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Dose response of continuous positive airway pressure on nasal symptoms, obstruction and inflammation *in vivo* and *in vitro*

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ABSTRACT: Obstructive sleep apnoea is a common condition associated with cardiovascular risk. Continuous positive airway pressure (CPAP) is an effective treatment but is associated with nasal side-effects, which hinder compliance and may result from inflammation.

We investigated whether CPAP was pro-inflammatory to human subjects *in vivo*, and to cultured bronchial epithelial cells *in vitro*. *In vivo*, we further investigated whether induction of nasal inflammation was associated with the development of systemic inflammation, nasal symptoms and changes in nasal mucociliary clearance.

***In vitro*, CPAP resulted in cytokine release from cultured BEAS-2B cells in a time- and dose (pressure)-dependent manner. *In vivo*, CPAP resulted in dose-dependent upregulation of nasal inflammatory markers associated with the development of nasal symptoms, and reduced mucociliary clearance. CPAP also upregulated selected markers of systemic inflammation.**

CPAP results in dose-dependent release of inflammatory cytokines from human epithelial cells *in vitro* and *in vivo*. *In vivo* responses were associated with systemic inflammation, reductions in nasal mucociliary function and the development of nasal symptoms. This emphasises the need for novel strategies to reduce nasal inflammation and therefore aid compliance.

KEYWORDS: Airway symptoms, bronchial epithelial cells, continuous positive airway pressure, inflammatory markers, mucociliary clearance

Obstructive sleep apnoea (OSA) syndrome is the most common sleep disorder, affecting up to a quarter of the western adult population [1]. It occurs when the pharyngeal airway becomes narrow due to the natural relaxation of muscles during sleep. Nasal continuous positive airway pressure (CPAP) has become the gold standard management of clinically significant OSA [2]. CPAP is a distending mechanical split-pressure applied at a continuous level throughout the respiratory cycle to maintain an open airway, preventing airway collapse during sleep [3, 4]. OSA is associated with significant excess cardiovascular risk [3, 4].

Despite its beneficial effects on airway patency, CPAP treatment is associated with a high prevalence of side-effects [5, 6]. Some patients adapt to the treatment within a few weeks, others struggle for longer periods, and some discontinue treatment entirely with consequent detriment to their health. Although the long-term compliance rate is generally good, 8–15% of OSA patients refuse treatment after a single night of use in the laboratory setting [6–9]. There are reports of many adverse symptoms occurring with CPAP use, including nasal congestion, sneezing, anosmia, itchy nose, dry nose, mouth, throat and eyes, blocked ears and dizziness [5]. Thus, initial experiences of the patient with CPAP may be of great importance in long-term treatment compliance.

The development of nasal symptoms with CPAP treatment may be related to the induction of nasal inflammation. Several clinical and experimental studies have reported on local and systemic inflammatory outcomes with ventilatory support [10–16], but little is known about the early induction of nasal inflammation with CPAP and how this relates to changes in nasal physiology, symptoms and therefore compliance. In addition, reported symptoms and inflammatory changes may be influenced by pre-existing conditions, such as OSA. Cell culture studies with CPAP are

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even scarcer and have mainly focused on stretch injury (barotrauma) rather than air pressure. We hypothesised that examining early symptoms and inflammatory changes after a short period of CPAP in CPAP-naïve healthy individuals *in vivo* and in epithelial cell cultures *in vitro* would provide complementary insights into the mechanisms associated with the development of adverse symptoms and inflammation.

This study aimed to: 1) investigate the short-term, dose-response effects of CPAP on airway and systemic inflammation, nasal symptoms and airway obstruction in CPAP-naïve healthy individuals *in vivo*, and 2) examine the dose-response secretion of two key interleukins by bronchial epithelial cell cultures (BEAS-2B) with CPAP during several hours of application *in vitro*.

MATERIALS AND METHODS

In vivo *study*

Study subjects and protocol

31 healthy nonsmokers (21 male and 10 female) with no prior history of nasal symptoms or disease were recruited for the study. The protocol was approved by the Research Ethics Committee at Royal Free Hampstead NHS Trust (study reference 09/H0720/24) and was conducted in accordance with the Declaration of Helsinki. Informed written consent was obtained from all subjects prior to their inclusion in the study.

One higher and one lower CPAP pressure (within the range of clinical use) was selected for the *in vivo* component of the study: 7.5 cmH_2O and 12.5 cmH_2O. 22 subjects received 3 h of standard CPAP (REMstar® Auto M Series with A-Flex™, Phillips Respironics Inc., Guildford, UK) at 7.5 cmH_2O pressure, without humidification, through a nasal mask. 31 subjects (11 of whom had received the 7.5 cmH_2O protocol 6 months previously) received 3 h of CPAP treatment at 12.5 cmH_2O, also without humidification. The subjects' mouths were closed to prevent leakage. Assessments were performed before and after intervention; thus the baseline measurement of each individual served as their own control. The following assessments were made: 1) nasal and systemic inflammation (interleukin (IL)-6, IL-8 and myeloperoxidase (MPO)) concentration in serum and nasal wash samples, and nasal wash leukocyte count; 2) functional assessments (spirometry, acoustic rhinometry and nasal mucociliary clearance); and 3) nasal symptoms.

Measurements

Nasal and systemic inflammation

Nasal wash samples were obtained and processed according to a technique that we have previously reported [17], modified from that described by HILDING [18]. In brief, a paediatric tracheostomy tube (Bivona Fome-Cuf, size I.D 2.5 mm; Smiths Medical, Kent, UK) was used to collect the nasal lavage. The recovered lavage from the two nostrils was pooled for analysis. To ensure standard conditions, all sampling procedures were performed by the same investigator.

Peripheral venous blood samples were obtained for serum measurements. A 5-mL sample of venous blood was collected into a sterile vacutainer, centrifuged at 224 $\times g$ for 10 min at 4°C, and the supernatant was stored at -80°C for later analysis of inflammatory mediators.

Measurements of the inflammatory cytokines (IL-6, IL-8 and MPO) in nasal wash supernatants and sera were performed by a standard ELISA technique (R&D Systems, Abingdon, UK). The detection limits were 0.70 $pg \cdot mL^{-1}$ for IL-6, 3.5 $pg \cdot mL^{-1}$ for IL-8 and 1.5 $ng \cdot mL^{-1}$ for MPO.

Physiological assessments

Acoustic rhinometry measurements were performed in accordance with a previously published protocol [19]. The device used was an A1 Acoustic Rhinometer with software version 0.5 (GM Instruments, Kilwinning, UK). All measurements were performed by the same operator in the same air-conditioned room to provide similar conditions with regard to temperature, humidity and ambient noise levels. The following five important acoustic rhinometry variables were assessed and examined separately: 1) outermost minimum cross-sectional area (MCA1); 2) the distance of the MCA1 from the nasal orifice (D-MCA1); 3) innermost minimum cross-sectional area (MCA2); 4) the distance of the MCA2 from the nasal orifice (D-MCA2); and 5) the volume of the nasal segment between the 2nd and 5th cm from the nasal orifice (V2–5). Mean area and volume values from the right and left nostrils were used to account for variations with the nasal cycle.

The best of three attempts at spirometry was recorded using a Vitalograph 2160 (Vitalograph, Maids Moreton, UK). A bronchodilator was not administered. We recorded forced expiratory volume in 1 s (FEV_1), forced vital capacity (FVC), FEV_1/FVC ratio and peak expiratory flow rate (PEFR).

Nasal mucociliary clearance was measured using the modified *in vivo* saccharin transit time (STT) technique described by RUTLAND and COLE [20]. Saccharin was applied on the inferior turbinate of the nasal cavity under direct visualisation, and the time at which the subject perceived a sweet taste on the tongue was recorded in seconds using a stopwatch.

Assessment of nasopharyngeal symptoms

The presence or absence of nasopharyngeal symptoms (including rhinorrhoea, post-nasal drip, nasal congestion, sneezing, reduced sense of smell and itchy nose) was assessed before and after CPAP intervention.

In vitro *study*

All chemicals and reagents were of tissue culture grade and were obtained from Sigma-Aldrich Chemical Co. (Poole, UK) unless otherwise stated. ELISA kits were obtained as above for the *in vivo* work.

Culture of bronchial epithelial cells

BEAS-2B cells, a virus-transformed human bronchial epithelial cell line, were obtained from the American Type Culture Collection (Manassas, VA, USA) [21]. After culturing, when the cells became fully confluent, the culture medium was removed, and the cells were washed with 10 mL of sterile PBS. The PBS was then discarded, after which 2–3 mL Trypsin/EDTA (0.25%, w/v) was added for 3–5 min to disperse the cells so that they could be transferred to 6-cm Falcon ''Primera'' culture dishes (Becton Dickinson, Oxford, UK). Cultures were then incubated (Galaxy R; Wolf Laboratories, York, UK) in 2 mL fresh, sterile, complete culture medium containing 10% fetal calf serum (Sigma-Aldrich), 5 mL antibiotic/antimycotic solution and 4 mL of each of the following: bovine pancreatic insulin (2.5 $\mu g \cdot mL^{-1}$), human transferrin (2.5 $\mu g \cdot mL^{-1}$), hydrocortisone (0.36 $\mu g \cdot mL^{-1}$) and

L-glutamine (0.02 mg·mL^{-1}) made up to a final volume of 500 mL in Medium 199 and filter-sterilised through a 0.22-μm syringe filter. The antibiotic/antimycotic solution contained 10,000 units penicillin G, 10 mg streptomycin and 25 μg amphotericin B per mL. Cells were then incubated for 1–3 days at 37°C in 95% air and 5% CO_2.

In vitro *CPAP exposure*

Cultured BEAS-2B cells were exposed *in vitro* to CPAP pressure in a chamber at 0, 4 and 7 cmH_2O for 1, 2, 3 or 4 h, after which the release of cytokine concentration was measured in the culture medium. The protocol is summarised in figure 1a.

10 tissue culture dishes (60 × 15 mm) were used at each time-point. The day prior to the experiment, the complete medium was removed and replaced with 2 mL of 199 medium/antibiotics and incubated at 37°C in 95% air and 5% CO_2 overnight. This was replaced the next morning before the experiment with 5 mL of fresh 199 medium/antibiotics. The cells were then incubated for 5 min, and 125 μL aliquots of cell culture supernatant were removed at 0 time (before any pressure application) and stored at -80°C for later analysis.

The experimental equipment is shown in figure 1b. CPAP (REMstar® Auto M Series with A-Flex™), at pressures of 4 or 7 cmH_2O, was applied by incubating the cells in an airtight chamber (4.6 L) for the appropriate time, with 10 replicates per time-point. The machine leak alarm was monitored to ensure that pressure was being delivered to the cells. The airtight chamber was placed inside a 60-L acrylic SI.60 incubator (Stuart Scientific, Redhill, UK) to ensure conditions of controlled temperature, reflecting the temperature in the nasal airway (31–33°C); humidity was maintained at approximately 98% by placing a 150-mm tissue culture dish with 20 mL of sterile water in the chamber. Humidity was monitored using a hygrothermometer. During each experiment, the modified chamber was tilted gently to an angle of 10° from horizontal in each quarter of the horizontal plane on a Luckham 4RT rocking table (Luckham Ltd, Burgess Hill, UK), thereby momentarily displacing approximately half the medium covering the surface of the culture plate during each tilt to directly expose the cells to pressurised, humidified air, mimicking intranasal physiological conditions.

At the end of each time-interval for the allocated pressure, and at which point the experiment was terminated, 1-mL aliquots of cell culture supernatant were removed. The remaining medium was then removed, and the cells were harvested by scraping them in 1 mL of 199 medium only. All experimental media and cell scrapes were stored at -80°C for later analysis. Control experiments that were not exposed to CPAP were run for each of the four time-points.

Measurements of IL-6 and IL-8 concentration

The cell culture supernatant samples were analysed for IL-6 and IL-8 by ELISA kits as described above.

To account for differences in the sizes of the cultures, cytokine concentrations were expressed corrected for total cellular protein concentration. Total cellular protein concentration was measured by a modified Lowry assay (Bio-Rad Laboratories Inc., Hercules, CA, USA) [22].

Statistical analysis

Data were analysed using GraphPad Prism 5 software (GraphPad Software Inc., La Jolla, CA, USA). The Kolmogorov–Smirnov test of normality was applied. For the *in vivo* study, paired t-tests were used to examine differences between baseline and post-CPAP therapy measurements. A one-way ANOVA was run to examine dose-response differences between treatments, followed by *post hoc* Tukey's multiple comparison tests. For the sub-group analysis, one-way repeated measures ANOVA for parametric data and Friedman tests for non-parametric data were run to examine dose-response differences between treatments as appropriate, followed by *post hoc* Tukey's multiple comparison tests. Pearson (r) and Spearman (ρ) correlations were conducted as appropriate to examine relationships between variables. A Chi-squared test was used to compare nasopharyngeal symptoms at baseline and after CPAP therapy. For the *in vitro* study, one-way ANOVAs were run to examine dose-response differences between cell culture responses to CPAP over several hours of application, followed by *post hoc* Tukey's multiple comparison tests. Multiple linear regression analyses were performed to determine a set of independent variables (time and pressure) that predicted *in vitro* cytokine productions. A p-value <0.05 was considered statistically significant.

Ethics statement

Written informed consent was received from participants prior to inclusion in the study, and the study had institutional approval as detailed previously.

RESULTS

In vivo *studies*

The baseline characteristics of the subjects enrolled in the study are reported in table 1. Both groups were healthy nonsmokers, with a mean age between 33 and 34 yrs.

Changes in nasal and systemic inflammation with CPAP

The changes in serum and nasal wash inflammatory markers in response to 3 h of nasal CPAP are reported in table 2. Both CPAP pressures resulted in significant increases in nasal inflammation as assessed by nasal wash leukocyte and MPO measurements. The increase in nasal IL-6 and IL-8 concentrations following CPAP was only statistically significant at the higher pressure. Both pressures also resulted in changes in systemic inflammatory markers, with significant increases in serum IL-6 concentrations and decreases in serum IL-8 concentrations following CPAP. There was no change in serum MPO concentration. The ANOVA test highlights the observed dose (pressure) responses for changes in inflammation with CPAP (fig. 2). When one-way repeated measures ANOVA was conducted for the subset of 11 subjects that underwent both experiments (7.5 and 12.5 cmH_2O), the changes in nasal wash IL-6 ($p=0.038$) and MPO ($p=0.027$) remained statistically significant.

Changes in physiology with CPAP

The changes in spirometry, rhinometry and nasal mucociliary clearance are reported in table 3. At both pressures, 3 h of nasal CPAP treatment resulted in a significant slowing of nasal clearance (*i.e.* increased saccharin transit time) without significant changes in rhinometry variables. At both pressures, CPAP was associated with small but significant changes in FVC and PEFR (but not FEV_1).

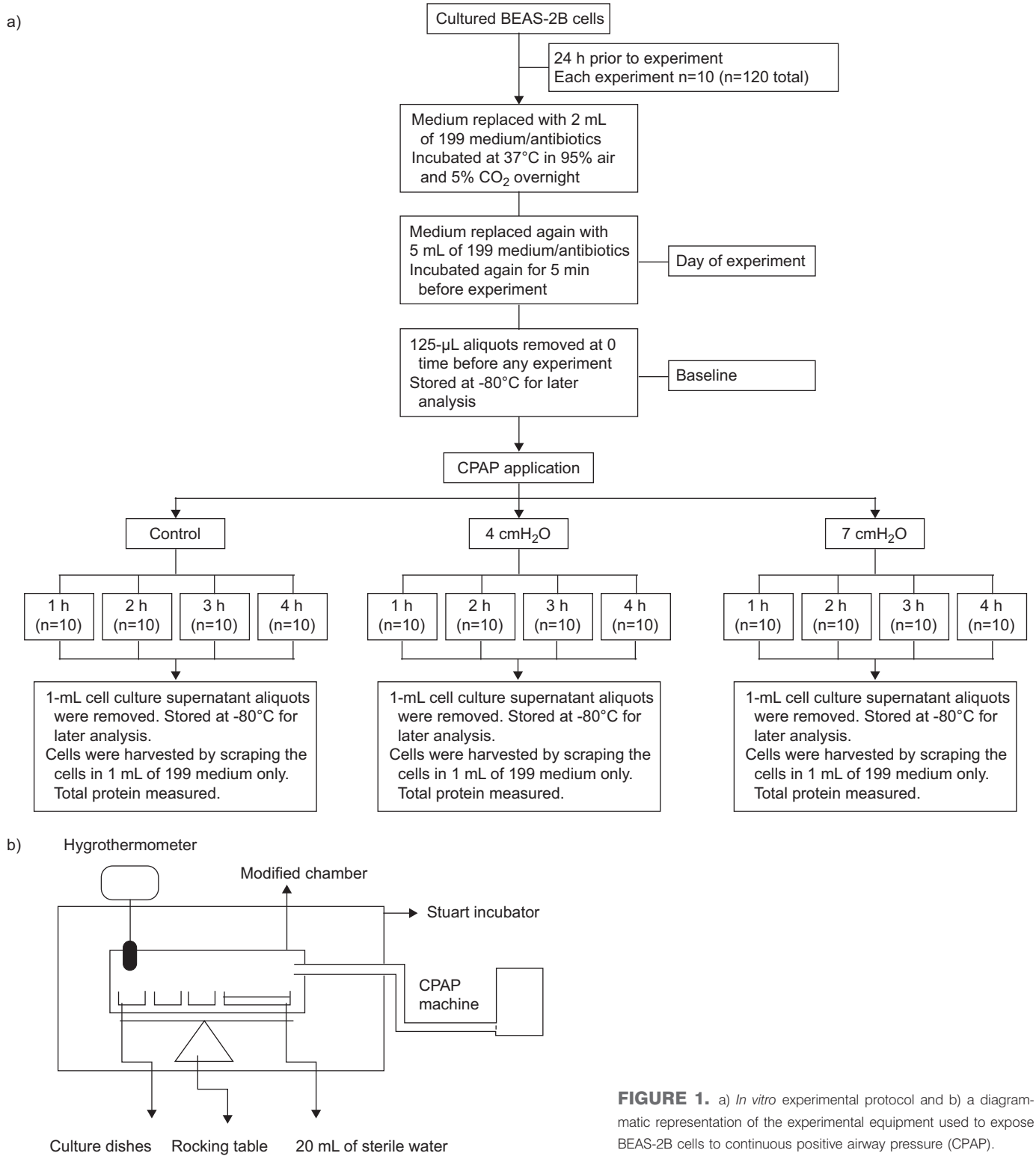

FIGURE 1. a) *In vitro* experimental protocol and b) a diagrammatic representation of the experimental equipment used to expose BEAS-2B cells to continuous positive airway pressure (CPAP).

Changes in nasopharyngeal symptoms with CPAP

Nasopharyngeal symptoms before and after nasal CPAP treatment are presented in table 4. None of the subjects had any upper airway symptoms before CPAP. The median number of nasopharyngeal symptoms increased significantly from 0 at baseline to 1 (0–3) after 7.5 cmH_2O CPAP ($p=0.002$) and to 2 (1–3) after 12.5 cmH_2O CPAP ($p<0.001$). After CPAP at 7.5 cmH_2O, 12 (55%) of 22 subjects experienced at least one nasal symptom; after CPAP at 12.5 cmH_2O, 21 (68%) of 31 subjects experienced at least one nasal symptom. There was an overall increase in the frequency of all of the symptoms during nasal CPAP treatment; the most common nasal symptom at

TABLE 1 Baseline information of subjects enrolled in the study[#]

Subject characteristics	7.5 cmH_2O	12.5 cmH_2O
Subjects	22	31
Age yrs	33.8±5.8	33.4±5.4
Weight kg	70.1±6.6	71.5±7.5
Height cm	168.8±7.1	169.7±7.1
BMI $kg \cdot m^{-2}$	25.12±1.84	24.82±2.01
Smoking history	Never smoker	Never smoker
Nasal symptoms	None	None
Medications	None	None

Data are presented as n or mean±SD. BMI: body mass index. [#]: 11 patients took part in both protocols.

both pressures was itchy nose. The higher the pressure, the more symptoms were recorded: Chi-squared (p=0.041).

Relationships between symptoms and changes in nasal physiology and inflammation

The data demonstrate relationships between the development of nasal symptoms, nasal inflammation and impaired nasal function *in vivo*. The greater the nasal symptoms with CPAP, the slower the nasal mucociliary clearance (r=0.40 p=0.025) and the greater the nasal inflammation as assessed by nasal wash IL-6 (r=0.43 p=0.045) (fig. 3a and b). The significant slowing in nasal clearance was also associated with the degree of nasal inflammation, as assessed by nasal MPO concentrations (r=0.42, p=0.049) (fig. 3c).

In vitro *studies*

Changes in IL-6 and IL-8 levels over time

CPAP was associated with both time- and dose (pressure)-dependent release of the inflammatory cytokines IL-6 and IL-8 from cultured BEAS-2B cells *in vitro*. These data are reported in table 5 and illustrated in figure 4.

In linear regression analysis, both pressure and time independently contributed to release of IL-6 and IL-8 (IL-6 adjusted R^2=0.55, p<0.001; IL-8 adjusted R^2=0.60, p<0.001; time β 0.278 and 0.399, pressure β 0.694 and 0.671, respectively).

DISCUSSION

We report that CPAP results in the release of inflammatory mediators from cultured human bronchial epithelial cells *in vitro*, in a time- and pressure-dependent manner. In addition, in healthy control subjects, CPAP was associated with dose (pressure)-response changes in nasal and systemic inflammatory markers, reduced nasal function and the development of nasal symptoms. The development of nasal symptoms related to the degree of functional impairment and nasal inflammatory response. To the best of our knowledge, this is the first report to examine the *in vitro* and *in vivo* effects of CPAP in this way, providing new data on the mechanisms of CPAP intolerance in the crucial early phase of therapy.

Findings from the *in vitro* component of this study showed cytokine (IL-6 and IL-8) secretion by bronchial epithelial cells in response to CPAP in a pressure- and time-dependent manner. IL-6 is an important pro-inflammatory cytokine. IL-8 is a chemokine which attracts neutrophils to the site of inflammation. Our *in vivo* findings are in line with the *in vitro* findings, particularly with regard to the neutrophilic nature of inflammation. Neutrophil chemotaxis following epithelial IL-8 release is the likely explanation of increased leukocyte count and elevated MPO activity in nasal wash fluid samples, since MPO is an enzyme abundantly present in neutrophils. The neutrophilic nature of CPAP-induced local inflammation has also been shown in a rat model in which early nasal inflammation was mediated by macrophage-induced inflammatory protein-2 and manifested as neutrophil extravasation following 5 h of 10 cmH_2O CPAP [10]. Paradoxically, in nasal wash fluid samples, IL-8 levels remained unchanged in this study at both

TABLE 2 Changes in inflammatory markers in serum and nasal wash fluid from baseline in response to 3 h of continuous positive airway pressure (CPAP) treatment

Inflammatory markers	7.5 cmH_2O[#]			12.5 cmH_2O[¶]			ANOVA[§]
	Baseline	After CPAP	p-value[+]	Baseline	After CPAP	p-value[+]	
Serum							
IL-6 $pg \cdot mL^{-1}$	4.6±3.5	6.3±3.5	0.010	8.7±4.2	11.3±5.8	0.037	<0.001
IL-8 $pg \cdot mL^{1}$	20.9±7.8	16.5±7.3	<0.001	14.9±5.9	13.0±5.6	0.002	0.022
MPO $ng \cdot mL^{-1}$	10.3±8.4	9.3±9.4	0.805	11.5±8.5	9.1±7.2	0.138	0.858
Nasal wash							
Leukocyte count ×1000 $cells \cdot mL^{-1}$	18.8±4.1	20.6±6.4	0.024	18.2±4.7	21.5±6.2	<0.001	0.039
IL-6 $pg \cdot mL^{-1}$	2.4±2.8	2.4±3.1	0.670	3.7±1.5	4.9±1.8	0.001	0.012
IL-8 $pg \cdot mL^{-1}$	86±114	81±98	0.689	164±90	206±164	0.281	0.006
MPO $ng \cdot mL^{-1}$	1.9±5.2	3.4±6.6	0.006	2.0±4.3	3.8±5.5	0.002	0.006

Data are expressed as the geometric mean±SD, unless otherwise stated. IL: interleukin; MPO: myeloperoxidase. [#]: n=22; [¶]: n=31; [+]: *versus* baseline, paired-samples t-test; [§]: compares the baseline (mean of the two baselines in the 11 subjects who had both pressures) with the 22 results at 7.5 cmH_2O and the 31 subjects at 12.5 cmH_2O nasal CPAP.

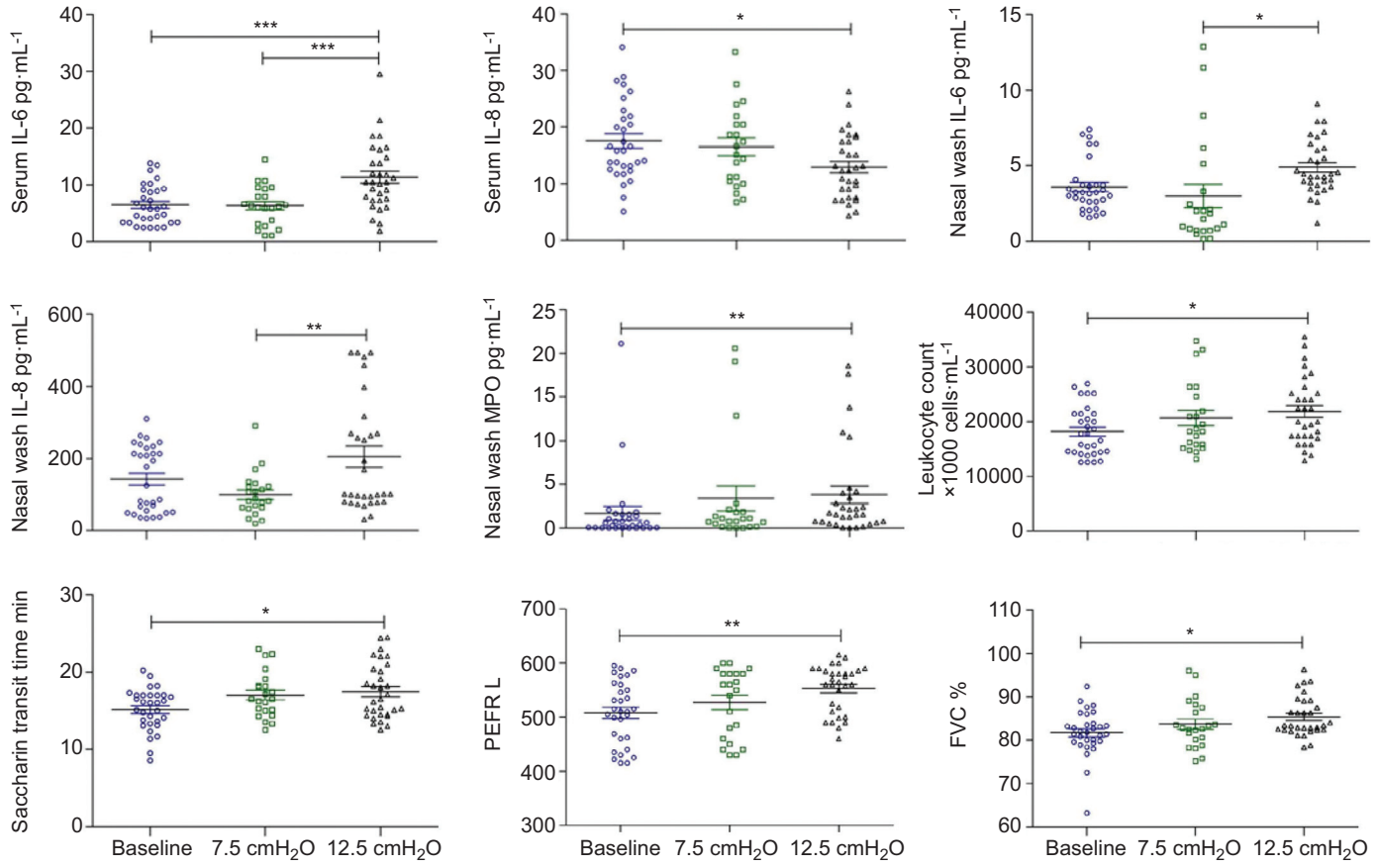

FIGURE 2. Nasal continuous positive airway pressure is associated with a pressure-dependent alteration in nasal and systemic inflammatory markers, and nasal mucociliary clearance. Significant differences illustrated using ANOVA with *post hoc* analysis. Lines represent mean±SE. IL: interleukin; MPO: myeloperoxidase; PEFR: peak expiratory flow rate; FVC: forced vital capacity. *: $p<0.05$; **: $p<0.01$; ***: $p<0.001$ (ANOVA).

pressures and an increase in IL-6 levels was only evident in response to the higher pressure. This may arise from rapid consumption and binding of interleukins before they cross the nasal epithelium. Our study therefore suggests that CPAP itself may be pro-inflammatory and that this effect occurs early after initiation of therapy.

In this study, even a brief period of CPAP application resulted in increased IL-6 levels in serum, suggesting a systemic inflammatory response. However, we did not observe an increase in systemic IL-8 concentration or MPO activity. The decrease in serum IL-8 levels may be due to local recruitment of leukocytes and increased consumption. Unaltered systemic MPO activity is plausible since MPO may predominately increase at the site of inflammation. Studies in OSA are even more complex, as the condition itself is associated with upregulated upper airway inflammation [23, 24] and in such circumstances CPAP may not upregulate this further [25]. The work complements previous findings in patients with OSA, where CPAP is known to increase nasal inflammation [11].

This *in vivo* nasal inflammatory response was associated with clinical and functional consequences in that we also demonstrated that CPAP reduced nasal clearance and was associated with a high prevalence of new nasal symptoms. It has been suggested that the presence of nasal inflammation predicts patients at greater risk of discontinuing CPAP therapy [26], and IL-8, a potent neutrophil chemoattractant that we have shown to be upregulated by CPAP *in vitro*, causes rhinorrhoea when directly instilled to the nose [27]. 3 h of CPAP decreased mucociliary clearance at both 7.5 and 12.5 cmH_2O in healthy individuals. Our findings are contrary to those reported by DE OLIVIERA *et al.* [28], who found significantly decreased STT after 20 min of CPAP in healthy individuals. This may be attributed to differences in the duration of CPAP treatment and suggests that CPAP may provide an initial improvement in nasal clearance that is followed by impairment due to inflammation. These inflammatory and functional changes may contribute to the high incidence of symptoms and adverse effects associated with CPAP treatment. In this study, more than half of the subjects experienced at least one nasal symptom after a single session. Previous studies have reported high incidences of side-effects during long-term therapy, which approached 97% in a large series [5]. To assess the duration of symptom changes, in a subsequent pilot experiment, we assessed nasal symptoms prior to and after 3 h of CPAP at 12 cmH_2O, then again at 3, 6, 9 and 24 h later. None of the subjects had nasal symptoms prior to CPAP and all had one or more symptom after. At 3, 6, 9 and 24 h post-CPAP the numbers

TABLE 3 Changes in lung function, rhinometry findings and nasal mucociliary clearance from baseline in response to 3 h of nasal continuous positive airway pressure (CPAP) treatment

Parameter	7.5 cmH_2O[#]			12.5 cmH_2O[¶]			ANOVA
	Baseline	After CPAP	p-value[+]	Baseline	After CPAP	p-value[+]	
Lung function test							
FEV L	3.03±0.52	3.05±0.50	0.093	3.07±0.50	3.09±0.51	0.596	0.751
FEV_1 % pred	86.31±5.94	86.22±5.75	0.506	85.74±5.58	86.90±4.62	0.091	0.370
FVC L	3.54±0.63	3.58±0.63	0.030	3.59±0.63	3.64±0.61	0.026	0.647
FVC % pred	83.06±5.62	83.71±5.53	0.052	83.01±6.18	85.36±4.54	0.071	0.022
FEV_1/FVC %	85.99±6.00	85.76±6.10	0.615	85.90±6.49	86.35±6.79	0.354	0.625
PEFR L	519.00±71.53	527.27±62.79	0.024	523.81±64.4	553.06±43.71	0.002	0.007
Acoustic rhinometry							
MCA1	0.57±0.31	0.60±0.38	0.527	0.61±0.31	0.59±0.31	0.175	0.894
D-MCA1	2.15±0.38	2.15±0.3	0.947	2.06±0.43	2.04±0.40	0.529	0.501
MCA2	1.62±0.54	1.58±0.45	0.488	1.55±0.53	1.53±0.50	0.531	0.938
D-MCA2	4.23±0.39	4.24±0.32	0.884	4.22±0.40	4.23±0.36	0.568	0.823
V2–5	4.43±1.05	4.51±1.08	0.482	4.73±0.93	4.82±0.89	0.113	0.362
Nasal mucociliary clearance							
STT min	16.31±2.71	17.41±3.31	0.035	15.30±3.56	16.35±3.34	0.045	0.011

Data are expressed as mean±SD, unless otherwise specified. FEV: forced expiratory volume; FEV_1: FEV in 1 s; % pred: % predicted; FVC: forced vital capacity; PEFR: peak expiratory flow rate; MCA1: outermost minimum cross-sectional area; D-MCA1: the distance of the MCA1 from the nasal orifice; MCA2: innermost minimum cross-sectional area; D-MCA2: the distance of the MCA2 from the nasal orifice; V2–5: the volume of the nasal segment between the 2nd and 5th cm from the nasal orifice; STT: saccharin transit time. [#]: n=22; [¶]: n=31; [+]: *versus* baseline, paired-samples t-test; [§]: compares the baseline (mean of the two baselines in the 11 subjects who had both pressures) with the 22 results at 7.5 cmH_2O and the 31 subjects at 12.5 cmH_2O CPAP.

remaining symptomatic were four out of 5, one out of 5, one out of 5 and none, respectively, suggesting that acute nasal symptoms typically last for between 3 and 6 h following initiation of CPAP.

In this study, CPAP treatment did not result in any change in acoustic rhinometry parameters in healthy individuals; thus, it did not alter nasal patency. This was unexpected given the apparent nasal inflammatory response. Nasal geometry has been reported to affect CPAP tolerability [29]. Although the effect of short- or long-term CPAP on acoustic rhinometry parameters has not been investigated previously, several studies have reported unaltered rhinomanometry results after long-term CPAP therapy [29, 30]. One study reported a reduction in airway resistance after an acute exposure to nasal CPAP for 6 h in healthy CPAP-naïve individuals [31]. In this study, a small but statistically significant improvement was identified in lung function parameters (*i.e.* FVC and PEFR). This suggests that the CPAP applied in our subjects had a demonstrable biological effect.

OSA is associated with increased cardiovascular risk [32, 33], as is increased systemic inflammation [34]. Whether CPAP reduces cardiovascular risk remains controversial [35–40], but the finding that CPAP itself, at least in the acute setting, is pro-inflammatory, is potentially important regarding the timing of

TABLE 4 Changes in nasopharyngeal symptoms following application of continuous positive airway pressure (CPAP) to healthy subjects *in vivo*

CPAP pressure	Subjects	Nasopharyngeal symptoms								
		Rhinorrhoea	PND	Sneezing	Congestion	Anosmia	Itchy nose	Dry nose	Dry mouth/ throat	Blocked ears
Baseline		0	0	0	0	0	0	0	0	0
7.5 cmH_2O	22	2 (9)	0 (0)	5 (23)	3 (14)	0 (0)	12 (54)	7 (32)	4 (18)	1 (4)
12.5 cmH_2O	31	5 (16)	2 (6)	5 (16)	7 (23)	0 (0)	17 (55)	15 (48)	11 (35)	2 (6)

Data are expressed as n or n (%). PND: post-nasal drip.

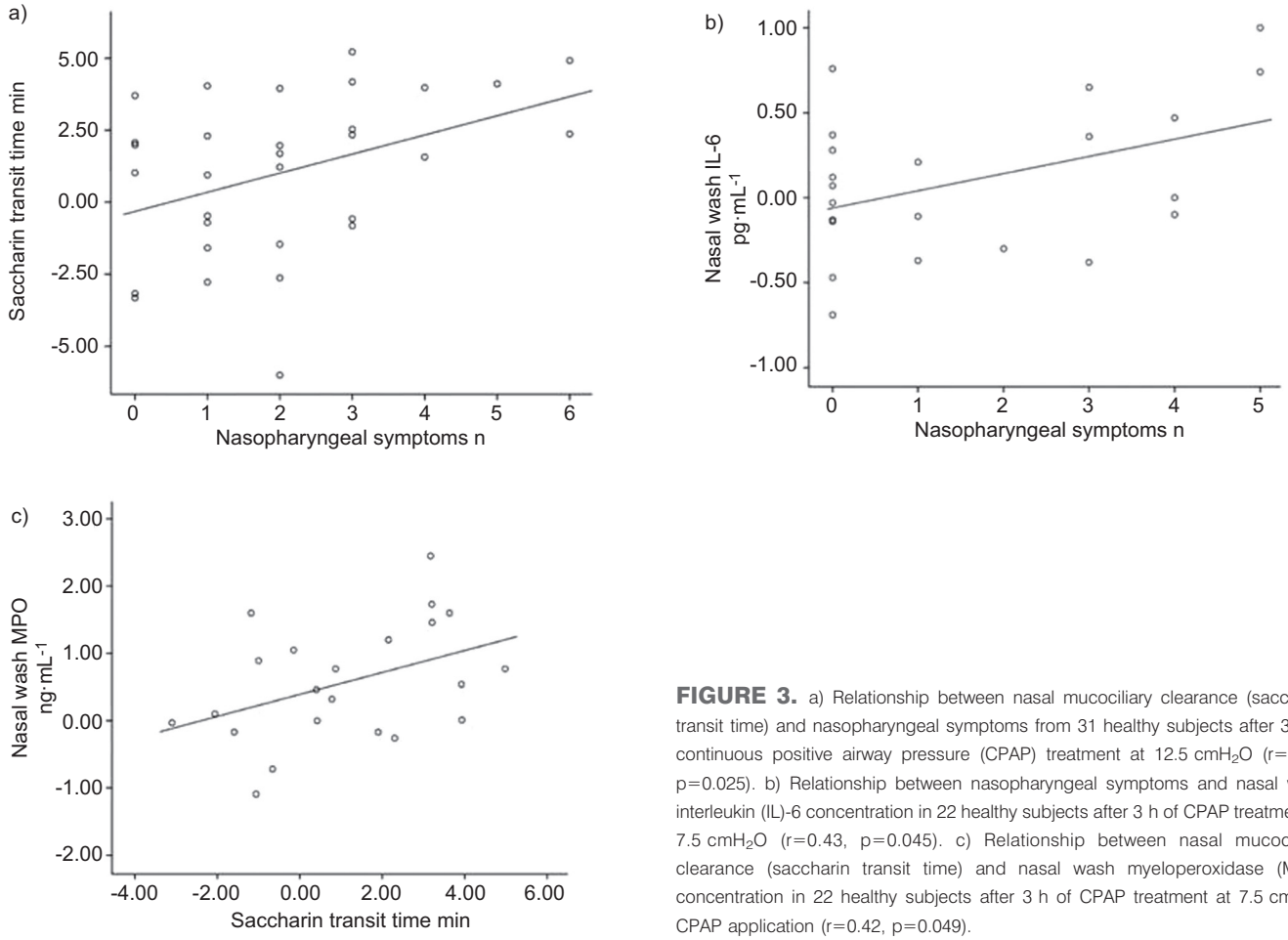

FIGURE 3. a) Relationship between nasal mucociliary clearance (saccharin transit time) and nasopharyngeal symptoms from 31 healthy subjects after 3 h of continuous positive airway pressure (CPAP) treatment at 12.5 cmH_2O ($r=0.40$; $p=0.025$). b) Relationship between nasopharyngeal symptoms and nasal wash interleukin (IL)-6 concentration in 22 healthy subjects after 3 h of CPAP treatment at 7.5 cmH_2O ($r=0.43$, $p=0.045$). c) Relationship between nasal mucociliary clearance (saccharin transit time) and nasal wash myeloperoxidase (MPO) concentration in 22 healthy subjects after 3 h of CPAP treatment at 7.5 cmH_2O CPAP application ($r=0.42$, $p=0.049$).

TABLE 5 Continuous positive airway pressure (CPAP) is associated with time- and pressure-dependent releases of interleukin (IL)-6 and IL-8 from cultured BEAS-2B cells *in vitro*

	1 h	2 h	3 h	4 h	ANOVA p-value[#]
IL-6 pg·μg^{-1} protein					
Baseline	0.01±0.001	0.01±0.002	0.01±0.015	0.01±0.002	0.334
Control no CPAP	0.01±0.002	0.01±0.003	0.01±0.004	0.01±0.002	0.743
CPAP 4 cmH_2O	0.01±0.005	0.02±0.005	0.02±0.005	0.02±0.008	0.017
CPAP 7 cmH_2O	0.03±0.005)	0.07±0.014	0.08±0.016	0.10±0.051	<0.001
ANOVA p-value	<0.001	<0.001	<0.001	<0.001	
Post hoc 4 cmH_2O *versus* control[¶]	0.012	0.016	0.09	0.649	
Post hoc 7 cmH_2O *versus* control[¶]	<0.001	<0.001	<0.001	<0.001	
Post hoc 4 cmH_2O *versus* 7 cmH_2O control[¶]	<0.001	<0.001	<0.001	<0.001	
IL-8 pg·μg^{-1} protein					
Baseline	0.01±0.003	0.01±0.004	0.02±0.02	0.01±0.004	0.470
Control no CPAP	0.01±0.011	0.01±0.004	0.01±0.006	0.02±0.009	0.569
CPAP 4 cmH_2O	0.04±0.03	0.09±0.022	0.11±0.014	0.15±0.040	<0.001
CPAP 7 cmH_2O	0.06±0.013	0.09±0.030	0.11±0.022	0.14±0.062	<0.001
ANOVA p-value	<0.001	<0.001	<0.001	<0.001	
Post hoc 4 cmH_2O *versus* control[¶]	0.005	<0.001	<0.001	<0.001	
Post hoc 7 cmH_2O *versus* control[¶]	<0.001	<0.001	<0.001	<0.001	
Post hoc 4 cmH_2O *versus* 7 cmH_2O control[¶]	<0.001	<0.001	<0.001	<0.001	

Data are presented as mean±SD, unless otherwise stated. [#]: p-values <0.05 were regarded as statistically significant; [¶]: *post hoc* Tukey's multiple comparison tests.

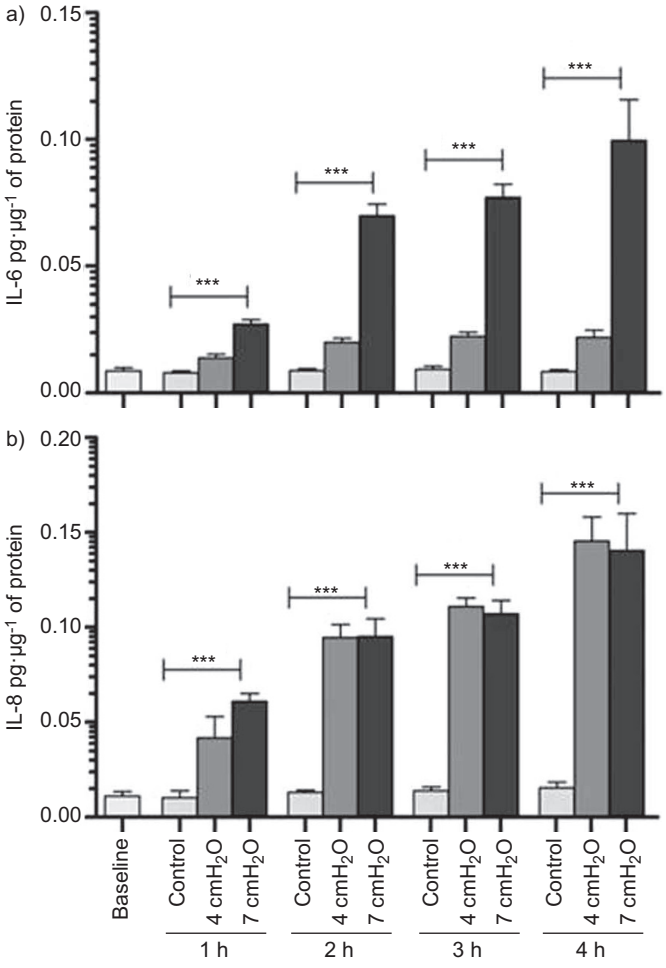

FIGURE 4. Dose (pressure)-dependent release of a) interleukin (IL)-6 and b) IL-8 from cultured human BEAS-2B cells *in vitro* with no pressure (control), and continuous positive airway pressure at 4 and 7 cmH_2O. ***: $p<0.001$.

the initiation of therapy. In the long-term, STEIROPOULOS *et al.* [41] reported significant improvements in systemic inflammatory markers, including total lymphocyte counts, CD4+ cells, tumour necrosis factor (TNF)-α levels and uric acid levels after 6 months in patients with good compliance to CPAP therapy; however, no such improvements were identified in patients with poor compliance. In contrast, KOHLER *et al.* [35] did not find any differences in systemic inflammatory markers, high-sensitive C-reactive protein (CRP), plasma IL-6, interferon-γ, and adiponectin levels between patients receiving therapeutic and sub-therapeutic CPAP treatments for 4 weeks. Thus, the relationships between CPAP, systemic inflammation and the duration of therapy are complex. We report small but statistically significant elevation in systemic IL-6 with CPAP and it is known that even small changes in long-term IL-6 concentration can be associated with excess cardiovascular risk [42]. The effects of acute changes are less well studied.

The mechanisms by which CPAP may be pro-inflammatory include airway drying (*i.e.* not using humidification) or direct distension. The possible benefits of humidification have been controversial, and our *in vitro* work, in which cells were exposed to high humidity, demonstrates that drying or the absence of humidification alone cannot be solely responsible for the pro-inflammatory changes observed. An experimental study in rats failed to demonstrate any beneficial effects of heated humidification on nasal inflammation [43], whereas clinical and experimental studies have reported conflicting results on the benefits of humidification [44, 45]. Most previous *in vitro* work has used direct distension [46–48], and a mouse model of airway stretch for ventilator-associated lung injury was associated with increased expression of the murine equivalent of IL-8 [13]. Stretch may affect inflammation *via* oxidative stress, as stretch-induced IL-6 and IL-8 production can be reduced by the use of antioxidants to increase intra-cellular glutathione; production can be increased with glutathione depletion [49]. Our data add to the literature by reporting a direct effect of pressure rather than stretch. It is unlikely that the nasal epithelium is able to accommodate stretch given the confines of the nose within the bony structures of the skull.

The strengths of our study include the careful and comprehensive assessment of symptoms, upper and lower airway function, and nasal and systemic inflammation, which demonstrated a dose response in healthy subjects. A further strength is the use of both *in vivo* and *in vitro* approaches to address the clinical problem. The pressures we selected for the *in vitro* work were necessarily different from the *in vivo* work, as higher pressures *in vitro* resulted in excessive evaporation of the cell culture fluid. Our results have important implications for clinical practice. In particular, by demonstrating a relationship between nasal symptoms, mucociliary clearance and inflammation, it should be possible to investigate strategies to reduce the nasal inflammation associated with CPAP treatment, which may reduce symptoms and, therefore, aid compliance. Approaches include anti-inflammatory agents or humidification (discussed previously), and we have provided further rationale for the development of strategies to mitigate nasal inflammation during CPAP therapy. This is particularly important as existing nasal corticosteroids appear clinically ineffective [50]. An alternative strategy might involve dose titration at the beginning of the therapy (*i.e.* a gradual increase of the pressure until optimal clinical benefits with minimal side-effects are obtained), as we have provided evidence that suggests that the inflammatory effects are dose (pressure)-dependent.

This study also has several limitations that should be considered. Ours was a relatively small sample. Associations observed in the *in vivo* study do not provide direct evidence for a causal relationship between CPAP and airway inflammation, although this is why we included the complementary *in vitro* work. The design of the study could have been more robust with the inclusion of a sham CPAP arm but we were concerned that even sham CPAP might affect nasal inflammation. We cannot comment on the timing of resolution of inflammation associated with CPAP as we were interested primarily in the induction of this response. Whilst we elected to measure IL-6 and IL-8 to provide consistency across the *in vitro* and *in vivo* work, there are alternative markers including CRP and TNF-α, that may have provided additional insight into cardiovascular risks associated with CPAP. Finally, as many of our analyses are hypothesis generating we have not attempted to correct analyses for multiplicity and the results should be interpreted in the light of this.

In conclusion, we report a high prevalence of nasal symptoms following CPAP therapy in healthy subjects associated with changes in nasal function and an inflammatory response in the nasal and systemic compartments. This study also suggests that CPAP triggers an early pressure-dependent inflammatory reaction, as evidenced by the increased secretion of inflammatory markers by cultured bronchial epithelial cells. These findings have implications for the adherence of patients to CPAP therapy, especially during the important initiation phase. Strategies to combat the initial side-effects of this treatment modality and to improve compliance and retention might target the epithelial lining of the respiratory system in an attempt to address the origin of the inflammatory response.

SUPPORT STATEMENT

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STATEMENT OF INTEREST

None declared.

REFERENCES

1 Young T, Palta M, Dempsey J, *et al.* The occurrence of sleep disordered breathing among middle-aged adults. *N Engl J Med* 1993; 323: 1230–1235.
2 Sullivan CE, Issa FG, Berthon-Jones M, *et al.* Reversal of obstructive sleep apnoea by continuous positive airway pressure applied through the nares. *Lancet* 1981; 1: 862–865.
3 American Association of Respiratory Care. Clinical Practice Guideline Respiratory Care. Use of positive pressure adjuncts to bronchial hygiene therapy. *Respir Care* 1993; 38: 516–521.
4 Wang CH, Lin HC, Huang TJ, *et al.* Differential effects of nasal continuous positive airway pressure on reversible or fixed upper and lower airway obstruction. *Eur Respir J* 1996; 9: 952–959.
5 Kalan A, Kenyon GS, Seemungal TA, *et al.* Adverse effects of nasal continuous positive airway pressure therapy in sleep apnoea syndrome. *J Laryngol Otol* 1999; 113: 888–892.
6 Pepin JL, Leger P, Veale D, *et al.* Side effects of nasal continuous positive airway pressure in sleep apnea syndrome. Study of 193 patients in two French sleep centers. *Chest* 1995; 107: 375–381.
7 Richards D, Bartlett DJ, Wong K, *et al.* Increased adherence to CPAP with a group cognitive behavioral treatment intervention: a randomized trial. *Sleep* 2007; 30: 635–640.
8 Waldhorn RE, Herrick TW, Nguyen MC, *et al.* Long-term compliance with nasal continuous positive airway pressure therapy of obstructive sleep apnea. *Chest* 1990; 97: 33–38.
9 Krieger J. Long-term compliance with nasal continuous positive airway pressure (CPAP) in obstructive sleep apnea patients and nonapneic snorers. *Sleep* 1992; 15: Suppl. 6, S42–S46.
10 Almendros I, Acerbi I, Vilaseca I, *et al.* Continuous positive airway pressure (CPAP) induces early nasal inflammation. *Sleep* 2008; 31: 127–131.
11 Skoczynski S, Ograbek-Krol M, Tazbirek M, *et al.* Short-term CPAP treatment induces a mild increase in inflammatory cells in patients with sleep apnoea syndrome. *Rhinology* 2008; 46: 144–150.
12 Malbouisson LM, Szeles TF, Barbalho L, *et al.* Lung hyperinflation stimulates the release of inflammatory mediators in spontaneously breathing subjects. *Braz J Med Biol Res* 2010; 43: 201–205.
13 Belperio JA, Keane MP, Burdick MD, *et al.* Critical role for CXCR2 and CXCR2 ligands during the pathogenesis of ventilator-induced lung injury. *J Clin Invest* 2002; 110: 1703–1716.
14 Vaneker M, Halbertsma FJ, van Egmond J, *et al.* Mechanical ventilation in healthy mice induces reversible pulmonary and systemic cytokine elevation with preserved alveolar integrity: an *in vivo* model using clinical relevant ventilation settings. *Anesthesiology* 2010; 107: 419–426.
15 Cheng KC, Zhang H, Lin CY, *et al.* Ventilation with negative airway pressure induces a cytokine response in isolated mouse lung. *Anesth Analg* 2002; 94: 1577–1582.
16 Lim LH, Wagner EM. Airway distension promotes leukocyte recruitment in rat tracheal circulation. *Am J Respir Crit Care Med* 2003; 168: 1068–1074.
17 AlAhmari MD, Sapsford RJ, Wedzicha JA, *et al.* Intersession repeatability of a novel nasal lavage technique. *Transl Res* 2011; 158: 163–168.
18 Hilding AC. Simple method for collecting near-normal human nasal secretion. *Ann Otol Rhinol Laryngol* 1972; 81: 422–423.
19 Hilberg O, Pedersen OF. Acoustic rhinometry: recommendations for technical specifications and standard operating procedures. *Rhinol Suppl* 2000; 16: 3–17.
20 Rutland J, Cole PJ. Nasal mucociliary clearance and ciliary beat frequency in cystic fibrosis compared with sinusitis and bronchiectasis. *Thorax* 1981; 36: 654–658.
21 Reddel RR, Ke Y, Gerwin BT, *et al.* Transformation of human bronchial epithelial cells by infection with SV40 or adenovirus 12-SV40 hybrid viruses, or transfection *via* strontium phosphate coprecipitation with a plasmid containing SV40 early region genes. *Cancer Res* 1988; 48: 1904–1909.
22 Lowry OH, Rosebrough NJ, Farr AL, *et al.* Protein measurement with the Folin phenol reagent. *J Biol Chem* 1951; 193: 265–275.
23 Boyd JH, Petrof BJ, Hamid Q, *et al.* Upper airway muscle inflammation and denervation changes in obstructive sleep apnea. *Am J Respir Crit Care Med* 2004; 5: 541–546.
24 Kimoff RJ, Hamid Q, Divangahi M, *et al.* Increased upper airway cytokines and oxidative stress in severe obstructive sleep apnoea. *Eur Respir J* 2011; 38: 89–97.
25 Lacedonia D, Salerno FG, Carpagnano GE, *et al.* Effect of CPAP-therapy on bronchial and nasal inflammation in patients affected by obstructive sleep apnea syndrome. *Rhinology* 2011; 49: 232–237.
26 Shadan FF, Jalowayski AA, Fahrenholz J, *et al.* Nasal cytology: a marker of clinically silent inflammation in patients with obstructive sleep apnea and a predictor of noncompliance with nasal CPAP therapy. *J Clin Sleep Med* 2005; 1: 266–270.
27 Douglass JA, Dhami D, Gurr CE, *et al.* Influence of interleukin-8 challenge in the nasal mucosa in atopic and nonatopic subjects. *Am J Respir Crit Care Med* 1994; 150: 1108–1113.
28 de Oliveira LR, Albertini Yagi CS, Figueiredo AC, *et al.* Short-term effects of nCPAP on nasal mucociliary clearance and mucus transportability in healthy subjects. *Respir Med* 2006; 100: 183–185.
29 Morris LG, Setlur J, Burschtin OE, *et al.* Acoustic rhinometry predicts tolerance of nasal continuous positive airway pressure: a pilot study. *Am J Rhinol* 2006; 20: 133–137.
30 Bossi R, Piatti G, Roma E, *et al.* Effects of long-term nasal continuous positive airway pressure therapy on morphology, function, and mucociliary clearance of nasal epithelium in patients with obstructive sleep apnea syndrome. *Laryngoscope* 2004; 114: 1431–1434.
31 Willing S, San Pedro M, Driver HS, *et al.* The acute impact of continuous positive airway pressure on nasal resistance: a randomized controlled comparison. *J Appl Physiol* 2007; 102: 1214–1219.
32 Peker Y, Hedner J, Norum J, *et al.* Increased incidence of cardiovascular disease in middle-aged men with obstructive sleep apnea: a 7-year follow-up. *Am J Respir Crit Care Med* 2002; 166: 159–165.
33 McNicholas WT, Bonsigore MR, Management Committee of EU COST Action B26. Sleep apnoea as an independent risk factor for cardiovascular disease: current evidence, basic mechanisms and research priorities. *Eur Respir J* 2007; 29: 156–178.
34 Shamsuzzaman AS, Gersh BJ, Somers VK. Obstructive sleep apnea: implications for cardiac and vascular disease. *JAMA* 2003; 290: 1906–1914.
35 Kohler M, Ayers L, Pepperell JC, *et al.* Effects of continuous positive airway pressure on systemic inflammation in patients

with moderate to severe obstructive sleep apnoea: a randomised controlled trial. *Thorax* 2009; 64: 67–73.
36 Yokoe T, Minoguchi K, Matsuo H, *et al.* Elevated levels of C-reactive protein and interleukin-6 in patients with obstructive sleep apnea syndrome are decreased by nasal continuous positive airway pressure. *Circulation* 2003; 107: 1129–1134.
37 Bazzano LA, Khan Z, Reynolds K, *et al.* Effect of nocturnal nasal continuous positive airway pressure on blood pressure in obstructive sleep apnea. *Hypertension* 2007; 50: 417–423.
38 Harsch IA, Schahin SP, Radespiel-Tröger M, *et al.* Continuous positive airway pressure treatment rapidly improves insulin sensitivity in patients with obstructive sleep apnea syndrome. *Am J Respir Crit Care Med* 2004; 169: 156–162.
39 Coughlin SR, Mawdsley L, Mugarza JA, *et al.* Cardiovascular and metabolic effects of CPAP in obese men with OSA. *Eur Respir J* 2007; 29: 720–727.
40 West SD, Nicoll DJ, Wallace TM, *et al.* The effect of CPAP on insulin resistance and HbA1c in males with obstructive sleep apnoea and type 2 diabetes. *Thorax* 2007; 62: 969–974.
41 Steiropoulos P, Kotsianidis I, Nena E, *et al.* Long-term effect of continuous positive airway pressure therapy on inflammation markers of patients with obstructive sleep apnea syndrome. *Sleep* 2009; 32: 537–543.
42 Danesh J, Kaptoge S, Mann A, *et al.* Long-term interleukin-6 levels and subsequent risk of coronary heart disease: two new prospective studies and a systematic review. *PLoS Med* 2008; 5: e78.
43 Martinez-Vidal B, Farre R, Montserrat JM, *et al.* Effects of heated humidification on nasal inflammation in a CPAP rat model. *Sleep Med* 2010; 11: 413–416.
44 Ruhle KH, Franke KJ, Domanski U, *et al.* Quality of life, compliance, sleep and nasopharyngeal side effects during CPAP therapy with and without controlled heated humidification. *Sleep Breath* 2011; 15: 479–485.
45 Koutsourelakis I, Vagiakis E, Perraki E, *et al.* Nasal inflammation in sleep apnoea patients using CPAP and effect of heated humidification. *Eur Respir J* 2011; 37: 587–594.
46 Haseneen NA, Vaday GG, Zucker S, *et al.* Mechanical stretch induces MMP-2 release and activation in lung endothelium: role of EMMPRIN. *Am J Physiol Lung Cell Mol Physiol* 2003; 284: L541–L547.
47 Vlahakis NE, Schroeder MA, Limper AH, *et al.* Stretch induces cytokine release by alveolar epithelial cells in vitro. *Am J Physiol* 1999; 277: L167–L173.
48 Yamamoto H, Teramoto H, Uetani K, *et al.* Cyclic stretch upregulates interleukin-8 and transforming growth factor-beta1 production through a protein kinase C-dependent pathway in alveolar epithelial cells. *Respirology* 2002; 7: 103–109.
49 Jafari B, Ouyang B, Li LF, *et al.* Intracellular glutathione in stretch-induced cytokine release from alveolar type-2 like cells. *Respirology* 2004; 9: 43–53.
50 Strobel W, Schlageter M, Andersson M, *et al.* Topical nasal steroid treatment does not improve CPAP compliance in unselected patients with OSAS. *Respir Med* 2011; 105: 310–315.