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Nasal inflammation in sleep apnoea patients using CPAP and effect of heated humidification

A randomized, sham-controlled, crossover study

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Abstract

Nasal continuous positive airway pressure (CPAP) can cause undesirable nasal symptoms such as congestion to obstructive sleep apnoea (OSA) patients, which symptoms can be attenuated by the addition of heated humidification. However, neither the nature of nasal symptoms nor the effect of heated humidification on nasal pathophysiology and pathology are convincingly known.

Twenty patients with OSA on nasal CPAP who exhibited symptomatic nasal obstruction were randomized to receive either 3 weeks of CPAP treatment with heated humidification or 3 weeks of CPAP treatment with sham-heated humidification, followed by 3 weeks of the opposite treatment, respectively. Nasal symptom score, nasal resistance, nasal lavage interleukin-6, interleukin-12 and tumor necrosis factor-a, and nasal mucosa histopathology were assessed at baseline and after each treatment arm.

Heated humidification in comparison with sham-heated humidification was associated with decrease in nasal symptomatology, resistance and lavage cytokines, and attenuation of inflammatory cell infiltration and fibrosis of the nasal mucosa.

In conclusion, nasal obstruction of OSA patients on CPAP treatment is inflammatory in origin, and the addition of heated humidification decreases nasal resistance and mucosal inflammation.

Trial Registration clinicaltrials.gov Identifier: NCT00850876

Introduction

Nasal continuous positive airway pressure (CPAP) is considered the standard of care for treating moderate to severe obstructive sleep apnoea (OSA) [1]. Its use has been convincingly associated with substantial improvement in quality of life [2] and somnolence [3], along with significant benefits to cardiovascular morbidity and mortality [4, 5]. The major clinical challenge however remains improving CPAP long-term adherence [6].

Among the side effects caused by CPAP, undesirable nasal symptoms such as congestion, dryness or rhinorrhea are particularly prevalent and can significantly compromise compliance [7]. Several mechanisms may be potentially involved in the development of nasal discomfort with CPAP but they have been described only in healthy volunteers [8, 9, 10] and in a rodent model [11]. Indeed, deliberate mouth leaks while normal subjects were using CPAP can cause high unidirectional nasal airflow with increased nasal airway resistance [8], as well as enhance nasal mucosal blood flux [9]. Similarly, TOGIAS *et al* documented a release of inflammatory mediators in nasal wash fluids when compressed cold and dry air was delivered through the nose [10]. More recently, ALMENDROS *et al* showed that CPAP employed in rats can trigger early nasal inflammation [11]. However, it remains unknown if the same mechanisms could be responsible for the nasal side effects of CPAP in OSA patients who frequently have increased nasal symptoms even in the absence of CPAP use [12]. Recognition of the inflammatory nature of the nasal side effects of CPAP in OSA would be important for implementation of effective treatment.

Of the variety of methods used to treat nasal symptoms during CPAP treatment, the most common is heated humidification of the inspired air [13]. Although several authors have studied the effect of different forms of humidification on the relative humidity of CPAP airflow, patient adherence and subjective outcomes [13, 14, 15], no study has examined so far the functional pathways by which heated humidified CPAP attenuates nasal symptoms in OSA patients [16].

Therefore, the purpose of this study was to examine the nature of nasal side effects of CPAP in OSA patients and the effect of heated humidification. A randomized, crossover, sham-controlled design was adopted, and nasal resistance, nasal wash

inflammatory markers and pathology of nasal mucosa were examined in OSA patients using CPAP and exhibiting nasal symptoms. Our hypothesis was that nasal side effects of CPAP are inflammatory in origin, and the addition of heated humidification attenuates nasal mucosal inflammation and decreases the elevated nasal resistance caused by the use of CPAP.

Methods

Study subjects

Patients who met the following criteria were considered eligible for enrollment: i) OSA with an apnoea-hypopnoea index > 15 events/h at diagnostic polysomnography; 2) initiation of CPAP therapy without humidification after an in-laboratory polysomnography for the manual titration of CPAP; 3) symptomatic nasal obstruction attributed to or exacerbated by initial nasal CPAP use; 4) no medication known to influence nasal resistance (e.g., antihistamines, vasoconstrictors, topical or systemic steroids); 5) no smoking for the last 6 months; 6) no upper or lower respiratory tract disease (e.g., upper respiratory tract infection, rhinitis, sinusitis, chronic obstructive pulmonary disease), including a history of nasal allergy; and 7) written informed consent from each patient. The exclusion criterion was poor compliance with CPAP therapy during the course of the study [mean CPAP daily use < 4 hours calculated after CPAP usage data were downloaded using proprietary software (Scan software; ResMed; Sydney, Australia)]. The study protocol was approved by the hospital ethics committee.

Protocol

Using a table of random numbers, subjects were randomized into two groups. The patients of the first group (HH_1; Figure 1) initially underwent 3-week nasal CPAP therapy (S7 Escape™; ResMed; Sydney, Australia) with heated humidity followed by 3-week nasal CPAP therapy with sham-heated humidity. The patients of the second group (HH_2; Figure 1) initially underwent 3-week nasal CPAP therapy with sham-heated humidity followed by 3-week nasal CPAP therapy with heated humidity. No washout period between the 3-week regimens was employed because it was considered unethical to withhold therapy. Treatment duration of 3 weeks was chosen because nasal symptoms are known to occur shortly after CPAP exposure [16]. Heated humidity was achieved by

passing air from the CPAP machine through a heating unit (HumidAire 2i™; ResMed; Sydney, Australia) consisting of a heated (37^{o} C) water chamber. Sham-heated humidity was achieved by turning off the heating unit, while water was not added to the chamber. Patients were wearing the same CPAP interface (nasal mask) in both arms of the study, and were contacted by an experienced technician after the first, sixth, eleventh and sixteenth day of each treatment arm in order to increase compliance and to ensure that patients were actually using the treatment modality they were prescribed.

Measurements

All patients underwent 3 assessments: at baseline, after the first 3-week regimen, and after the second 3-week regimen, as shown in Fig. 1. Each assessment took place at a specific time (between 10 $_{A.M.}$ and 11 $_{A.M.}$) and consisted of the filling of the nasal symptoms questionnaire, and of performing anterior rhinomanometry in seated and supine positions, nasal lavage and nasal mucosa biopsies.

Nasal symptoms questionnaire

Nasal symptoms were assessed using a five point nasal scale score, as previously described [17]. Accordingly, rhinorrhea, post-nasal drip, sneezing, impaired sense of smell and nasal blockage were binary coded as present/increased over baseline (1) or absent/not increased (0) and summed to yield a total nasal score between zero and five.

Rhinomanometry

For each subject, nasal resistance to airflow was measured during wakefulness first in the upright seated position and then in supine position by active anterior rhinomanometry (PDD-301/r, Piston, Budapest, Hungary) using a standard protocol [18]. In brief, patients wore a closely fitting face mask which didn't distort the nostrils or the nasal valve and breathed through one only nostril (first the left and afterwards the right) with the mouth closed. The pressure probe was placed at the opening of the contralateral occluded nostril not being tested. Total resistance was then automatically calculated from the 2 unilateral measurements. Nasal resistance was given at a pressure difference of 150 Pa across the nasal passage. Nasal resistance values below 3.0 $cmH_20 \cdot L^{-1} \cdot s$ were considered within normal limits [18].

Nasal lavage procedure and analysis

Nasal isotonic saline lavages were carried out using a technique previously described [17]. In brief, a 12-F Foley catheter (Bard, Crawley, UK), modified by removal of the tip distal to the balloon, was inserted into the nostril and inflated with sufficient air to form a comfortable seal (typically 7–10 ml). With the patients head flexed 45^{o} forward, 7 ml of warmed 0.9% saline was instilled through the catheter and washed in and out of the nasal cavity three times. The lavage fluids from both nostrils were centrifuged (325 x g, 10 minutes, 4^{o}C) and samples were obtained from the supernatant and frozen (-80^{o}C) awaiting subsequent analysis of inflammatory mediators. The inflammatory mediators interleukin (IL)-6, IL-12 and tumor necrosis factor (TNF)-a were quantified using commercial sandwich high sensitivity ELISA kits (R&D systems, Inc; MN, USA). The limits of detection were 0.70, 5.0 and 1.6 pg/ml for IL-6, IL-12 and TNF-a, respectively.

Nasal biopsies

Paired nasal biopsies were obtained. Topical anesthesia was applied using a solution of lidocain and naphazolin delivered first by a spray device and thereafter by a cotton swab. Nasal biopsies were extracted from the inferior aspects of the inferior nasal turbinate about 0.5 cm from its anterior margin, taking care not to crush the tissue. A cutting punch forcep (diameter: 2mm) with a drilled out punch was used for this purpose. The specimens were immersed in 4% buffered formaldehyde, dehydrated, and embedded in paraffin. Sections cut at 4μm were stained with hematoxylin-eosin and periodic acid Schiff and analyzed in a blinded fashion by two independent pathologists (C.M. and M.K.) with a Zeis Axiophot microscope (Zeiss, Oberkochen, Germany). Semiquantitive grading was performed for the degree of inflammatory cell infiltration and fibrosis and the density of submucosal mucous glands according to the criteria of MAURIELLO *et al* [19]. In brief, the degree of inflammatory cell infiltration or fibrosis and the density of submucosal mucous glands were considered: i) absent when they could not be detected in the specimen, ii) mild and moderate when they were confined to the upper one-third and half of the subepithelial mucosa, respectively, and iv) severe when they were extended beyond the upper half or involved the full thickness of the subepithelial mucosa [19].

Statistical analysis

Statistical procedures were based on recommendations for analysis of crossover trials [20] and included analyses of heated humidification dependent and sham-heated humidification dependent effects (see Figure 1). Non-parametric statistics were applied. Differences between group characteristics at baseline in quantitative and qualitative data were evaluated by the Mann-Whitney U and Fisher's exact tests, respectively. Within groups changes of variables were analyzed with Wilcoxon-matched paired test, when indicated. The treatment effect of heated humidification (individual crossover difference between heated humidification and sham-heated humidification) was analyzed by comparing the values at the end of each period with Wilcoxon-matched paired test with 'heated humidification dependent" p-value (p_{hhd}) indicating significance. The possibility of a carryover period or other treatment effect was assessed by comparing the values of the differences at the end of each period between HH_1 and HH_2 groups with Mann-Whitney U signed rank sum test with 'heated humidification independent' p-value (p_{hhi}) indicating significance (Figure 1). The Bonferroni correction was used for multiple comparisons. Reported *p*-values are two-sided and pertain to the variables that remained statistically significant after the Bonferroni correction. An a priori power analysis was performed with GPower 3.1. The minimum sample size was calculated based on 90% power and a two-sided 0.05 significance level. Sample size capable of detecting between-condition difference of 0.3 $cmH_2O \cdot L^{-1} \cdot s$ was estimated for the decrease in nasal resistance using data from previous studies [8, 21]. The critical sample size was estimated to be 20 patients. Statistical significance was accepted at $p < 0.05$. Data are presented as median (interquartile range).

Results

Of the 28 patients (21 men) consecutively examined for eligibility, 20 patients were enrolled in the study, and 8 patients were not included (3 patients with respiratory tract infections, 3 patients with a history of nasal allergy or sinusitis and topical steroid use, and 2 patients with chronic obstructive pulmonary disease). Ten patients were randomly assigned to the HH_1 group and 10 patients to the HH_2 group; all of them finished the study uneventfully

Baseline characteristics

No significant differences were evident between the two groups (table 1).

Nasal score

Nasal score decreased after using heated humidification in comparison with sham-heated humidification [2.30 (2.0-3.00) versus 3.40 (3.00-4.00), $p_{hhd}<0.001$]. Nasal score decreased in both periods of administration of heated humidification, whereas sham-humidification had no effect on nasal score in HH_2 and increased the already decreased by the use of heated humidification nasal score in HH_1, which reached to a level comparable to that of baseline (Figure 1B).

Nasal resistance

Nasal resistance in supine position decreased after administration of heated humidification in comparison with sham-heated humidification [2.3 (2.00-2.50) versus 2.8 (2.4-3.5) $cmH_20 \cdot L^{-1} \cdot s$, $p_{hhd}<0.001$]. Nasal resistance decreased in both periods of applying heated humidification, whereas sham-humidification had no effect on nasal resistance in HH_2 and increased the previously decreased by the application of heated humidification nasal resistance in HH_1, which reversed to levels similar to that of baseline (Figure 2). Results of nasal resistance in seated position were identical.

Nasal lavage inflammatory mediators

Baseline nasal lavage levels of IL-6, IL-12 and TNF-a were not different between HH_1 and HH_2 groups, and levels of these cytokines decreased after administration of heated humidification in comparison with sham-heated humidification [1.2 (1.0-1.5) versus 1.5 (1.3-1.6) $pg \cdot ml^{-1}$ for IL-6, $p_{hhd}<0.001$; 7.2 (6.2-7.9) versus 8.3 (7.4-9.1) $pg \cdot ml^{-1}$ for IL-12, $p_{hhd}=0.005$; and 1.8 (1.7-2.1) versus 2.6 (1.9-2.7) $pg \cdot ml^{-1}$ for TNF-a, $p_{hhd}=0.001$]. All cytokine levels decreased in both periods of using heated humidification, whereas sham-humidification had no effect on cytokine levels in HH_2 and increased the previously decreased by the application of heated humidification cytokine levels in HH_1, which reversed to levels similar to that at baseline (Figure 3).

Nasal mucosa pathology

Nasal mucosa specimens revealed a chronic subepithelial inflammatory cell infiltration consisting predominantly of lymphocytes and plasma cells. At baseline, the degree of inflammation and fibrosis and the density of the submucosal mucous glands

were ~~almost~~ identical between HH_1 and HH_2 groups (data not shown). The administration of heated humidification was associated with a significant decrease in the degree of inflammation and fibrosis in comparison with sham-heated humidification (table 2). Representative specimens are shown in Fig. 4.

Carryover effect

Carryover or treatment effect of the application of heated humidification on the result of sham-heated humidification in HH_1 group and of the application of sham-heated humidification on the result of heated humidification in HH_2 group was not significant for nasal score, nasal resistance, and nasal lavage cytokines (Figures 1B, 2 and 3, respectively). Therefore, it can be assumed that possible carryover period or treatment effect could not have influenced the results.

Discussion

The data of this study demonstrated: 1) the inflammatory nature of nasal side effects of CPAP in OSA patients, and 2) that a 3-week administration of CPAP with heated humidification in comparison with sham-heated humidification was associated with decrease in nasal symptomatology, resistance and lavage cytokines (IL-6, IL-12, TNF-a), and attenuation of inflammatory and fibrotic changes of the nasal mucosa.

Nasal discomfort is among the most common side effects that CPAP can cause in OSA patients and nasal side effects can compromise long-term compliance to CPAP therapy [6]. Previous studies have assessed the role of CPAP on nasal airway of healthy volunteers [8, 9, 10, 16] and in a rodent model [11], and have also evaluated the effect of different forms of humidification on nasal symptoms and adherence in OSA patients [13, 14, 15, 22]. However, the effect of CPAP (with and without humidification) on nasal airway pathophysiology of OSA patients remains unknown. The current study adds to the literature by demonstrating that CPAP with heated humidification reduces pre-existing nasal airway inflammation and nasal symptomatology presumably attributed to previous use of CPAP without heated humidification. OSA patients on CPAP therapy exhibiting nasal obstruction were recruited and nasal airway pathophysiology and pathology were evaluated. This subgroup of OSA patients was selected because it is in these patients that the application of CPAP had already provoked clinical problems and thus the effect of

continuation of CPAP either with sham-heated humidification or heated humidification on nasal airway would be more apparent and clinically relevant.

Only two previous studies that included a small number of patients have provided limited, albeit conflicting, data regarding the effect of CPAP therapy on nasal pathophysiology of OSA patients [21, 23]. Indeed, CONSTANTINIDIS *et al* [23] demonstrated prolonged mucociliary transport time after 3-10 months of CPAP use along with a desiccation of the epithelial surface and an inflammatory cell infiltration of the mucosa, whereas BOSSIE *et al* [21] failed to discern any change in mucociliary transport time or in nasal resistance after 6 months of CPAP use. However, although measures of mucociliary clearance are simple to perform, they have poor reproducibility and consequently their incorporation into clinical trials is considered questionable [24].

Nasal symptomatology during CPAP therapy has been studied in OSA patients in crossover studies, and it was demonstrated a marked decrease in upper airway symptoms after the application of CPAP with heated humidification in comparison with CPAP with placebo humidification [14, 22]. Interestingly, ALMENDROS *et al* in an effort to explain nasal symptoms following CPAP proposed the presence of deficiency of adaptive anti-inflammatory mechanisms because of preexisting conditions such as nasal obstruction [11]. In such a case the application of CPAP would act as a second-hit source of injury [11]. The results obtained in the current study suggest that the addition of heated humidification could compensate for the potential deficiency associated with nasal obstruction and could thus ameliorate nasal symptomatology.

Heated humidification was associated with a decrease in the level of nasal lavage pro-inflammatory cytokines. IL-6 and TNF-a are important in the induction of the acute phase response of inflammation, whereas IL-12 mediates an enhancement of the cytotoxic activity of cytotoxic T lymphocytes [25]. Although the pathophysiologic mechanisms by which CPAP causes cytokine release are unknown, some speculations can be made. It is likely that airflow of cold and dry air has a toxic effect on nasal epithelium [26] and mediator containing cells of the nasal mucosa [10]. The addition of heated humidification could eventually prevent this effect, thus avoiding epithelial detachment and shedding and mediator release. Furthermore, CPAP airflow may lead to water evaporation and an increase in the osmolarity of the extracellular fluid surrounding

the mast cells that is sufficient to induce mediator release [27]. This effect could also eventually be avoided by heated humidification, as ARAUJO *et al* [28] have demonstrated a decreased dehydration effect of the inspired air when humidification was added to CPAP therapy.

As far as the histopathology of the nasal mucosa is concerned, the present study demonstrated an attenuated nasal mucosa inflammation after the addition of heated humidification in comparison with sham-heated humidification. This finding supports the results of a previous study, which provided evidence that CPAP can disrupt the equilibrium of the mucous membrane, giving rise to shedding and inflammation of the nasal mucosa [23]. Interestingly and in accordance with our results, CRUZ *et al* demonstrated that it was cold and dry air and not warm and moist air that can lead to epithelial cell damage suggesting that this effect does not result from the sheer force stimulus exerted by the airflow [26]. These authors further hypothesized that the epithelial detachment following cold and dry air is likely to be linked to some form of a mucosal water transportation defect [26]. This suggestion could also provide an explanation for the attenuation of nasal inflammation after the application of heated humidification observed in our study.

Combining the results of nasal mucosa pathology with the other measurements it is noteworthy to add that the apparition of increased nasal mucosa inflammation after application of CPAP with sham-heated humidification in HH_1 group was associated with increased nasal scale score, elevated nasal resistance and increased nasal lavage inflammatory mediators (Figures 1B, 2 and 3, respectively). In contrast, the vanishing of nasal mucosa inflammation after application of CPAP with heated humidification in HH_1 and HH_2 groups was associated with a decreased nasal scale score, nasal resistance and nasal lavage inflammatory mediators (Figures 1B, 2 and 3, respectively). Therefore, it is plausible to suggest that the time response of nasal mucosa pathology to the application of CPAP is closely reflected by the fluctuations of nasal score, nasal resistance and nasal lavage mediators.

Some methodological issues require consideration in the current study. Firstly, blinding of the patients on the therapy used could not have been optimal. Therefore, in an attempt to control for a placebo effect, all study patients were naive to the use of heated

humidification while told that the purpose of the study was to compare the effect of CPAP treatment at "two levels of humidity". However, the objective assessment of CPAP use by the CPAP data card did not differ between arms supporting the view that patients believed they were in fact getting a similar amount of treatment. Secondly, recommendations for nasal wash technique are not currently available. However, the reproducibility of the nasal wash technique that was employed in the present study for the assessment of inflammatory cytokines has been tested previously [17]. Additionally, the nasal symptom questionnaire that was used in the current study, albeit not validated, is considered a practical, rapid and useful indication of upper airway involvement and has been shown to correlate well with the more complex sino-nasal outcome test-20 (SNOT-20) questionnaire [17]. The SNOT-20 is one of the most widely used quality-of-life instruments for a validated evaluation of sino-nasal conditions and is intended for populations with rhinosinusitis [17]. Lastly, the sample size of the current study could be considered limited, especially in comparison with recently published data [29]. Yet, in accordance with the results of the present study, RYAN *et al* [29] demonstrated that the addition of heated humidification is associated with a decrease in the frequency of nasal symptoms in OSA patients. However, this decrease does not lead to improved adherence to CPAP [30].

In conclusion, the present study provided evidence about the inflammatory effect of CPAP on nasal mucosa of OSA patients and that CPAP with heated humidification is associated with decrease in nasal symptomatology, resistance and lavage cytokines, and attenuation of inflammation and fibrosis of the nasal mucosa. It is thus plausible to suggest that clinical response to anti-inflammatory drugs might be anticipated. Further studies are warranted in this direction.

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Table 1. Patient characteristics at baseline

	HH_1 group (n=10)	HH_2 group (n=10)
Age, yrs	61.5 (51.0-67.0)	62.0 (48.8-68.5)
Male sex, n	6	6
Body mass index, $kg \cdot m^{-2}$	31.5 (28.8-35.3)	30.5 (28.8-33.3)
Apnoea-hypopnoea index, $events \cdot h^{-1}$	32.4 (26.8-38.3)	34.0 (28.7-39.5)
Nasal resistance supine, $cmH_2O \cdot L^{-1} \cdot s$	2.7 (2.4-3.2)	2.7 (2.3-3.3)
Nasal resistance seated, $cmH_2O \cdot L^{-1} \cdot s$	2.6 (2.3-3.2)	2.5 (2.3-3.1)
Nasal score	3.5 (2.8-4.0)	3.5 (2.8-4.0)
CPAP pressure, cmH_2O	7.5 (6.3-8.5)	7.2 (6.1-8.2)
Time of CPAP use at enrollment to the study, days	11 (5-20)	9 (5-17)

Data are presented as median (interquartile range). HH_1 group: the group of patients who initially underwent 3 weeks of CPAP therapy with heated humidification followed by 3 weeks of CPAP therapy with sham-heated humidification; HH_2 group: the group of patients who initially underwent 3 weeks of CPAP therapy with sham-heated humidification followed by 3 weeks of CPAP therapy with heated humidification; Nasal score: a five point nasal symptom scale score based on a nasal symptoms questionnaire, and yielding a score between zero and five [17].

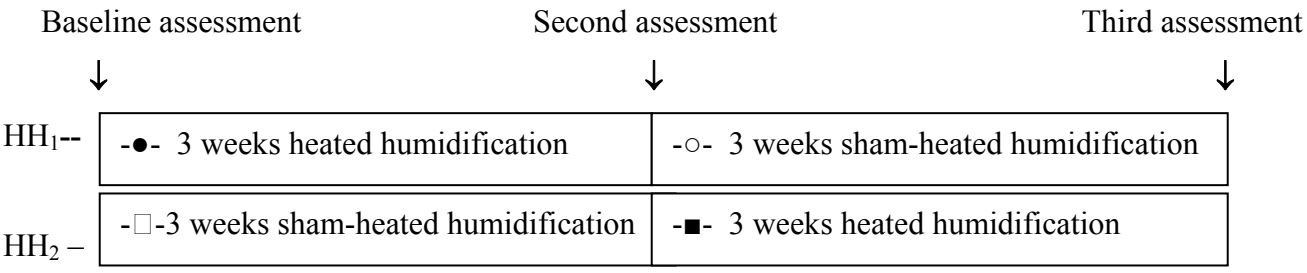
Table 2. Histopathologic features of nasal mucosa biopsy specimens after sham-heated humidification and heated humidification.

	Inflammation		Fibrosis		Mucous glands	
	S-HH	HH	S-HH	HH	S-HH	HH
Absent, n (%)	0 (0)	8 (40)#	0 (0)	6 (30)*	0 (0)	4 (20)
Mild, n (%)	6 (30)	8 (40)	10 (50)	6 (30)	8 (40)	10 (50)
Moderate, n (%)	8 (40)	4 (20)	6 (30)	8 (40)	8 (40)	6 (30)
Severe, n (%)	6 (30)	0 (0)*	4 (20)	0 (0)	4 (20)	0 (0)

S-HH: Sham-Heated Humidification (n = 20); HH: Heated Humidification (n = 20); *: $p<0.05$ versus S-HH; #: $p<0.01$ versus S-HH. For explanation and further definitions see footnote of Table 2.

Figure 1

A)


Baseline assessment
Second assessment
Third assessment
HH1--
-●- 3 weeks heated humidification
-○- 3 weeks sham-heated humidification
HH2 –
-□-3 weeks sham-heated humidification
-■- 3 weeks heated humidification

B)

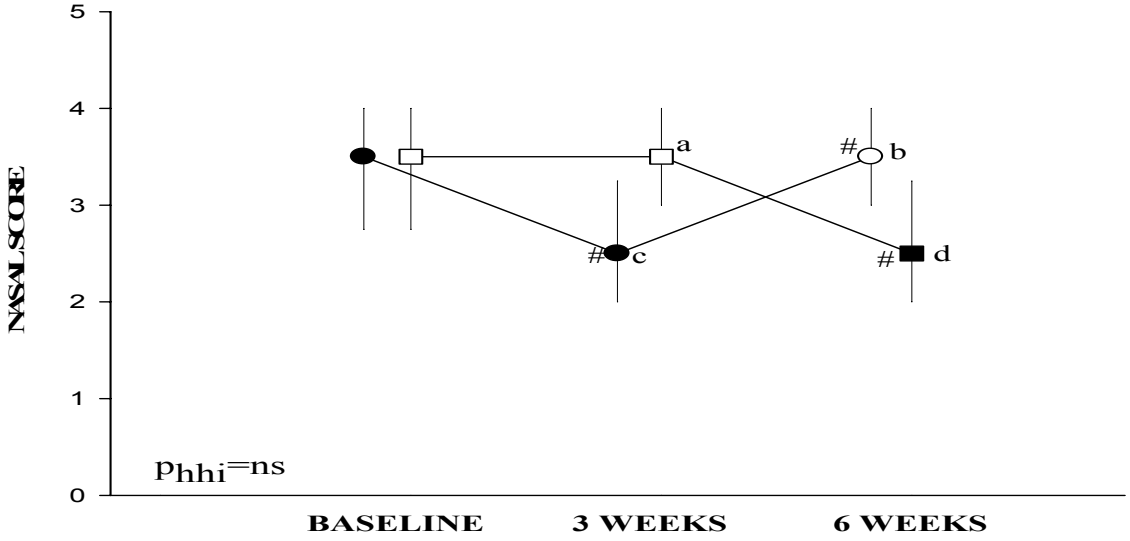

5
4
3
2
1
0
a
#
b
#
c
#
d
phhi=ns
BASELINE
3 WEEKS
6 WEEKS

Figure 2

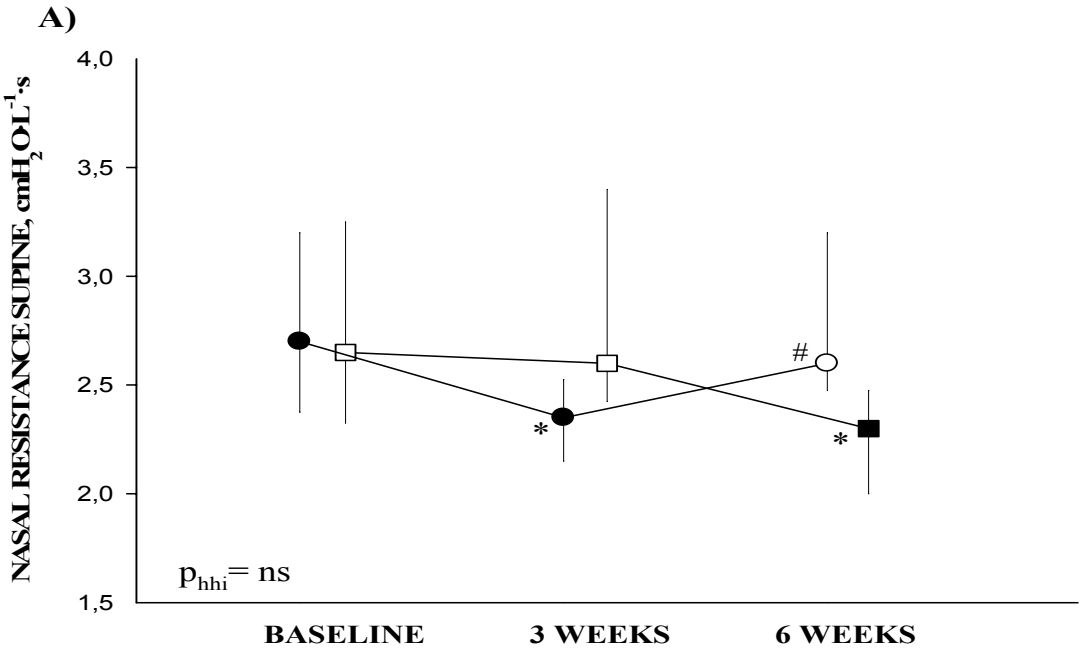

A)
NASAL RESISTANCE SUPINE, cmH2OL-1·s
4,0
3,5
3,0
2,5
2,0
1,5
#
*
*
phhi= ns
BASELINE
3 WEEKS
6 WEEKS

Figure 3A

A)

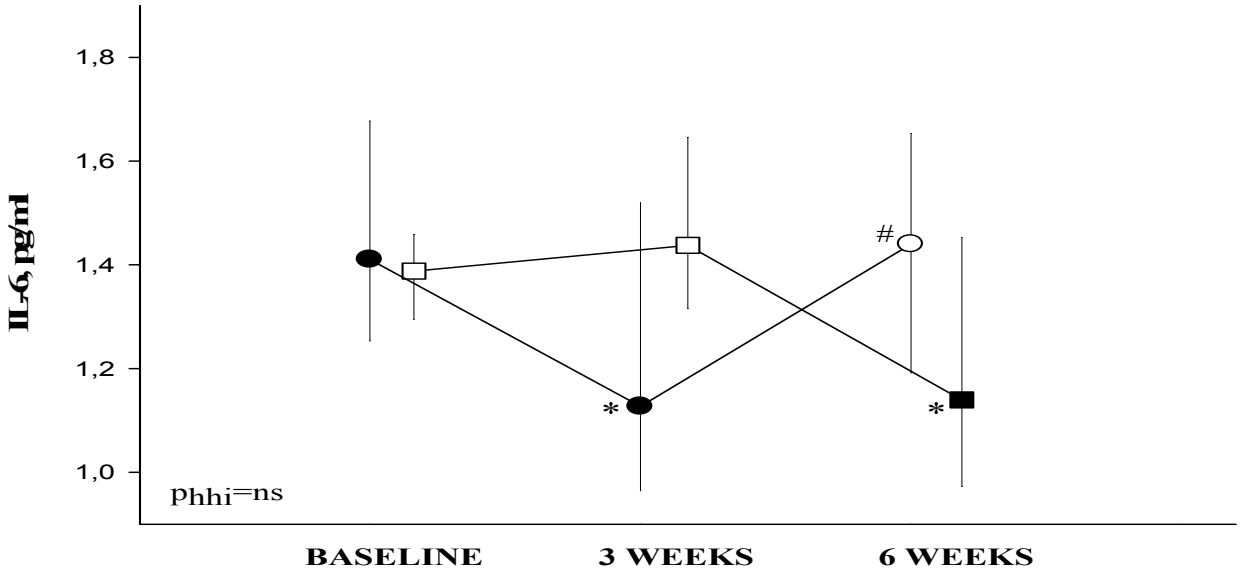


Figure 3B

B)

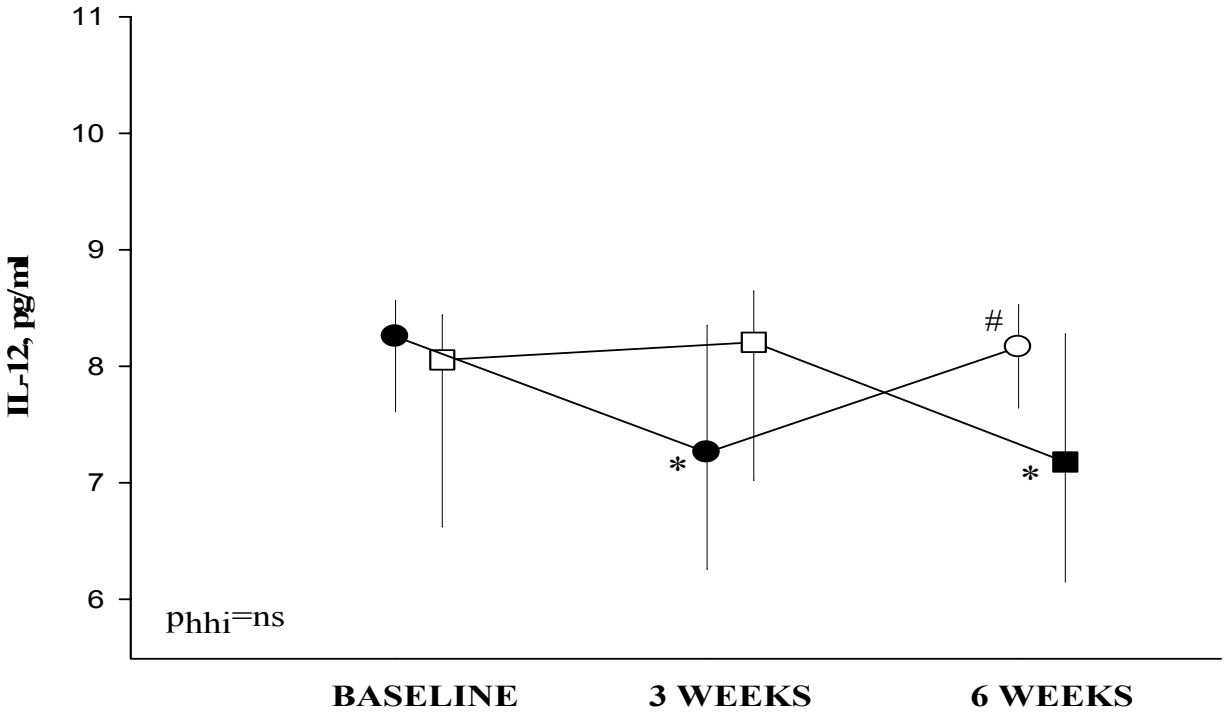


Figure 3C

C)

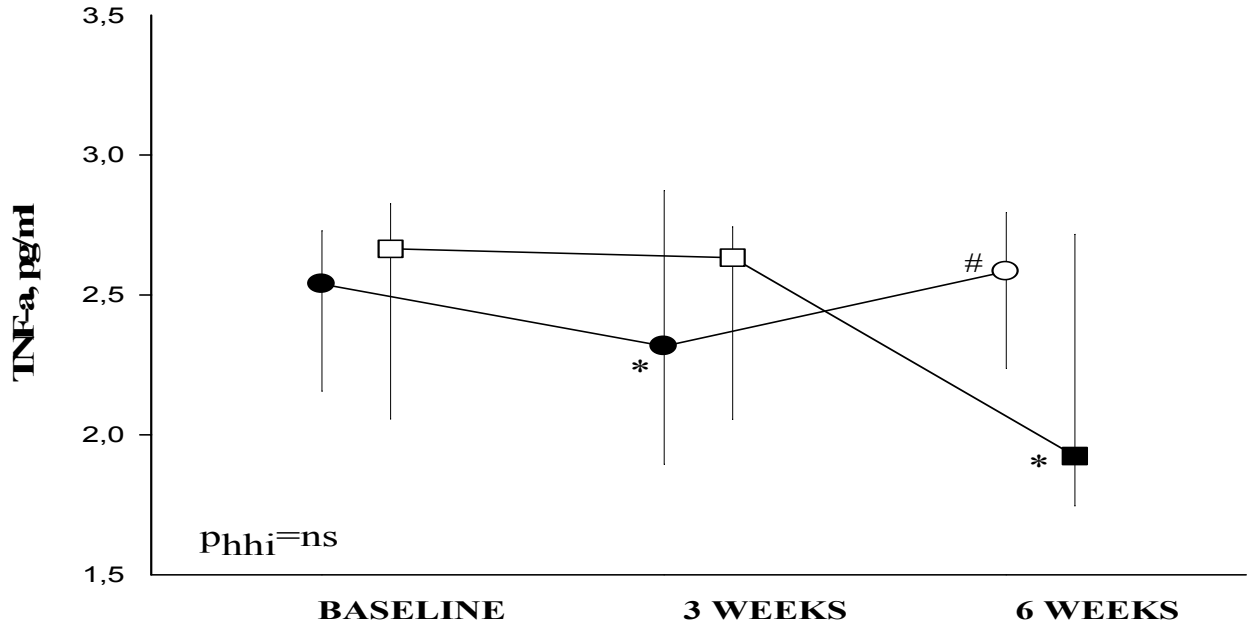


Figure 4

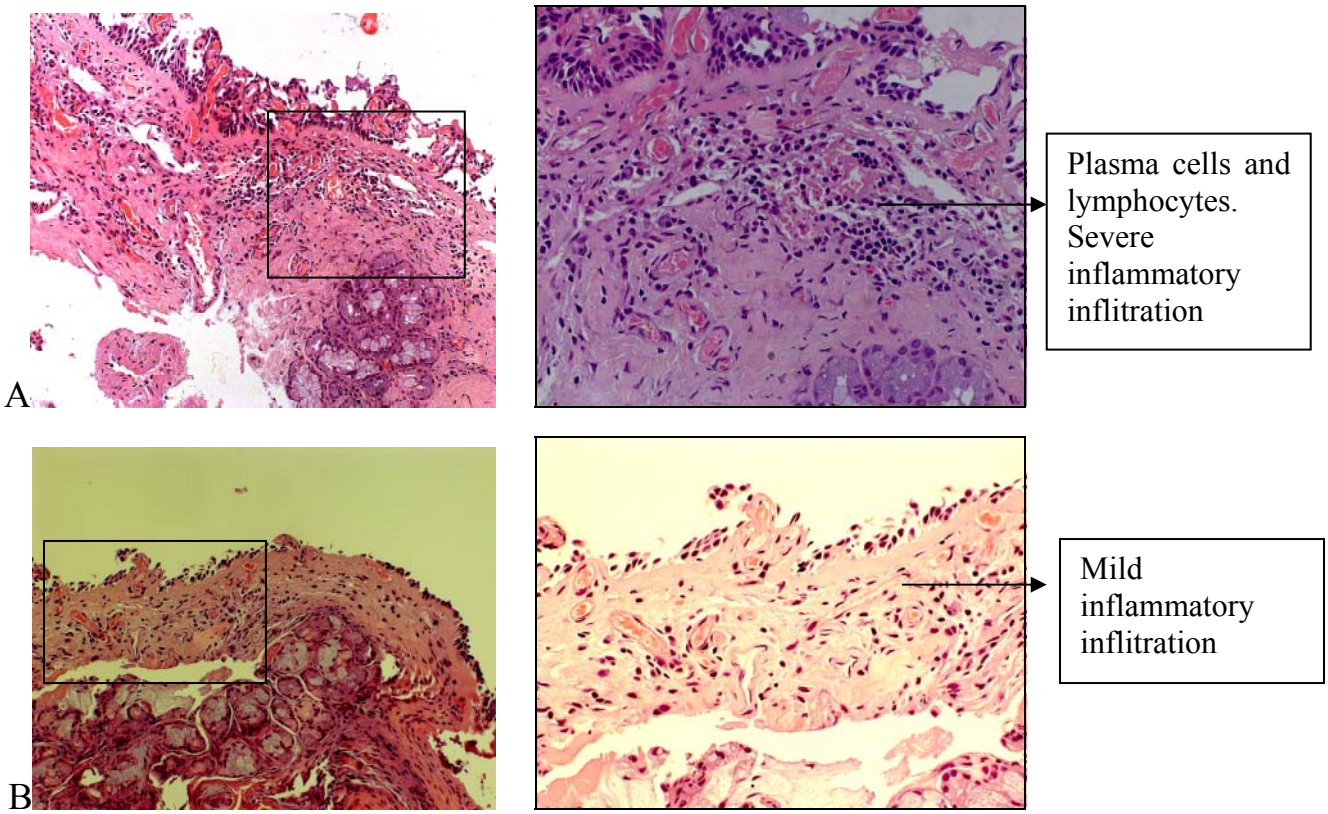

A
Plasma cells and lymphocytes. Severe inflammatory inflitration
B
Mild inflammatory inflitration

Figure legends

Figure 1 (A-B) Study design and nasal score values. (A) Crossover study design. After baseline assessment, the 10 patients of the first group (HH_1) underwent 3 weeks of CPAP therapy with heated humidification (closed circle) followed by 3 weeks of CPAP therapy with sham-heated humidification (open circle), whereas the 10 patients of the second group (HH_2) underwent 3 weeks of CPAP therapy with sham-heated humidification (open square) followed by 3 weeks of CPAP therapy with heated humidification (closed square). (B) Nasal score decreased in both periods of administration of heated humidification, whereas sham-humidification had no effect on nasal score in HH_2 and increased the already decreased by the use of heated humidification nasal score in HH_1, which reached to a level comparable to that of baseline. Values are median and interquartile range. #: $p<0.05$ *versus* previous assessment value. The possibility of a carryover period or other treatment effect was assessed by comparing the values of the differences at the end of each period between HH_1 and HH_2 groups with Mann-Whitney U signed rank sum test (*d* minus *a* versus *c* minus *b*) with 'heated humidification independent' p-value (p_{hhi}) indicating significance. The treatment effect of heated humidification (individual crossover difference between heated humidification and sham-heated humidification) was analyzed by comparing the values at the end of each period (*a* plus *b versus c* plus *d*) with Wilcoxon-matched paired test.

Figure 2 Nasal resistance values. ~~A)~~ Nasal resistance decreased in both periods of applying heated humidification, whereas sham-humidification had no effect on nasal resistance in HH_2 group and increased the previously decreased by the application of heated humidification nasal resistance in HH_1 group, which reversed to levels similar to that of baseline. Circles: HH_1 group; squares: HH_2 group; closed symbols: heated humidification; open symbols: sham heated humidification. *: $p<0.01$ *versus* previous assessment value; #: $p<0.05$ *versus* previous assessment value. For further explanation see legend of Figure 1.

Figure 3 IL-6, IL-12, TNF-a values in nasal lavage fluids. All cytokine levels decreased in both periods of using heated humidification, whereas sham-humidification had no effect on cytokine levels in HH_2 group and increased the previously decreased by the application of heated humidification cytokine levels in HH_1 group, which reversed to levels similar to that at baseline. Circles: HH_1 group; squares: HH_2 group; closed symbols: heated humidification; open symbols: sham heated humidification. *: $p<0.01$ *versus* previous assessment value; #: $p<0.05$ *versus* previous assessment value. For further explanation see legend of Figure 1.

Figure 4 Histopathologic features of nasal mucosa biopsy specimens obtained after: A) the administration of 3-weeks of CPAP therapy with sham-heated humidification, at low magnification (hematoxylin-eosin stain, original magnification x 200) *(left panel)* and high magnification of the black boxed area on the left panel (original magnification x 400) *(right panel)*. Specimen is characterized by epithelial detachment and severe inflammatory cell infiltration; B) the administration of 3-weeks of CPAP therapy with heated humidification, at low magnification (hematoxylin-eosin, original magnification x 200) *(left panel)* and high magnification of the black boxed area on the left panel (original magnification x 400) *(right panel)*. Specimen shows mild inflammatory cell infiltration.