Author's response to reviews

Title: No evidence of enhanced oxidant production in blood obtained from patients with obstructive sleep apnea

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The answer to the Reviewer’s number 1 report

We thank you for reviewing the article “No evidence of enhanced oxidant production in blood obtained from patients with obstructive sleep apnea.” In efforts to improve our manuscript, we implemented the suggestions proposed by the Reviewers.

To elucidate on the study protocol, the control data for the morning results of the OSAS and CPAP-OSAS group were reflective of their own outcome data from the evening.

Additionally, 11 volunteers free of OSAS were involved, creating a separate control group.

This allowed the capability of comparing all results in two aspects: firstly, evening to morning data within each group, secondly, evening to evening as well as morning-to-morning results between each group. Due to this, placebo treated controls were not considered. Nevertheless, the study may contribute as a pilot
The following sentences were removed from the introduction to the “materials and methods”

A luminol enhanced whole blood chemiluminescence (LBCL) technique was employed as a measure of ROS production by circulating phagocytes [17,18] in order to avoid any priming and/or activation of oxidative cell response due to isolation procedures [18]. Moreover, to avoid any possible bias related to patients’ interindividual variability and differences in comorbidity and pharmacological treatment, LBCL was measured before (evening) and just after polysomnography-controlled sleep (morning) in a matched manner. Therefore, evening results served as reference values designed for morning data, presumably affected by apnea/hypopnea episodes in addition to subsequent IH during sleep.

A method of standardization was utilized in reporting the results. Parameters including white blood cells, hematocrit and chemiluminescence are currently evident in tables. We also added the severe OSAS outcome data to tables 5 and 6.

For differences between means the 95% confidence intervals were calculated. Illustrations of this data have been implemented in tables. The additional sentence was added in the statistical analysis - a part of “Materials and methods”: (…) For differences between means the 95% confidence intervals were calculated.

Discussion was condensed by eliminating the following sentences:

LBCL is a valuable and sensitive technique in its determination of ROS production by means of circulating phagocytes. Avoidance of any possible artifacts related to cell isolation procedures and rapid measurement are the major advantages of this technique [17,18].

Obesity as well as the age of blood donors may alter the function of PMNs in conjunction with the intensity of respiratory burst [17,25,26]. In addition, cigarette smoke can sensitize circulating PMNs in the direction of agonist stimulation [27]. These protocol inaccuracies may elucidate the discrepancies between our investigation and that of Schultz et al. [14] and Dyugovskaya et al. [15]. However, our results establish concurrence with the investigative report of Müns and coworkers, describing a voided enhancement in ROS production from Escherichia coli-activated PMNs in OSAS patients [13].

An overnight stay at 3196 m above sea level results in blood desaturation by 4 to 5%, increasing resting and fMLP-stimulated ROS production via PMNs. Simultaneous strenuous exercise prevented this phenomenon, maximizing inhibition with epinephrine and norepinephrine reaching the highest possible plasma levels [36], suggestive of catecholamines as the chief mediator.
Although the sleep time of our patients was shorter than the duration of hypobaric hypoxia, it is rather not a causative factor concerning the negative results obtained, particularly, in part, due to the ability of a 20 min. hypobaric hypoxic activation of circulating PMNs in healthy subjects [10].

Three articles were struck out from the references and one was added (number 34).

Table 5 (previously table 4) contains one font.

In presenting the results in table 1, 4, 5 and 6, we reported the mean and standard deviation, eliminating the median and interquartile range.

It is well known that interquartile range (IQR) is the difference between the first quartile and third quartile of a set of data. This is one approach in describing the spread of a set of data. With this definition obtained from www.mathwords.com, we present an example where the data 2, 5, 6, 9, 12 minimum is 2, first quartile is 3.5, median is 6, third quartile is 10.5 and maximum is 12. The IQR for these data is 10.5 – 3.5 = 7. Thus, IQR is 7. We thereby stand firm in presenting the IQR as one number.

It is noteworthy to mention that prior to the submission of the manuscript; Jeffrey E. de Graft-Johnson, M.D., M.P.A., M.S., a native American English speaker, conducted an extensive review of the manuscript. Taking into consideration, the suggestions proposed by the Reviewers, further review of the manuscript has been conducted.

The answer to the Reviewer’s number 2 report

We thank you for the review, in efforts to improve our paper all attempts were made in implementing your suggestions.

Although OSAS as well as CPAP-OSAS patients observed elevated chemiluminescence parameter levels in comparison to controls (Table 5), we did not expect a significantly diverse difference with the incorporation of OSAS patients mainly with an elevated AHI (AHI ≥ 30).

Among 11 patients with a high AHI, almost all chemiluminescence parameters were lower than that represented in OSAS patients.

Our decision stems from a study conducted in our department (Rysz et al. Arch Immunol Ther Exp 54, 2006). The study involved three groups: 26 hemodialyzed patients, 11 non-dialyzed with chronic renal failure (CRF) and 20 gender- and age-matched healthy controls.

Although the total number of patients were lower than our study, (57 versus 60), the results were nevertheless evident. Median rCl, pCl and tCl were respectively 1.5, 3.0 and 2.8 times higher in hemodialyzed patients before dialysis than in healthy controls.

Due to the fact that COPD patients did not take part in our study as a group with elevated hydrogen peroxide levels in exhaled breath condensate (Kostikas et al. Chest 124, 2003), an oversight in not enumerating COPD among other exclusion criteria (chapter: study population and polysomnography) has been corrected.

Furthermore, the additional sentences were added in the discussion:

(…) The morning and evening chemiluminescence parameters like rCL, pCL and tCL as well as FRAP were higher in OSAS patients in comparison with controls. (…)

(…)The results of our study suggest that circulating phagocytes (PMNs and monocytes) are not the main culprit of OSAS consequences in the human body. It doesn’t exclude augmented ROS production and activation of the systemic ROS signaling system [7, 14, 34]. The oxidative stress can take place in the nearness or exactly in the blood vessels endothelium what can significantly accelerate the atherosclerosis development. Circulating phagocytes probably do not take a part in oxidative stress what doesn’t synonymously reject the oxidative stress presence in OSAS patients.

Sincerely,

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