Respiratory Physiology & Neurobiology 266 (2019) 73–81

Contents lists available at ScienceDirect

Respiratory Physiology & Neurobiology

journal homepage: www.elsevier.com/locate/resphysiol

An act of balance: Interaction of central and peripheral chemosensitivity with inflammatory and anti-inflammatory factors in obstructive sleep apnoea

Christopher Earing[b], Julian Owen[a], Claire Griffith-Mcgeever[a], Damian McKeon[b], Stefan Engeli[c], Jonathan Moore[a], Hans-Peter Kubis[a,*]

[a] College of Human Sciences, Bangor University, United Kingdom
[b] Pulmonary and Sleep Department, Ysbyty Gwynedd Hospital, Bangor, United Kingdom
[c] Clinical Pharmacology, Medical School, Hanover, Germany

ARTICLE INFO

Keywords:
Obstructive sleep apnoea
Obesity
Inflammation
Endocannabinoids
C-reactive protein
Chemosensitivity

ABSTRACT

Objectives: Central and peripheral chemosensitivity i.e. ventilatory response to CO_2 and O_2 are thought to be decisive for ventilatory control instability in obstructive sleep apnoea (OSA). Obesity is associated with chronic low level inflammation. Whether body mass related inflammatory and anti-inflammatory factors influencing peripheral and central chemosensitivity differentially is unclear.

Methods: Ventilatory response to hypercapnic-hyperoxic and hypercapnic-hypoxic gas mixtures in patients with OSA (n = 46) and healthy individuals (n = 45) was measured. C-reactive protein (CRP), leptin, adiponectin, and endocannabinoids 2-arachidonoylglycerol (2-AG) and anandamide (AEA) were measured in blood samples.

Results: Mediation analysis revealed that association of chemoresponse to CO_2 with apnoea hypopnea index (AHI) was fully mediated by body mass index (BMI). Regression analysis showed that CRP and leptin levels explained ˜25% and ˜15% of the variance in central CO_2 response, while 2-AG explained ˜42% of the variance in peripheral response to hypoxia.

Conclusion: Inflammatory and anti-inflammatory factors could explain differential alterations in peripheral and central ventilatory chemoresponse in patients with OSA.

1. Introduction

Obstructive sleep apnoea (OSA) is a breathing disorder characterized by cyclical pharyngeal collapse to the point of ventilatory constraint accompanied by hypopnoea and apnoea periods during sleep. OSA is suggested to promote hypertension, stroke, myocardial infarction and has a high risk of all-cause mortality (Marshall et al., 2008). Obesity, hypertension and male gender are strongly associated with OSA prevalence (Young et al., 2004).

In OSA, oversensitive ventilatory response to apnoeic events and the instability of breathing during sleep has been suggested to be caused by an elevation of loop gain (Wellman et al., 2008). High loop gain, which is connected to the resulting large increase in ventilation after a former reduction in ventilation below the eucapnic status due to obstruction, is shown to be critically dependent on chemosensitivity (part of the controller gain) (Dempsey and Smith, 2014).

The ventilatory response to hypoxia and hypercapnia has been investigated in OSA patients with various techniques and outcomes in wakefulness and during sleep. Outcomes of ventilatory response to carbon dioxide in wakefulness have been very variable: decreased (Osanai et al., 1999) or similar (Sin et al., 2000; Narkiewicz et al., 1999; Verbraecken et al., 2000), while ventilatory responses to hypoxia were lower (Osanai et al., 1999) or higher (Narkiewicz et al., 1999) compared with healthy individuals. However, most studies did not use mixed hyperoxic/hypercapnic and hypoxic/hypercapnic gases for differentiation of peripheral and central response to carbon dioxide (Duffin, 2007). Chemosensitivity during sleep has been more consistently showing an elevation of ventilatory response to hypoxic/hypercapnic gas mixtures (Edwards et al., 2012; Salloum et al., 2010; Xie et al., 2001; Younes et al., 2007). However, only in about ˜30% of OSA patients an elevated controller gain is detected, and other factors, like altered plant gain, arousal threshold, and dilator muscle recruitment of airways seem to be relevant producing a more complex picture (Dempsey et al., 2014). It is clear from models based on experimental

* Corresponding author at: College of Human Sciences, Bangor University, Bangor, LL57 2PZ, United Kingdom.
E-mail address: pes203@Bangor.ac.uk (H.-P. Kubis).

https://doi.org/10.1016/j.resp.2019.05.002
Received 6 February 2019; Received in revised form 30 April 2019; Accepted 2 May 2019
Available online 03 May 2019

data, peripheral and central chemosensitivity should be of substantial influence on OSA symptoms (Francis et al., 2000). OSA is strongly associated with obesity, and often patients develop obesity hypoventilation syndrome (OHS) in addition to OSA (Shetty and Parthasarathy, 2015). The prevalence of OHS is about 20%–30% in OSA and even higher in BMI > 50 patients (Mokhlesi et al., 2007). OHS is characterized by BMI > 30 with awake arterial blood pCO_2 > 45 mmHg unexplained by other disorders (Shetty and Parthasarathy, 2015); the ventilatory response to carbon dioxide and hypoxia is reduced in OHS (Piper and Grunstein, 2010). However, many OSA patients may share similar changes in chemosensitivity without reaching levels of daytime hypercapnia.

This opens the question, how obesity is able to influence chemosensitivity resulting in such different outcomes in ventilatory responses to hypoxia and hypercapnia in connection with OSA and OHS? In obesity, mechanical factors may play a role with oxygen cost of breathing increased in combination with reduced forced vital capacity (FVC) (Rochester and Enson, 1974). However, the variability of the ventilatory response to hypoxia and hypocapnia in OSA suggests more complex mechanisms, which might involve changes of central and peripheral chemosensitivity.

Biochemical factors associated with obesity are known in great detail and are often related to poor health outcome like cardiovascular disease, type 2 diabetes, cancer etc. (Kahn et al., 2006; Van Gaal et al., 2006). Some of the factors are reported to influence, or being associated, with ventilatory drive and chemosensitivity. The adipokine leptin has been shown to be an important regulator of central respiratory drive and leptin resistance is suggested to be an important contributor to reduced ventilatory drive in obesity and OHS (Bassi et al., 2015; Cundrle et al., 2014; Malli et al., 2010) but also adiponectin, which is reduced in obesity, is reported to be associated with AHI in OSA (Lacedonia et al., 2016). Moreover, systemic low level inflammation is connected with obesity; inflammatory factors like CRP, IL6 and TNF alpha are known to be associated with cardiovascular disease (Van Gaal et al., 2006), but also shown to be connected with pulmonary diseases like chronic obstructive pulmonary disease (COPD) (Gan et al., 2004) and OSA (Guilleminault et al., 2004). In theory, peripheral chemosensitivity could be by influenced by inflammatory cytokines as it is shown that hypoxia leads to increased immune cell invasion and cytokine expression in carotid body in rats (Liu et al., 2009). Furthermore, an activation of the peripheral endocannabinoid system is known in obesity (Engeli et al., 2005); the action of endocannabinoids anandamide (AEA) and 2-arachidonoylglyceraol (2-AG) are mainly recognized in energy homeostasis and metabolism (Di Marzo, 2008) but also have anti-inflammatory properties (de Lago et al., 2012). Indeed, recent findings show additional effects of endocannabinoids on chemosensitivity of the carotid body (Kim et al., 2009) extending their possible role for breathing disorders.

Consequently, our study had the objective to investigate the association of obesity related body characteristics and biochemical factors with alterations of peripheral and central chemosensitivity in OSA patients. Firstly, we measured the ventilatory response to hypercapnia and hypoxia in wakefulness with gas mixtures, enabling a better differentiation between central and peripheral chemosensitivity (Duffin, 2007; Earing et al., 2014), body characteristics and pulmonary function in newly diagnosed OSA patients and 'healthy' individuals. Secondly, we measured obesity related biochemical factors leptin, adiponectin, CRP, AEA, and 2-AG to investigate their possible interaction with changes of peripheral and central chemosensitivity and apnoea hypopnoea index (AHI) in OSA patients.

We hypothesized that OSA patients reveal a reduced ventilatory response to carbon dioxide and hypoxia in wakefulness compared with healthy individuals. We expected that BMI would contribute most to central ventilatory hypercapnic response in regression models and that distinct BMI associated biochemical factors, related to inflammatory and anti-inflammatory responses, would explain a significant proportions in the variance of peripheral and central chemosensitivity, i.e. ventilatory responses to hypercapnia and hypoxia.

2. Methods

2.1. Participants

The study was approved by the North Wales Research Ethics Committee (No. 11/WNO: 01/02) and the departmental research ethics committee, Bangor University, UK, according to the Declaration of Helsinki for research on human subjects.

Participants were adult males diagnosed with obstructive sleep apnoea (OSA) (N = 48) and healthy adult male individuals (HI) (N = 49). Participants with OSA were recruited from newly diagnosed patients from the Pulmonary Department and Sleep Clinic at Ysbyty Gwynedd, Bangor. Patients were diagnosed using an unattended home sleep study (respiratory polygraphy; Embletta®Gold, Embla Systems, USA). Measures of pulse oximetry, nasal airflow, thoracic and abdominal movements were analysed using RemLogic software. Diagnosis of OSA was performed by either an experienced registered Clinical Physiologist, or experienced Sleep Technologist. OSA patients' medical records were reviewed and patients with documented respiratory disease or with FEV1/FVC < 70%, or taking medications known to affect their ventilatory drive (i.e. opioid-analgetics) were excluded. Additionally, potential participants were excluded if they had a body mass index (BMI) ≥ 50 kg/m^2 to avoid inclusion of individuals with predominately obesity hypoventilation syndrome. Written informed consent was obtained from all participants prior to testing and participants with OSA were tested within two weeks prior to their treatment with Continuous Positive Airway Pressure (CPAP). Healthy individuals were recruited from general population from the Bangor area, North Wales, who reported no medical conditions, BMI < 50, taking no medication suppressing ventilation, and reported no sleep problems according to the Epworth Sleepiness Scale (< 10) (ESS) and Pittsburgh Sleep Quality Index (< 5) (PSQI). Epworth Sleepiness Scale is widely used for pre-screening purposes for OSA; however, validation in targeted OSA patient populations is lacking (Gamaldo et al., 2018). We used both questionnaires to exclude participants from the 'healthy' group with potential sleep disorders such as OSA.

2.2. General procedures

2.2.1. Body characteristics

Participants' height was measured using a stadiometer (Bodycare Products, Southam, United Kingdom). A digital scale (Seca; Vogel & Halke, Hamburg, Germany) was used to measure body weight. Participants were weighed in a non-fasting state while they wore minimal clothing. Circumferences were measured using a tape measure; waist circumference was measures after normal expiration at noticeable waist narrowing. Hip circumference measured at the level of symphysis pubis and largest guteal protuberance, and neck circumference taken three inches above the collarbone and in line with where the shoulder meets the neck.

2.2.2. Pulmonary function

Pulmonary function of OSA patients was assessed during diagnostic examination in the Pulmonary Department of Ysbyty Gwynedd hospital using standard clinical equipment for spirometry. A trained physiologist at Bangor University assessed healthy participants' pulmonary function data; a MicroLoop spirometer (Micro Medical Ltd., Basingstoke, UK) was used following ATS/ERS guidelines (Miller et al., 2005).

2.2.3. Ventilatory response to gas mixtures

Measurements of the ventilatory response to carbon dioxide and oxygen mixtures were performed according to the method by Earing et al. (Earing et al., 2014). In brief, the gas mixtures were ambient air,

Table 1
Body characteristics of patients with OSA and healthy individuals (HI).

Parameter:	OSA	HI	Mann & Whitney U P value
N	46	45	
Age (yrs.)	53.2 ± 10.9 (45.5|54.5|63.5)	33.1 ± 9.0 (26.0|34.0|41.0)	< 0.0001*
Height (cm)	174.5 ± 7.1 (169.3|174.4|181.0)	178.8 ± 7.0 (174.0|179.4|183.3)	0.001*
Mass (kg)	106.0 ± 21.1 (90.0|100.5|119.5)	80.2 ± 13.5 (72.5|77.0|83.8)	< 0.0001*
Neck (cm)	44.3 ± 3.8 (41.8|43.5|47.0)	38.7 ± 2.4 (37.4|38.5|40.0)	< 0.0001*
Waist (cm)	114.0 ± 14.7 (103.0|109.3|126.0)	87.4 ± 9.5 (81.0|86.3|90.0)	< 0.0001*
BMI (kg/m^2)	35.0 ± 6.5 (29.8|33.7|39.6)	25.0 ± 3.4 (22.5|24.2|25.9)	< 0.0001*

Values represented mean ± standard deviation, Tukey's Hinges in brackets. *, significant using Benjamini-Hochberg procedure for multiple comparisons with false discovery rate of 0.10.

25% O_2 /6% CO_2, 13% O_2, and 13% O_2 / 6% CO_2; all gas mixtures were balanced with N_2 (BOC Ltd., England). Ambient air was used to assess baseline minute ventilation. The volume transducer and gas sampling port of the metabolic cart (MetaMax®3B, Cortex Biophysik, Germany) were attached to a two-way valve allowing gas to be inspired from a 250 l Douglas bag and expired into the atmosphere. All measurement were performed with the metabolic cart calibrated prior to each testing session. Participants were blinded to the order of tests, however, the order of gas mixtures were the same for all participants (see above). Seated participants were breathing each gas mixture until a plateau in minute ventilation was achieved (up to 5 min), while focussing on a non-dramatic video to avoid conscious control of ventilation (Eynan et al., 2003). Expired gas concentrations and minute ventilation data were averaged over 5 s periods. Data of minute ventilation at ambient air over 2 min were used for baseline. For the gas mixtures, the plateau of minute ventilation was detected using a moving average filter and the maximal ventilation over a period of 30 s was selected from the plateau and averaged excluding any outliers. Ambient air minute ventilation results were subtracted from minute ventilation data at plateau for the specific gas mixtures to calculate minute ventilation changes for the specific gas mixtures ($\Delta \dot{V}$ X% CO_2 / Y% O_2). All minute ventilation data were normalized on body surface area (BSA) to cater for mass related individual differences (Menitove et al., 1984). BSA was estimated using the Mostellers equation as previously recommended as the most valid for use with obese individuals (Verbraecken et al., 2006).

2.2.4. Blood samples

Venous blood samples were only taken from patients with OSA. 10 ml of venous blood was drawn by antecubital venepuncture into two 6 ml Vacutainer® EDTA- plasma tubes after overnight fast. Plasma was produced by centrifugation (4000 rpm at 4 °C (Universal 320R, Hettich Centrifuge, Germany) for ten minutes) within five minutes after blood drawing to avoid contamination of blood plasma with endocannabinoids produced after drawing of blood (Engeli et al., 2012). Plasma was immediately snap frozen in liquid nitrogen and stored at −80 °C for batch analysis.

Endocannabinoids, 2-arachidonoylglycerol (2-AG) and anandamide (AEA) were measured in plasma samples at the Department of Clinical Pharmacology, Medical School Hanover, Germany, according to a method by Zoerner et al. (Zoerner et al., 2012) using a Waters ACQUITY UPLC–MS/MS system consisting of a solvent delivery device, an autosampler, a column thermostat and the tandem quadrupole mass spectrometer XEVO TQ MS (Waters, Milford, MA, USA). Further, adiponectin, CRP, and leptin were measured by enzyme-linked immunosorbent assay (ELISA) (Biovendor, Czech Republic). Adiponectin: intra- and interassay coefficient of variation of 5.4% and 19.7%, respectively; leptin: with intra- and interassay coefficient of variation of 6.4% and 4.2%; CRP: intra- and interassay coefficient of variation of 10.0% and 16.8%.

2.2.5. Data analysis

Pulmonary and ventilatory response measurements are expressed in body temperature and pressure saturated units (BTPS) with mean ± standard deviation (SD). Several outcome variables were not normally distributed, therefore for comparison between groups a non-parametric Mann & Whitney *U* test was used. Parameters which were normal distributed were used in Student's *t*-test Correlation analysis was performed using bivariate Spearman's analysis. Linear regression, multiple linear regression (backward method). Additionally, mediation analysis using PROCESS 3.0 with SPSS was conducted. Mediation analysis is a sequential regression analysis to test a potential indirect association between two variables, which may be caused by a third variable (mediator) (MacKinnon et al., 2007). Non-normal parameters (i.e. FVC, BMI, end-tidal P_{CO2}, AHI, CRP, leptin) were successfully log-transformed for the former analyses. Non-parametric data are displayed with Tukey's hinges (25, 50, and 75 percentiles) and mean and SD. Data were analysed using Statistical Package for the Social Science (IBM SPSS) version 24. Significance levels were reported if lower than $p < 0.05$. For reduction of type I errors due to multiple comparisons, False Discovery Rate (FDR) procedure by Benjamini & Hochberg (Benjamini and Hochberg, 2000) was performed with a false discovery rate of 0.10, and significant comparisons marked by *.

3. Results

Body characteristics of patients with obstructive sleep apnoea (OSA) (n = 46) and healthy individuals (n = 45) are shown in Table 1; groups were significantly different in their body measures. OSA patients were older and had larger weight, BMI, neck, waist. Based on means, OSA patients belonged to the obese category (BMI > 29.9), while healthy individuals to the overweight category (BMI > 24.9).

As expected, pulmonary function parameters for OSA patients were lower than for HI, although patients' values were comparable with age-, sex- and height-predictions (Table 2). FEV1/FVC of OSA patients' group were in the age, sex, and height predicted range (Roca et al., 1998), and seemed not pathologically altered. However, FVC was negatively correlated with BMI (*rho* = -0.374, p = 0.032) in the OSA group, as expected. OSA patients had a wide range of apnoea hypopnoea index (AHI), with most of the patients having moderate to severe symptoms (Table 2). No significant difference in end-tidal P_{CO2} was reported between OSA patients and HI. Moreover, HI were asymptomatic for daytime sleepiness (Epworth Sleepiness Score = 4.43 ± 2.94) and sleep quality scores (Pittsburgh Sleep Quality Index = 4.13 ± 2.31).

Measurements of the ventilatory responses to carbon dioxide and oxygen mixtures indicated that OSA patients had significant lower ventilatory responses to hypercapnic and hypoxic gas mixtures than HI (Table 2). However, the percentage alteration of the ventilatory response to 6% carbon dioxide between hyperoxic and hypoxic gas mixtures was not different between groups.

Correlation analysis of AHI with pulmonary function parameters and ventilatory responses to gas mixtures showed that AHI was significantly negatively correlated with the ventilatory response to hyperoxic carbon dioxide gas mixture ($\Delta \dot{V}$ 6% CO_2 / 25% O_2) (Table 3). Patients with higher OSA severity were centrally less sensitive to carbon

Table 2
Pulmonary function data, ventilatory response to carbon dioxide and hypoxia in OSA patients (OSA) and healthy individuals (HI).

Parameter:	OSA	HI	P value
N	46	45	
AHI	34.64 ± 25.50 (13.30|29.7|49.45)	N/A	N/A
ESS	N/A	4.43 ± 2.94 (2.0|5.0|6.0)	N/A
PSQI	N/A	4.13 ± 2.31 (2.0|3.0|6.0)	N/A
FEV1 (l)	3.30 ± 0.58	4.38 ± 0.77	< 0.0001*
FVC (l)	4.22 ± 0.75	5.16 ± 0.84	< 0.0001*
FEV1/FVC (%)	78.53 ± 7.14	85.78 ± 15.60	< 0.0001*
End-tidal P_{CO2} (mmHg)	43.60 ± 7.81 (36.89|43.25|51.18)	41.89 ± 4.26 (39.60|41.00|44.02)	0.724
Ambient air; minute ventilation (l/min) per BSA (m^2)	4.66 ± 1.24	5.86 ± 0.73	< 0.0001*
$\Delta\dot{V}$ 6% CO_2 / 25% O_2	4.79 ± 2.17	6.99 ± 2.75	< 0.0001* *t*
$\Delta\dot{V}$ 13 % O_2	0.93 ± 0.76	1.73 ± 0.98	< 0.0001* *t*
$\Delta\dot{V}$ 6% CO_2 / 13 % O_2	5.76 ± 2.54	8.49 ± 2.93	< 0.0001* *t*
Percentage change in $\Delta\dot{V}$ 6% CO_2 from 25% O_2 to 13 % O_2	37.34 ± 73.21 (-1.47|19.62|78.54)	36.05 ± 67.18 (-1.53|22.42|49.87)	0.881

Values represented mean ± standard deviation, Tukey's Hinges in brackets. P values are according to Mann & Whitney U tests with exception of *t* labeled data tested with Student's *t*-test *, significant using Benjamini-Hochberg procedure for multiple comparisons with false discovery rate of 0.10.

Table 3
Correlation of pulmonary function, ventilatory response to carbon dioxide and oxygen gas mixtures, and body characteristics within OSA patients.

Parameter: (N = 46)	AHI	P value	Parameter: (N = 46)	AHI	P value
$\Delta\dot{V}$ 6% CO_2 / 25% O_2	−0.329*	0.026	Neck (cm)	0.555*	< 0.0001
$\Delta\dot{V}$ 13 % O_2	0.013		Waist (cm)	0.456*	0.001
$\Delta\dot{V}$ 6% CO_2 / 13 % O_2	−0.155		BMI (kg/m^2)	0.483*	0.001

Data represents bivariate Spearman's *rho*; P values < 0.05 are listed. *, significant using Benjamini-Hochberg procedure for multiple comparisons with false discovery rate of 0.10.

dioxide than patients with mild OSA. In contrast, AHI did not correlate with responses to hypoxic-hypercapnic gas mixture. Additionally, end-tidal P_{CO2} was not associated with AHI; however, $\Delta\dot{V}$ 6% CO_2 / 25% O_2 and $\Delta\dot{V}$ 6% CO_2 / 13% O_2 were negatively correlated with end-tidal P_{CO2}; *rho* = -0.620, p < 0.001 and *rho* = -0.515, p = 0.001, respectively. Patients with reduced sensitivity to carbon dioxide had higher end-tidal carbon dioxide values during ambient air breathing. $\Delta\dot{V}$ 6% CO_2 / 25% O_2 and $\Delta\dot{V}$ 6% CO_2 / 13% O_2 were also significantly correlated with end-tidal P_{CO2} for the combined groups (OSA plus HI) (n = 85), *rho* = -0.414, p < 0.001, and *rho* = -0.361, p = 0.001, respectively. No association between end-tidal P_{CO2} with ventilator response to hypoxic gas was found.

Body characteristics were positively correlated with AHI in the OSA group, in particularly with neck and waist circumference, as well as with BMI (Table 3), as expected.

To further investigate the influence of body characteristic parameters on the ventilatory response to hypercapnic and hypoxic gas mixtures, we analysed the ventilatory response data from OSA and HI together (Table 4). Correlation analysis revealed that the response to hypercapnic gasses was negatively correlated with body characteristics; with increasing BMI and body circumferences the response to carbon dioxide declined. Results with hypoxic gas showed very low association with body characteristics for the collapsed groups' data; however, for OSA patients, significant low positive correlation were found with BMI and waist.

In the combined group of male participants, multiple regression analysis, using body and age characteristics as predictor variables, showed that BMI was the most significant predictor explaining about 28% of the variance in ventilatory response to carbon dioxide (i.e. $\Delta\dot{V}$ 6% CO_2 / 25% O_2, $R^2 = 0.276$, F (1/85) = 32.0, p < 0.0001); participants with higher BMI revealed a lower $\Delta\dot{V}$ 6% CO_2 / 25% O_2 (β = -0.525). Lower values were reported with neck circumference as predictor (not shown). Moreover, within the OSA group, multiple regression analysis confirmed the importance of BMI for the prediction of $\Delta\dot{V}$ 6% CO_2 / 25% O_2 ; about 30% of the variance of the central carbon dioxide response was explained by BMI ($R^2 = 0.308$, F(1/45) = 18.247, p < 0.0001; β = -0.555).

To investigate the connection between AHI and the observed reduced ventilatory response to carbon dioxide, as well as their association with body characteristics in OSA patients, we performed a mediation analysis using regression with bootstrapping (5000 samples); we entered $\Delta\dot{V}$ 6% CO_2 / 25% O_2 as the outcome variable, AHI as predictor variable, and BMI as the mediator (Fig. 1). Confirming a possible direct effect, AHI was a significant predictor of $\Delta\dot{V}$ 6% CO_2 / 25% O_2, b = -2.2871, SE = 0.8955, 95% LLCI: -4.0956, UPCI: -0.4786. However, AHI was no longer a significant predictor of $\Delta\dot{V}$ 6% CO_2 / 25% O_2 after controlling for BMI, b = -0.9201, SE = 0.8999, 95% LLCI: -2.7389, UPCI: 0.8987, ns, consistent with full mediation. Additionally, BMI was a significant predictor of $\Delta\dot{V}$ 6% CO_2 / 25% O_2, b = -15.648, SE = 3.108, 95% LLCI: -21.490, ULCI: -9.291, and AHI was a significantly regressed on BMI, b = 0.0995, SE = 0.0305, 95% LLCI: 0.0380, ULCI: 0.1610. Approximately 33% of the variance in $\Delta\dot{V}$ 6% CO_2 / 25% O_2 was accounted for by the predictors ($R^2 = 0.326$, p < 0.001). Bootstrap estimation of the indirect effect indicated the indirect coefficient was significant, b = -1.3670, SE = 0.569, 95% LLCI = -2.6881, ULCI = -0.4763. These results reveal that the effect of AHI on ventilatory response to carbon dioxide was fully mediated by BMI.

To further explore reasons for the dual influence of BMI on AHI and ventilatory responses to the various gas mixtures in OSA patients, we investigated a possible involvement of cytokines and adipokines, which are known to be typically associated with high body mass. We assessed leptin, adiponectin, CRP and the two endocannabinoids, 2-arachidonoylglycerol (2-AG) and anandamide (AEA), in venous blood samples (Table 5). Correlation analysis showed that neither of the factors was significantly associated with AHI. However, $\Delta\dot{V}$ 6% CO_2 / 25% O_2 was negatively correlated with CRP and leptin, however, not significant after FDR procedure for multiple comparisons (Table 5); no correlations were found with $\Delta\dot{V}$ 6% CO_2 / 13% O_2. Linear regression analysis showed that about 25% of $\Delta\dot{V}$ 6% CO_2 / 25% O_2 variance could be explained by CRP levels in OSA patients ($R^2 = 0.263$, p = 0.017; β = -0.513), while leptin explained about 15% ($R^2 = 0.156$, p = 0.038; β = -0.395).

Consequently, a considerable proportion of the effect of BMI on $\Delta\dot{V}$ 6% CO_2 / 25% O_2 can be explained by CRP and leptin but no contribution was found for AHI.

Ventilatory response to hypoxia, $\Delta\dot{V}$ 13% O_2, was positively correlated with CRP and 2-AG (Table 5). Particularly, 2-AG was strongly correlated and regression showed that it could explain 42% of the $\Delta\dot{V}$ 13% O_2 ($R^2 = 0.418$, p < 0.001), while CRP could explain 19% of the ventilatory response to 13% oxygen ($R^2 = 0.190$, p = 0.048).

Table 4
Correlation of body characteristics with change in minute ventilation to hypercapnic and hypoxic gas mixtures from ambient air levels (OSA plus HI) and OSA.

Parameter:	Age (yrs.)	Neck (cm)	Waist (cm)	BMI (kg/m^2)
OSA plus HI (N = 86)				
$\Delta\dot{V}$ 6% CO_2 / 25% O_2	−0.303* (0.005)	−0.491* (< 0.0001)	−0.496* (0.0001)	−0.482* (< 0.0001)
$\Delta\dot{V}$ 13 % O_2	−0.154	−0.203	−0.248* (0.021)	−0.238* (0.027)
$\Delta\dot{V}$ 6% CO_2 / 13 % O_2	−0.273* (0.011)	−0.482* (< 0.0001)	−0.519* (< 0.0001)	−0.498* (< 0.0001)
OSA (N = 46)				
$\Delta\dot{V}$ 6% CO_2 / 25% O_2	−0.096	−0.395* (0.0009)	−0.354	−0.516* (0.0004)
$\Delta\dot{V}$ 13 % O_2	0.299	0.265	0.254	0.259
$\Delta\dot{V}$ 6% CO_2 / 13 % O_2	0.083	−0.185	−0.310 (0.043)	−0.374* (0.013)

Data represents bivariate Spearman's *rho*; P values < 0.05 in brackets. *, significant using Benjamini-Hochberg procedure for multiple comparisons with false discovery rate of 0.10.

Moreover, biochemical factors were associated with body characteristics. In particular, leptin and CRP were positively correlated with BMI, while CRP and 2-AG were negatively correlated with pulmonary function FVC and FEV1. In particular, high associations between pulmonary function parameters and ventilatory response to hypoxia were apparent for 2-AG.

Based on the former finding that 2-AG explained the largest proportion of variance in $\Delta\dot{V}$ 13% O_2 and CRP lost its significance in a multiple regression model with 2-AG as predictor variables of hypoxic ventilatory response (not shown), we focused on 2-AG in relation to hypoxic response.

To interpret the importance of 2-AG for the connection between increased ventilatory response to hypoxia with lower FVC (*rho* = -0.359, p = 0.021), we performed an additional mediation analysis using regression with bootstrapping (5000 samples); we entered $\Delta\dot{V}$ 13% O_2 as the outcome variable, FVC as predictor variable, and 2-AG as the mediator (Fig. 2). Results indicated that FVC was a significant predictor of 2-AG, b = -8.246, SE = 1.941, 95% LLCI: -12.303, ULCI: -5.564. Additionally, 2-AG was a significant predictor of $\Delta\dot{V}$ 13% O_2, b = 0.281, SE = 0.077, 95% LLCI: 0.150, ULCI: 0.456. Confirming a possible direct effect, FVC was a significant predictor of $\Delta\dot{V}$ 13% O_2, b = -3.877, SE = 1.295, 95% LLCI: -6.570, UPCI: -1.184. However, FVC was no longer a significant predictor of $\Delta\dot{V}$ 13% O_2 after controlling for 2-AG, b = -1.074, SE = 1.514, 95% LLCI: -4.232, UPCI: 2.084, ns, consistent with full mediation. Approximately 49% of the variance of $\Delta\dot{V}$ 13% O_2 was accounted for by the predictors (R^2 = 0.494, p < 0.001). Bootstrap estimation of the indirect effect indicated the indirect coefficient was significant, b = -2.802, SE = 1.625, 95% LLCI = -6.265, ULCI = -0.190. These results reveal that the effect of FVC on ventilatory response to hypoxia was fully mediated by 2-AG. Further, mediation analysis revealed that the association of CRP with $\Delta\dot{V}$ 13% O_2 was not significant in a mediation model (not shown). Consequently, higher 2-AG levels contributed strongly to the enhancement of hypoxic response in OSA patients.

4. Discussion

In the first part of this study, we measured the ventilatory response to carbon dioxide and oxygen mixtures to investigate central and peripheral chemosensitivity during wakefulness in obstructive sleep apnoea patients and healthy individuals and their connection with body characteristics. Importantly, we selected newly diagnosed, untreated OSA patients, due to the reported influence of continuous positive airway pressure (CPAP) treatment on chemosensitivity (Loewen et al., 2009; Salloum et al., 2010). Our results show that patients with OSA possess a reduced ventilatory response to hypercapnia and hypoxia in wakefulness in comparison with healthy individuals, suggesting that central and peripheral chemosensitivity is reduced in OSA patients. In agreement with our findings, former studies with OSA patients revealed reduced ventilator response to carbon dioxide (Gold et al., 1993; Javaheri et al., 1994; Littner et al., 1984), which was more often found in patients with elevated P_{aCO2} (Ayappa et al., 2002; Han et al., 2001). Additionally, Osanai et al. (Osanai et al., 1999) found a reduction of peripheral chemosensitivity in OSA patients compared with controls. However, former studies did not use the combination of CO_2 gasses with different oxygen concentrations in an attempt to differentiate central and peripheral responses as recommended by Duffin (Duffin, 2007).

A very consistent finding in former studies is the association of higher BMI for OSA patients with reduced response to carbon dioxide (Han et al., 2001; Javaheri et al., 1994), which was even stronger in patients with daytime hypercapnia (Resta et al., 2000). In contrast, in a

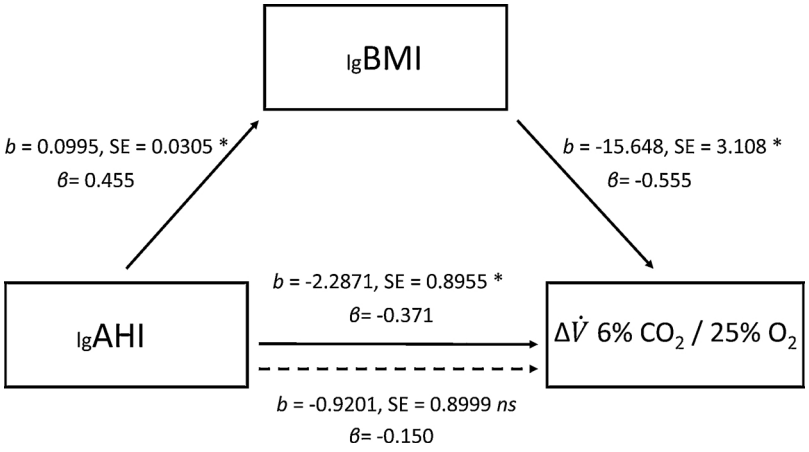

Fig. 1. Mediation Analysis for the effect of AHI on chemosensitivity to carbon dioxide with BMI as mediator. Beta values (*b*) and standard errors (SE), and standardized beta values (β) from regression analysis next to solid lines between variables shown in boxes; values below dashed line depicts beta value (*b*)and standard error (SE) after controlling for indirect effects. *, p < 0.05; ns, not significant.

Table 5
Cytokine and hormone levels and their correlations with respiratory characteristics in patients with OSA.

Parameter: Mean ± SD (Tukey's Hinges)	Leptin (ng/ml) (N = 31) 31.41 ± 21.51 (13.5|21.2|40.4)	Adiponectin (ng/ml) (N = 32) 8.87 ± 3.92	CRP (μg/ml) (N = 24) 4.61 ± 3.29 (2.0|4.0|5.6)	AEA (nM) (N = 30) 0.95 ± 0.27	2-AG (nM) (N = 30) 3.51 ± 1.47
	Leptin	Adiponectin	CRP	AEA	2-AG
Adiponectin	−0.118				
CRP	0.553* (0.008)	−0.092			
AEA	0.111	0.156	0.237		
2-AG	0.197	−0.178	0.471 (0.027)	0.159	
AHI	0.243	0.001	−0.019	0.136	−0.034
$\Delta\dot{V}$ 6% CO_2 / 25% O_2	−0.354	−0.074	−0.434 (0.046)	−0.088	0.076
$\Delta\dot{V}$ 13 % O_2	0.153	−0.035	0.474 (0.030)	0.036	0.626* (0.0005)
$\Delta\dot{V}$ 6% CO_2 / 13 % O_2	−0.184	−0.222	−0.501 (0.021)	−0.263	0.053
BMI	0.822* (< 0.0001)	−0.172	0.652* (0.001)	0.186	0.318
FVC	−0.124	0.029	−0.366	−0.107	−0.657* (0.0004)
FEV1	−0.301	−0.088	−0.437	−0.143	−0.609* (0.001)

Data represents bivariate Spearman's *rho*; P values < 0.05 in brackets. *, significant using Benjamini-Hochberg procedure for multiple comparisons with false discovery rate of 0.10.

study comparing OSA patients with BMI matched controls, the ventilatory response to carbon dioxide was still significantly reduced compared with controls but not the hypoxic response (Gold et al., 1993) suggesting that a reduced response to carbon dioxide may be independent of BMI in OSA. However, in our study, within patients with OSA, as well as in the combined data with healthy individuals, a negative association of carbon dioxide response with BMI was found (about 30% variance of hypercapnic response explained by BMI in regression analyses).

Interestingly, the severity of OSA, i.e. AHI scores, was negatively associated with the ventilatory response to carbon dioxide (hyperoxic gas mixture) but not with the hypoxic response in OSA patients, suggesting that this association was mainly driven by central chemoresponse. Other polysomnography parameters (not shown), like average oxygen saturation and frequency below 85% saturation were highly correlated with AHI and did not show stronger associations with chemosensitivity parameters. Therefore, AHI was used throughout the manuscript.

An early hypothesis in relation to the mechanisms responsible for OSA was that nocturnal hypercapnia and hypoxia would lead to gradual adaptation of central and peripheral chemoreceptors (Dempsey and Forster, 1982). Conversely, our mediation analysis showed that the effect of AHI scores on carbon dioxide response was not direct, but fully mediated by BMI. This suggests that BMI related mechanisms, possibly via biochemical and mechanical factors may be more relevant for the observed decline in CO_2 response in OSA patients than the frequency of apnoea-hypopnoea periods. Indeed, findings of reduced ventilator response to carbon dioxide are known in patients with obesity hypoventilation syndrome (OHS) (Shetty and Parthasarathy, 2015). It is reported that 90% of OHS patients have been suggested to have some degree of OSA (Cooksey and Mokhlesi, 2016). While the OSA patients in our study were not diagnosed with OHS, not meeting the clinical criteria (Al Dabal and BaHammam, 2009), the endtidal pCO_2 was not significantly different compared to the HI group. However, significant negative correlation of ventilatory hypercapnic response with endtidal pCO_2 was found. Additionally, endtidal pCO_2 was also positively correlated with BMI in OSA patients, as well as in the collapsed data of both groups. Undoubtedly, endtidal pCO_2 is not as strong as arterial blood pCO_2 for indication of hypercapnia in OHS; however, its clinical validity is supported by studies even with patients with increased dead space (Donald and Paterson, 2006; McSwain et al., 2010). Consequently, our data supports a crucial importance of high BMI for the reduction of the ventilatory response to CO_2 in OSA patients in wakefulness. Considering our observed association of mainly central ventilatory CO_2 response with AHI, it was the question what importance this finding may have as a contributing factor for obstructive sleep apnoea together with peripheral chemosensitivity.

According to Dempsey et al. (Dempsey et al., 2014), two sleep-induced changes are significant in OSA: the changes in the mechanics of the upper airways and the importance of chemosensitivity for the control of respiratory motor output. In particular, differences in the involvement of peripheral and central chemoreceptors balance are suggested in OSA (Dempsey, 2005). Indeed, peripheral chemoreflex was stronger in OSA patients without comorbidities (Narkiewicz et al., 1999) and was particularly elevated below eupnea during sleep (Salloum et al., 2010). Work by Xie et al (Xie et al., 2013) highlighted the importance of the peripheral chemoreceptor by showing that hyperoxia prolonged the apnoea length in OSA patient during sleep, while

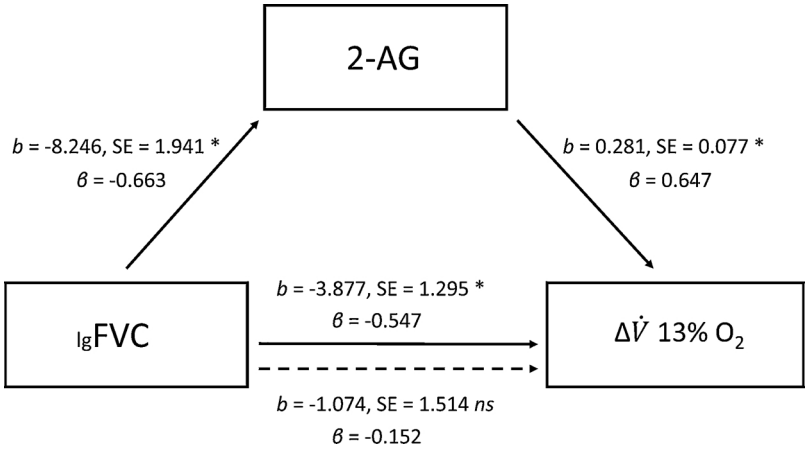

Fig. 2. Mediation Analysis for the effect of FVC on chemosensitivity to hypoxia with 2-AG as mediator. Beta values (*b*) and standard errors (SE), and standardized beta values (β) from regression analysis next to solid lines between variables shown in boxes; values below dashed line depicts beta value (*b*)and standard error (SE) after controlling for indirect effects. *, p < 0.05; ns, not significant.

supplemental oxygen could reduce AHI in OSA patients with high loop gain (Wellman et al., 2008). A shifted importance of chemosensitivity from central during wakefulness to the periphery might explain that reduced chemosensitivity in wakefulness can coexist with elevated controller gain during sleep.

In the second part of this study, we investigated biochemical factors, which could explain the strong associations of OSA and alterations in chemosensitivity with BMI. Measuring BMI associated biochemical factors suggested to be influencing ventilatory chemosensitivity, we found that a significant proportion of the variance of ventilatory CO_2 response could be explained by leptin (15%) and CRP (25%). Indeed, OSA patients with higher leptin and CRP had a stronger reduction of ventilatory response to CO_2 than patients with lower levels. Earlier, CRP was shown to be positively associated with AHI in OSA patients (Shamsuzzaman et al., 2002), which was independent of visceral obesity (Lui et al., 2009). CRP was shown to be linked with IL-6 in inflammatory processes seen in cardiovascular diseases (Van Gaal et al., 2006) and COPD (Gan et al., 2004). Indeed, influence of inflammatory factors like IL6 and TNF alpha has been shown to influence chemoreceptor sensitivity in the carotid bodies of rats linking these factors to adaptation to chronic hypoxia (Liu et al., 2009). Moreover, inflammatory factors are linked to sleep disordered breathing in OHS and OSA (Al Dabal and BaHammam, 2009; Ryan, 2017; Zamarron et al., 2008).

A positive association of leptin with suppression of CO_2 response in OSA patients is reported in connection with OSA and OHS (Malli et al., 2010). Reduced leptin sensitivity is known to be strongly associated with obesity and elevated leptin levels were associated with reduced respiratory drive and hypercapnic response (Campo et al., 2007; O'Donnell et al., 1999). Possible mechanisms between leptin and breathing disorders have been recently investigated in animal models, showing that leptin acts on central chemosensitivity areas via the brain melanocortin system (Bassi et al., 2015). Indeed, our finding of the association of CRP and leptin with reduced ventilator response to CO_2 could be linked; inflammatory factors can acerbate leptin resistance (Chen et al., 2006; Hribal et al., 2014) and would therefore lead to the association of CRP with CO_2 response without directly influencing chemosensitivity.

Further factors which could be relevant for the regulation of chemosensitivity are endocannabinoids (Kim et al., 2009) and are also known to be potent anti-inflammatory factors (de Lago et al., 2012). Indeed, our data show a strong positive correlation between ventilatory response to hypoxia and 2-AG, which was also negatively correlated with FVC. OSA patients with higher levels of 2-AG had a stronger ventilator response to hypoxia. Mediation analysis revealed that the association between FVC and hypoxic response was fully mediated by 2-AG. Moreover, 2-AG levels were positively correlated with CRP levels in our OSA patient group, suggesting that the elevation of 2-AG could be partly a response to the systemic low-level inflammation seen in obesity. In support of an involvement of endocannabinoids in the upregulation of hypoxic response seen in our OSA patients, it was recently shown that endocannabinoids can increase chemosensitivity in carotid body glomus cells (Kim et al., 2009). Relevant for this response seems to be TASK-like potassium channels shown to play a central role in oxygen sensing of the carotid body (Buckler, 2007); various endocannabinoids can block TASK-like potassium channels relevant for neuronal response to chemical stimuli (Kim et al., 2009; Maingret et al., 2001). In this respect, AEA has been more investigated than 2-AG; however, we could not find strong association between AEA and the ventilatory response to hypoxia in our OSA patient group. Nonetheless, further target receptors are reported to be involved in endocannabinoid response of the carotid bodies (Roy et al., 2012). AEA and 2-AG have been shown to increase pulmonary arterial pressure via CB1 receptors in rat lungs and lung tissue seems to be also involved in endocannabinoid metabolism (Wahn et al., 2005). Consequently, an involvement of endocannabinoids in the upregulation of peripheral chemosensitivity in OSA seems likely.

Summarized, we found inflammatory factor CRP and leptin negatively associated with ventilatory hypercapnic response and anti-inflammatory factor 2-AG positively associated with ventilatory response to hypoxia in OSA patients. To interpret our findings in connection with a mechanistic link between obesity related biochemical factors and chemosensitivity in wakefulness for OSA, it could be hypothesized, that high adipocyte mass increases levels of adipokines and mediates chronic low-level inflammation. Inflammatory factors could contribute to leptin resistance and would reduce central chemosensitivity. Accordingly, anti-inflammatory response i.e. endocannabinoids, which might be more elevated if pulmonary function is reduced, would lead to an upregulation of peripheral chemosensitivity in the carotid bodies. In wakefulness, this shift towards higher oxygen and CO_2 sensitivity of carotid bodies in face of a reduced CO_2 response in the centre might be a beneficial alteration. However, during sleep, where the responsibility for the respiratory drive shifts more to the periphery, the imbalance in the regulatory systems might lead to increased breathing instability and exaggerated compensatory response after apnoeic periods, in particular connected to loop gain. Both OSA and OHS would therefore share mechanisms, which are ultimately connected with obesity. Indeed, weight loss has been shown to have beneficial effects on both, OSA and OHS (Foster et al., 2009; Piper and Grunstein, 2010) and the involvement of the above mentioned biochemical factors might also open a novel pharmaceutical approach for OSA and OHS treatment.

Our study has several limitations, we have only measured the ventilatory response to hypercapnia and hypoxia in wakefulness, the additional measurement in sleep would have strengthened the interpretation of our data. Moreover, 'healthy' individuals where assessed by ESS and PSQI and not by polysomnography for exclusion of participants with OSA, which adds a risk of inclusion of undiagnosed participants with mild OSA in this group.

This study is correlative and therefore interpretations of causative relationships between parameters need to be taken as a starting point. However, our study suggests connections between biochemical factors related to obesity, central and peripheral chemosensitivity in wakefulness, and AHI in OSA patients, which could explain some of the overlapping and divergent findings in the literature regarding the involvement of chemosensitivity in wakefulness and sleep in OSA.

5. Conclusion

Our outcomes show that the reduced central chemoresponse to carbon dioxide in OSA patients could be partially explained by obesity related inflammatory factors. Anti-inflammatory factors, being strongly positively associated with an elevation of ventilatory response to hypoxia, suggest a shift of ventilatory regulation towards the periphery. A shift of balance in the ventilatory regulation towards the periphery is suggested to contribute to the exacerbation of OSA.

Declarations of interest

None.

References

Al Dabal, L., BaHammam, A.S., 2009. Obesity hypoventilation syndrome. Ann. Thorac. Med. 4, 41–49.

Ayappa, I., Berger, K.I., Norman, R.G., Oppenheimer, B.W., Rapoport, D.M., Goldring, R.M., 2002. Hypercapnia and ventilatory periodicity in obstructive sleep apnea syndrome. Am. J. Respir. Crit. Care Med. 166, 1112–1115.

Bassi, M., Nakamura, N.B., Furuya, W.I., Colombari, D.S.A., Menani, J.V., do Carmo, J.M., da Silva, A.A., Hall, J.E., Colombari, E., 2015. Activation of the brain melanocortin system is required for leptin-induced modulation of chemorespiratory function. Acta Physiol. 213, 893–901.

Benjamini, Y., Hochberg, Y., 2000. On the adaptive control of the false discovery fate in multiple testing with independent statistics. J. Educ. Behav. Stat. 25, 60–83.

Buckler, K.J., 2007. TASK-like potassium channels and oxygen sensing in the carotid body. Respir. Physiol. Neurobiol. 157, 55–64.

Campo, A., Fruhbeck, G., Zulueta, J.J., Iriarte, J., Seijo, L.M., Alcaide, A.B., Galdiz, J.B.,

Salvador, J., 2007. Hyperleptinaemia, respiratory drive and hypercapnic response in obese patients. Eur. Respir. J. 30, 223–231.
Chen, K., Li, F.H., Li, J., Cai, H.B., Strom, S., Bisello, A., Kelley, D.E., Friedman-Einat, M., Skibinski, G.A., McCrory, M.A., Szalai, A.J., Zhao, A.Z., 2006. Induction of leptin resistance through direct interaction of C-reactive protein with leptin. Nat. Med. 12, 425–432.
Cooksey, J., Mokhlesi, B., 2016. Postoperative Complications in Obesity Hypoventilation Syndrome and Hypercapnic OSA CO2 Levels Matter!. Chest 149, 11–13.
Cundrle, I., Somers, V.K., Singh, P., Johnson, B.D., Scott, C.G., van der Walt, C., Olson, L.J., 2014. Leptin deficiency promotes central sleep apnea in patients with heart failure. Chest 145, 72–78.
de Lago, E., Moreno-Martet, M., Cabranes, A., Ramos, J.A., Fernandez-Ruiz, J., 2012. Cannabinoids ameliorate disease progression in a model of multiple sclerosis in mice, acting preferentially through CB1 receptor-mediated anti-inflammatory effects. Neuropharmacology 62, 2299–2308.
Dempsey, J.A., 2005. Crossing the apnoeic threshold: causes and consequences. Exp. Physiol. 90, 13–24.
Dempsey, J.A., Forster, H.V., 1982. Mediation of ventilatory adaptations. Physiol. Rev. 62, 262–346.
Dempsey, J.A., Smith, C.A., 2014. Pathophysiology of human ventilatory control. Eur. Respir. J. 44, 495–512.
Dempsey, J.A., Xie, A.L., Patz, D.S., Wang, D., 2014. Physiology in Medicine: obstructive sleep apnea pathogenesis and treatment-considerations beyond airway anatomy. J. Appl. Physiol. 116, 3–12.
Di Marzo, V., 2008. Endocannabinoids: synthesis and degradation. Rev. Physiol. Biochem. Pharmacol. 160 (160), 1–24.
Donald, M.J., Paterson, B., 2006. End tidal carbon dioxide monitoring in prehospital and retrieval medicine: a review. Emerg. Med. J. 23, 728–730.
Duffin, J., 2007. Measuring the ventilatory response to hypoxia. J. Physiol.-London 584, 285–293.
Earing, C.M.N., McKeon, D.J., Kubis, H.P., 2014. Divers revisited: The ventilatory response to carbon dioxide in experienced scuba divers. Respir. Med. 108, 758–765.
Edwards, B.A., Sands, S.A., Eckert, D.J., White, D.P., Butler, J.P., Owens, R.L., Malhotra, A., Wellman, A., 2012. Acetazolamide improves loop gain but not the other physiological traits causing obstructive sleep apnoea. J. Physiol.-London 590, 1199–1211.
Engeli, S., Bohnke, J., Feldpausch, M., Gorzelniak, K., Janke, J., Batkai, S., Pacher, P., Harvey-White, J., Luft, F.C., Sharma, A.M., Jordan, J., 2005. Activation of the peripheral endocannabinoid system in human obesity. Diabetes 54, 2838–2843.
Engeli, S., Bluher, M., Jumpertz, R., Wiesner, T., Wirtz, H., Bosse-Henck, A., Stumvoll, M., Batkai, S., Pacher, P., Harvey-White, J., Kunos, G., Jordan, J., 2012. Circulating anandamide and blood pressure in patients with obstructive sleep apnea. J. Hypertens. 30, 2345–2351.
Eynan, M., Daskalovic, Y.I., Arieli, Y., Arieli, R., Shupak, A., Eilender, E., Kerem, D.H., 2003. Training improves divers' ability to detect increased CO2. Aviat. Space Environ. Med. 74, 537–545.
Foster, G.D., Borradaile, K.E., Sanders, M.H., Millman, R., Zammit, G., Newman, A.B., Wadden, T.A., Kelley, D., Wing, R.R., Pi-Sunyer, F.X., Reboussin, D., Kuna, S.T., Sleep, A.R.G., Look, A.R.G., 2009. A randomized study on the effect of weight loss on obstructive sleep apnea among obese patients with type 2 diabetes the sleep AHEAD study. Arch. Intern. Med. 169, 1619–1626.
Francis, D.P., Willson, K., Davies, L.C., Coats, A.J.S., Piepoli, M., 2000. Quantitative general theory for periodic breathing in chronic heart failure and its clinical implications. Circulation 102, 2214–2221.
Gamaldo, C., Buenaver, L., Chernyshev, O., Derose, S., Mehra, R., Vana, K., Walia, H.K., Gonzalez, V., Gurubhagavatula, I., Gamaldo, C., Gurubhagavatula, I., Heald, J.L., Hwang, D., OSA Assessment Tools Task Force of the American Academy of Sleep Medicine, 2018. Evaluation of clinical tools to screen and assess for obstructive sleep apnea. J. Clin. Sleep Med. 14, 1239–1244.
Gan, W.Q., Man, S.F.P., Senthilselvan, A., Sin, D.D., 2004. Association between chronic obstructive pulmonary disease and systemic inflammation: a systematic review and a meta-analysis. Thorax 59, 574–580.
Gold, A.R., Schwartz, A.R., Wise, R.A., Smith, P.L., 1993. Pulmonary-Function and respiratory chemosensitivity in moderately obese patients with sleep-apnea. Chest 103, 1325–1329.
Guilleminault, C., Kirisoglu, C., Ohayon, M.M., 2004. C-reactive protein and sleep-disordered breathing. Sleep 27, 1507–1511.
Han, F., Chen, E.Z., Wei, H.L., He, Q.Y., Ding, D.J., Strohl, K.P., 2001. Treatment effects on carbon dioxide retention in patients with obstructive sleep apnea-hypopnea syndrome. Chest 119, 1814–1819.
Hribal, M.L., Fiorentino, T.V., Sesti, G., 2014. Role of C reactive protein (CRP) in leptin resistance. Curr. Pharm. Des. 20, 609–615.
Javaheri, S., Colangelo, G., Lacey, W., Gartside, P.S., 1994. Chronic hypercapnia in obstructive sleep apnea-hypopnea syndrome. Sleep 17, 416–423.
Kahn, S.E., Hull, R.L., Utzschneider, K.M., 2006. Mechanisms linking obesity to insulin resistance and type 2 diabetes. Nature 444, 840–846.
Kim, D., Cavanaugh, E.J., Kim, I., Carroll, J.L., 2009. Heteromeric TASK-1/TASK-3 is the major oxygen-sensitive background K+ channel in rat carotid body glomus cells. J. Physiol.-London 587, 2963–2975.
Lacedonia, D., Nigro, E., Matera, M.G., Scudiero, O., Monaco, M.L., Polito, R., Carpagnano, G.E., Barbaro, M.P.F., Mazzarella, G., Bianco, A., Daniele, A., 2016. Evaluation of adiponectin profile in Italian patients affected by obstructive sleep apnea syndrome. Pulm. Pharmacol. Ther. 40, 104–108.
Littner, M., Young, E., McGinty, D., Beahm, E., Riege, W., Sowers, J., 1984. Awake abnormalities of control of breathing and of the upper airway - occurrence in healthy older men with nocturnal disordered breathing. Chest 86, 573–579.
Liu, X., He, L., Stensaas, L., Dinger, B., Fidone, S., 2009. Adaptation to chronic hypoxia involves immune cell invasion and increased expression of inflammatory cytokines in rat carotid body. Am. J. Physiol.-Lung Cell. Mol. Physiol. 296, L158–L166.
Loewen, A., Ostrowski, M., Laprairie, J., Atkar, R., Gnitecki, J., Hanly, P., Younes, M., 2009. Determinants of ventilatory instability in obstructive sleep apnea: inherent or acquired? Sleep 32, 1355–1365.
Lui, M.M.S., Lam, J.C.M., Mak, H.K.F., Xu, A.M., Ooi, C., Lam, D.C.L., Mak, J.C.W., Khong, P.L., Ip, M.S.M., 2009. C-Reactive protein is associated with obstructive sleep apnea independent of visceral obesity. Chest 135, 950–956.
MacKinnon, D.P., Fairchild, A.J., Fritz, M.S., 2007. Mediation analysis. Annu. Rev. Psychol. 593–614.
Maingret, F., Patel, A.J., Lazdunski, M., Honore, E., 2001. The endocannabinoid anandamide is a direct and selective blocker of the background K+ channel TASK-1. EMBO J. 20, 47–54.
Malli, F., Papaioannou, A.I., Gourgoulianis, K.I., Daniil, Z., 2010. The role of leptin in the respiratory system: an overview. Respir. Res. 11.
Marshall, N.S., Wong, K.K.H., Liu, P.Y., Cullen, S.R.J., Knuiman, M.W., Grunstein, R.R., 2008. Sleep apnea as an independent risk factor for all-cause mortality: The Busselton Health Study. Sleep 31, 1079–1085.
McSwain, S.D., Hamel, D.S., Smith, P.B., Gentile, M.A., Srinivasan, S., Meliones, J.N., Cheifetz, I.M., 2010. End-tidal and arterial carbon dioxide measurements correlate across all levels of physiologic dead space. Respir. Care 55, 288–293.
Menitove, S.M., Rapoport, D.M., Epstein, H., Sorkin, B., Goldring, R.M., 1984. Co2 Rebreathing and exercise ventilatory responses in humans. J. Appl. Physiol. 56, 1039–1044.
Miller, M.R., Hankinson, J., Brusasco, V., Burgos, F., Casaburi, R., Coates, A., Crapo, R., Enright, P., van der Grinten, C.P.M., Gustafsson, P., Jensen, R., Johnson, D.C., MacIntyre, N., McKay, R., Navajas, D., Pedersen, O.F., Pellegrino, R., Viegi, G., Wanger, J., 2005. Standardisation of spirometry. Eur. Respir. J. 26, 319–338.
Mokhlesi, B., Tulaimat, A., Faibussowitsch, I., Wang, Y., Evans, A.T., 2007. Obesity hypoventilation syndrome: prevalence and predictors in patients with obstructive sleep apnea. Sleep Breath. 11, 117–124.
Narkiewicz, K., van de Borne, P.J.H., Pesek, C.A., Dyken, M.E., Montano, N., Somers, V.K., 1999. Selective potentiation of peripheral chemoreflex sensitivity in obstructive sleep apnea. Circulation 99, 1183–1189.
O'Donnell, C.P., Schaub, C.D., Haines, A.S., Berkowitz, D.E., Tankersley, C.G., Schwartz, A.R., Smith, P.L., 1999. Leptin prevents respiratory depression in obesity. Am. J. Respir. Crit. Care Med. 159, 1477–1484.
Osanai, S., Akiba, Y., Fujiuchi, S., Nakano, H., Matsumoto, H., Ohsaki, Y., Kikuchi, K., 1999. Depression of peripheral chemosensitivity by a dopaminergic mechanism in patients with obstructive sleep apnoea syndrome. Eur. Respir. J. 13, 418–423.
Piper, A.J., Grunstein, R.R., 2010. Big breathing: the complex interaction of obesity, hypoventilation, weight loss, and respiratory function. J. Appl. Physiol. 108, 199–205.
Resta, O., Foschino-Barbaro, M.P., Bonfitto, P., Talamo, S., Legari, G., De Pergola, G., Minenna, A., Giorgino, R., 2000. Prevalence and mechanisms of diurnal hypercapnia in a sample of morbidly obese subjects with obstructive sleep apnoea. Respir. Med. 94, 240–246.
Roca, J., Burgos, F., Sunyer, J., Saez, M., Chinn, S., Anto, J.M., Rodriguez-Roisin, R., Quanjer, P.H., Nowak, D., Burney, P., Grp European Community Resp Hlth, S, 1998. References values for forced spirometry. Eur. Respir. J. 11, 1354–1362.
Rochester, D.F., Enson, Y., 1974. Current concepts in pathogenesis of obesity-hypoventilation-syndrome - mechanical and circulatory factors. Am. J. Med. 57, 402–420.
Roy, A., Mandadi, S., Fiamma, M.N., Rodikova, E., Ferguson, E.V., Whelan, P.J., Wilson, R.J.A., 2012. Anandamide modulates carotid sinus nerve afferent activity via TRPV1 receptors increasing responses to heat. J. Appl. Physiol. 112, 212–224.
Ryan, S., 2017. Adipose tissue inflammation by intermittent hypoxia: mechanistic link between obstructive sleep apnoea and metabolic dysfunction. J. Physiol.-London 595, 2423–2430.
Salloum, A., Rowley, J.A., Mateika, J.H., Chowchuri, S., Omran, Q., Badr, M.S., 2010. Increased propensity for central apnea in patients with obstructive sleep apnea effect of nasal continuous positive airway pressure. Am. J. Respir. Crit. Care Med. 181, 189–193.
Shamsuzzaman, A.S.M., Winnicki, M., Lanfranchi, P., Wolk, R., Kara, T., Accurso, V., Somers, V.K., 2002. Elevated c-reactive protein in patients with obstructive sleep apnea. Circulation 105, 2462–2464.
Shetty, S., Parthasarathy, S., 2015. Obesity hypoventilation syndrome. Curr. Pulmonol. Rep. 4, 42–55.
Sin, D.D., Jones, R.L., Man, G.C., 2000. Hypercapnic ventilatory response in patients with and without obstructive sleep apnea - Do age, gender, obesity, and daytime PaCO2 matter? Chest 117, 454–459.
Van Gaal, L.F., Mertens, I.L., De Block, C.E., 2006. Mechanisms linking obesity with cardiovascular disease. Nature 444, 875–880.
Verbraecken, J., Willemen, M., De Cock, W., Wittesaele, W., Govaert, K., Van de Heyning, P., De Backer, W., 2000. Influence of longterm CPAP therapy on CO2 drive in patients with obstructive sleep apnea. Respir. Physiol. 123, 121–130.
Verbraecken, J., Van de Heyning, P., De Backer, W., Van Gaal, L., 2006. Body surface area in normal-weight, overweight, and obese adults. A comparison study. Metab.-Clin. Exp. 55, 515–524.
Wahn, H., Wolf, J., Kram, F., Frantz, S., Wagner, J.A., 2005. The endocannabinoid arachidonyl ethanolamide (anandamide) increases pulmonary arterial pressure via cyclooxygenase-2 products in isolated rabbit lungs. Am. J. Physiol.-Heart Circ.y Physiol. 289, H2491–H2496.
Wellman, A., Malhotra, A., Jordan, A.S., Stevenson, K.E., Gautam, S., White, D.P., 2008. Effect of oxygen in obstructive sleep apnea: role of loop gain. Respir. Physiol. Neurobiol. 162, 144–151.

Xie, A.L., Skatrud, J.B., Dempsey, J.A., 2001. Effect of hypoxia on the hypopnoeic and apnoeic threshold for CO2 in sleeping humans. J. Physiol.-London 535, 269–278.
Xie, A.L., Teodorescu, M., Pegelow, D.F., Teodorescu, M.C., Gong, Y.S., Fedie, J.E., Dempsey, J.A., 2013. Effects of stabilizing or increasing respiratory motor outputs on obstructive sleep apnea. J. Appl. Physiol. 115, 22–33.
Younes, M., Ostrowski, M., Atkar, R., Laprairie, J., Siemens, A., Hanly, P., 2007. Mechanisms of breathing instability in patients with obstructive sleep apnea. J. Appl. Physiol. 103, 1929–1941.
Young, T., Skatrud, J., Peppard, P.E., 2004. Risk factors for obstructive sleep apnea in adults. Jama-J. Am. Med. Assoc. 291, 2013–2016.
Zamarron, C., Paz, V.G., Riveiro, A., 2008. Obstructive sleep apnea syndrome is a systemic disease. Current evidence. Eur. J. Intern. Med. 19, 390–398.
Zoerner, A.A., Batkai, S., Suchy, M.T., Gutzki, F.M., Engeli, S., Jordan, J., Tsikas, D., 2012. Simultaneous UPLC-MS/MS quantification of the endocannabinoids 2-arachidonoyl glycerol (2AG), 1-arachidonoyl glycerol (1AG), and anandamide in human plasma: minimization of matrix-effects, 2AG/1AG isomerization and degradation by toluene solvent extraction. J. Chromatogr. B-Anal. Technol. Biomed. Life Sci. 883, 161–171.