcholine with diacyl residue sum C38:1
PC aa C38:3	Phosphatidylcholine with diacyl residue sum C38:3
PC aa C38:4	Phosphatidylcholine with diacyl residue sum C38:4
PC aa C38:5	Phosphatidylcholine with diacyl residue sum C38:5
PC aa C38:6	Phosphatidylcholine with diacyl residue sum C38:6
PC aa C40:1	Phosphatidylcholine with diacyl residue sum C40:1
PC aa C40:2	Phosphatidylcholine with diacyl residue sum C40:2
PC aa C40:3	Phosphatidylcholine with diacyl residue sum C40:3
PC aa C40:4	Phosphatidylcholine with diacyl residue sum C40:4
PC aa C40:5	Phosphatidylcholine with diacyl residue sum C40:5
PC aa C40:6	Phosphatidylcholine with diacyl residue sum C40:6
PC aa C42:0	Phosphatidylcholine with diacyl residue sum C42:0
PC aa C42:1	Phosphatidylcholine with diacyl residue sum C42:1
PC aa C42:2	Phosphatidylcholine with diacyl residue sum C42:2
PC aa C42:4	Phosphatidylcholine with diacyl residue sum C42:4
PC aa C42:5	Phosphatidylcholine with diacyl residue sum C42:5
PC aa C42:6	Phosphatidylcholine with diacyl residue sum C42:6
PC ae C30:0	Phosphatidylcholine with acyl-alkyl residue sum C30:0
PC ae C30:1	Phosphatidylcholine with acyl-alkyl residue sum C30:1
PC ae C30:2	Phosphatidylcholine with acyl-alkyl residue sum C30:2
PC ae C32:1	Phosphatidylcholine with acyl-alkyl residue sum C32:1
PC ae C32:2	Phosphatidylcholine with acyl-alkyl residue sum C32:2
PC ae C34:0	Phosphatidylcholine with acyl-alkyl residue sum C34:0

PC ae C34:1	Phosphatidylcholine with acyl-alkyl residue sum C34:1
PC ae C34:2	Phosphatidylcholine with acyl-alkyl residue sum C34:2
PC ae C34:3	Phosphatidylcholine with acyl-alkyl residue sum C34:3
PC ae C36:0	Phosphatidylcholine with acyl-alkyl residue sum C36:0
PC ae C36:1	Phosphatidylcholine with acyl-alkyl residue sum C36:1
PC ae C36:2	Phosphatidylcholine with acyl-alkyl residue sum C36:2
PC ae C36:3	Phosphatidylcholine with acyl-alkyl residue sum C36:3
PC ae C36:4	Phosphatidylcholine with acyl-alkyl residue sum C36:4
PC ae C36:5	Phosphatidylcholine with acyl-alkyl residue sum C36:5
PC ae C38:0	Phosphatidylcholine with acyl-alkyl residue sum C38:0
PC ae C38:1	Phosphatidylcholine with acyl-alkyl residue sum C38:1
PC ae C38:2	Phosphatidylcholine with acyl-alkyl residue sum C38:2
PC ae C38:3	Phosphatidylcholine with acyl-alkyl residue sum C38:3
PC ae C38:4	Phosphatidylcholine with acyl-alkyl residue sum C38:4
PC ae C38:5	Phosphatidylcholine with acyl-alkyl residue sum C38:5
PC ae C38:6	Phosphatidylcholine with acyl-alkyl residue sum C38:6
PC ae C40:1	Phosphatidylcholine with acyl-alkyl residue sum C40:1
PC ae C40:2	Phosphatidylcholine with acyl-alkyl residue sum C40:2
PC ae C40:3	Phosphatidylcholine with acyl-alkyl residue sum C40:3
PC ae C40:4	Phosphatidylcholine with acyl-alkyl residue sum C40:4
PC ae C40:5	Phosphatidylcholine with acyl-alkyl residue sum C40:5
PC ae C40:6	Phosphatidylcholine with acyl-alkyl residue sum C40:6
PC ae C42:0	Phosphatidylcholine with acyl-alkyl residue sum C42:0
PC ae C42:1	Phosphatidylcholine with acyl-alkyl residue sum C42:1
PC ae C42:2	Phosphatidylcholine with acyl-alkyl residue sum C42:2

PC ae C42:3	Phosphatidylcholine with acyl-alkyl residue sum C42:3
PC ae C42:4	Phosphatidylcholine with acyl-alkyl residue sum C42:4
PC ae C42:5	Phosphatidylcholine with acyl-alkyl residue sum C42:5
PC ae C44:3	Phosphatidylcholine with acyl-alkyl residue sum C44:3
PC ae C44:4	Phosphatidylcholine with acyl-alkyl residue sum C44:4
PC ae C44:5	Phosphatidylcholine with acyl-alkyl residue sum C44:5
PC ae C44:6	Phosphatidylcholine with acyl-alkyl residue sum C44:6

Sphingomyelins (n=15)

|	
SM (OH) C14:1	Hydroxysphingomyelin with acyl residue sum C14:1
SM (OH) C16:1	Hydroxysphingomyelin with acyl residue sum C16:1
SM (OH) C22:1	Hydroxysphingomyelin with acyl residue sum C22:1
SM (OH) C22:2	Hydroxysphingomyelin with acyl residue sum C22:2
SM (OH) C24:1	Hydroxysphingomyelin with acyl residue sum C24:1
SM C16:0	Sphingomyelin with acyl residue sum C16:0
SM C16:1	Sphingomyelin with acyl residue sum C16:1
SM C18:0	Sphingomyelin with acyl residue sum C18:0
SM C18:1	Sphingomyelin with acyl residue sum C18:1
SM C20:2	Sphingomyelin with acyl residue sum C20:2
SM C22:3	Sphingomyelin with acyl residue sum C22:3
SM C24:0	Sphingomyelin with acyl residue sum C24:0
SM C24:1	Sphingomyelin with acyl residue sum C24:1
SM C26:0	Sphingomyelin with acyl residue sum C26:0
SM C26:1	Sphingomyelin with acyl residue sum C26:1

Supplementary table S2. Metabolites' average Cohen's f value computed from ranked general linear modelling with repeated measures for different apnoea-hypopnea index (AHI) thresholds and the number of symptomatic individuals with AHI values below the AHI threshold and equal to or above the AHI threshold. At AHI threshold of zero, the "No of participants <AHI threshold" had an AHI of exactly zero and the „No of participants ⩾AHI threshold" had AHI values above zero. A total of 65 individuals were included into the study.

AHI threshold (events/h)	Cohen's f	No of participants <AHI threshold	No of participants ⩾AHI threshold
0	0.115	1	64
1	0.125	4	61
2	0.156	7	58
3	0.118	11	54
4	0.136	12	53
5	0.161	14	51
6	0.141	17	48
7	0.145	18	47
8	0.154	20	45
9	0.128	21	44
10	0.128	21	44
15	0.132	32	33
20	0.114	40	25
25	0.078	44	21
30	0.112	48	17
35	0.120	52	13
40	0.131	54	11

|			
45	0.089	57	8
50	0.133	60	5
60	0.135	61	4
70	0.113	63	2
80	0.103	64	1
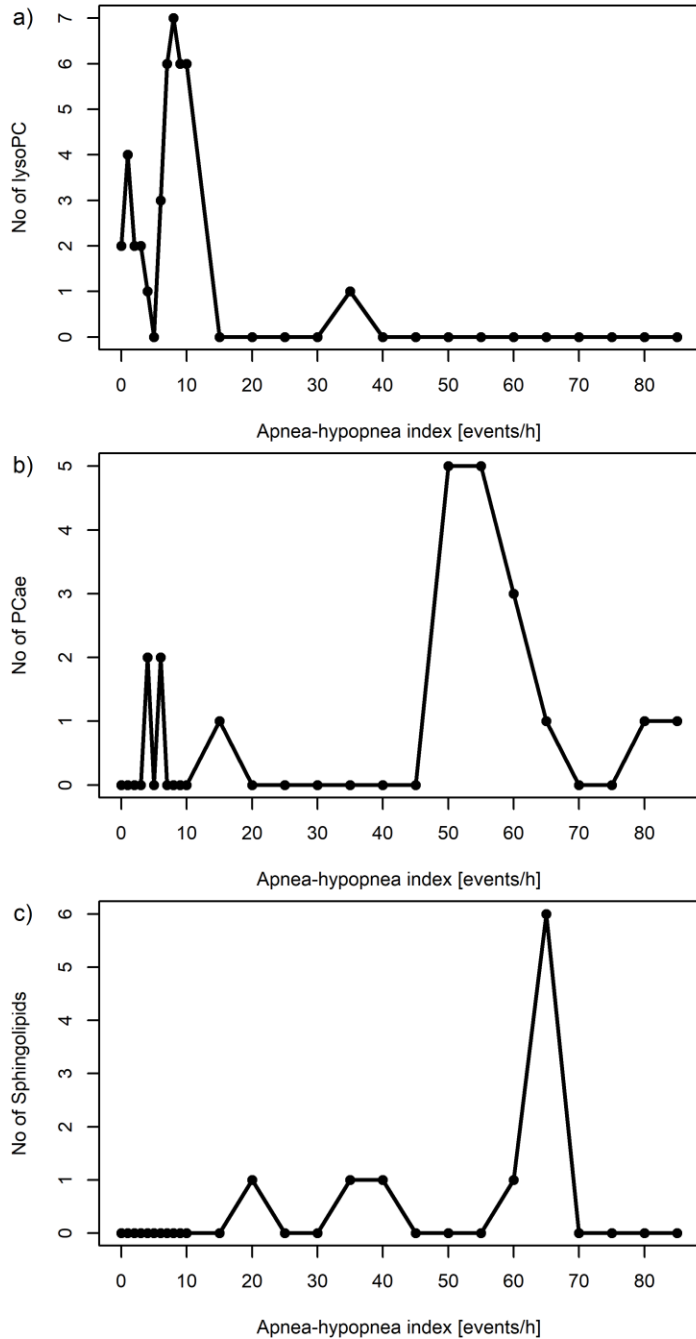
Supplementary figure S1. Numbers of metabolites with significantly different concentrations between the sera of symptomatic individuals with apnoea-hypopnoea index below the metabolomic threshold (<5 events/h) (n=14) and those with apnoea-hypopnoea index at or above the metabolomic threshold (≥5 events/h) (n=51) for different classes of metabolites. Comparisons between the groups were done using

ranked general linear model with repeated measures. a) lysophosphatidylcholines (LysoPC); b) phosphatidylcholines with acyl-alkyl residues (PCae); c) sphingolipids.
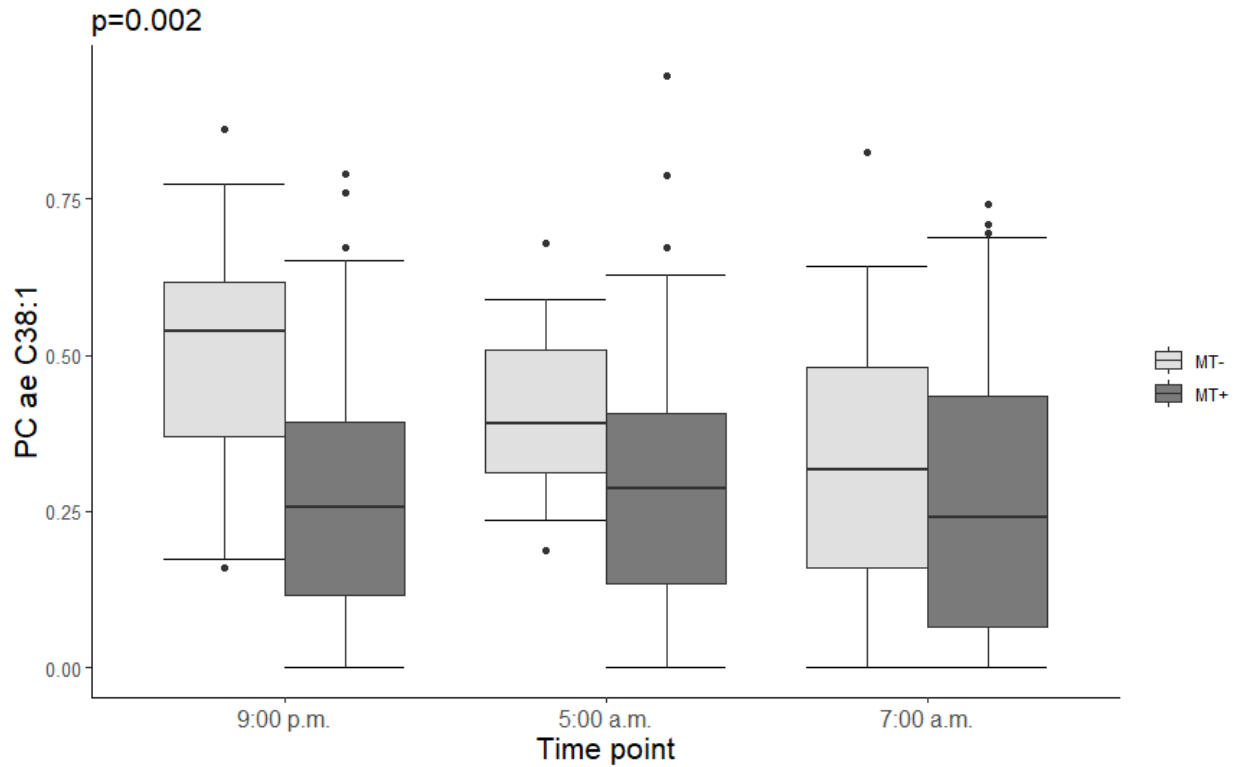
Supplementary figure S2. Peripheral blood concentrations of PC ae C38:1 (phosphatidylcholine with acyl-alkyl residue sum C38:1) were reduced overnight in the group of symptomatic individuals with apnoea-hypopnea index (AHI) below the metabolomic threshold (AHI <5/h, MT-) (n=14), but remained relatively unchanged in the group of patients with AHI above the metabolomic threshold (AHI ⩾5/h, MT+) (n=51), p=0.002. Individuals with symptoms characteristic of sleep-disordered breathing (n=65) underwent targeted analysis of their peripheral blood metabolome and comparisons were done using ranked general linear model with repeated measures.
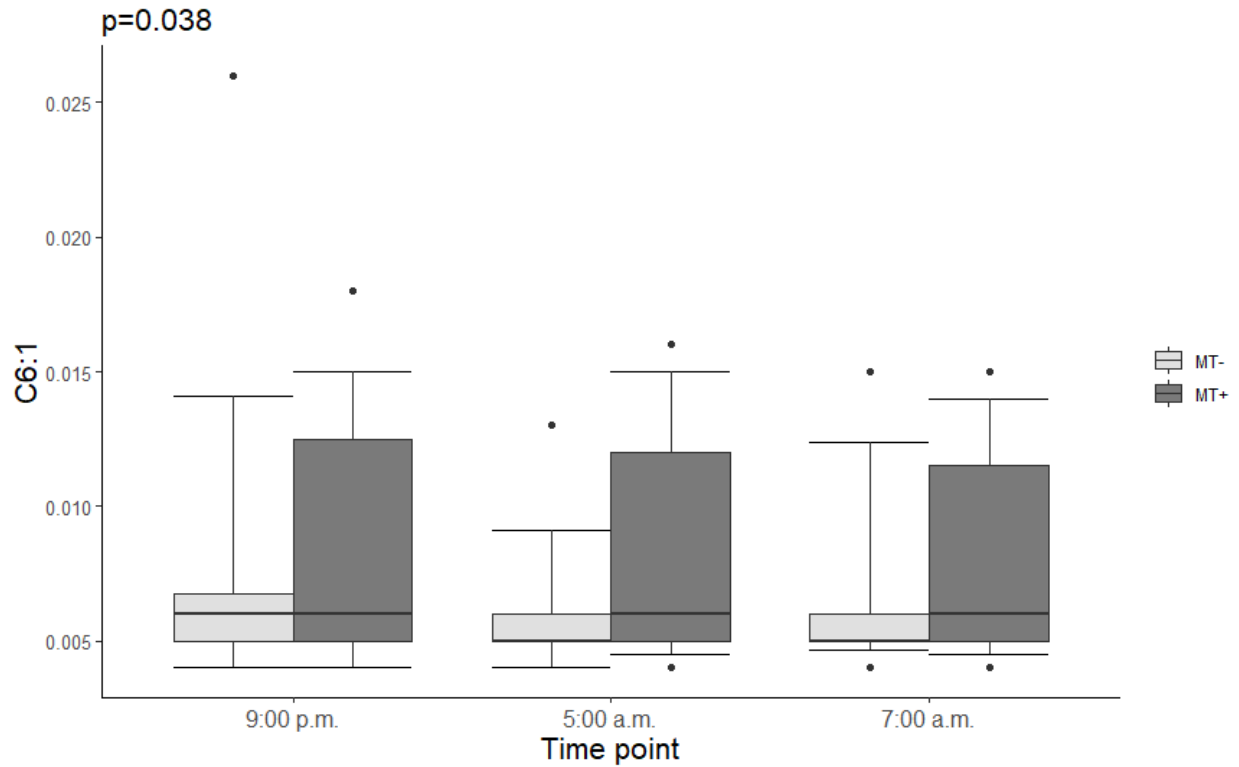
Supplementary figure S3. Peripheral blood concentrations of C6:1 (hexenoylcarnitine) were reduced overnight in the group of symptomatic individuals with apnoea-hypopnea index (AHI) below the metabolomic threshold (AHI <5/h, MT-) (n=14), but remained relatively unchanged in the group of patients with AHI above the metabolomic threshold (AHI ⩾5/h, MT+) (n=51), p=0.038. Individuals with symptoms characteristic of sleep-disordered breathing (n=65) underwent targeted analysis of their peripheral blood metabolome and comparisons were done using ranked general linear model with repeated measures.