Author response to reviewer comments

EDITORIAL COMMENTS (CAPITAL LETTERS)

Authors reply (lower case letters)

- AS THE PRESENT STUDY ASSESSED PREVALENCE, PLEASE USE THIS TERM THROUGHOUT, RATHER THAN INCIDENCE.

Reply: Done as recommended

- ALTHOUGH THE SAMPLE SIZE IS MENTIONED AS A POTENTIAL LIMITATION, SOME DETAIL ON HOW THE SAMPLE SIZE WAS DETERMINED WOULD BE USEFUL IN THE METHODS SECTION; FOR EXAMPLE, WAS ANY PROSPECTIVE POWER CALCULATION PERFORMED WITH REGARD TO THE GROUP SIZES?

Reply: We certainly acknowledge that this issue should be further clarified. The sample size selected for our investigation was based on previously reported data (Arias MA. Circulation. 2005;112(3):375-383) on the prevalence of diastolic dysfunction in an OSAS population quite similar to ours. In that study (Arias MA. Circulation. 2005;112(3):375-383), in a population without any cardiovascular disease as well, the prevalence of diastolic dysfunction was approximately 20% in controls and 55% in OSAS patients (175% increase in prevalence, P=0.020). In this respect, to depict a clinically meaningful difference (a similar increase with Arias et al) in the frequency of diastolic dysfunction in OSAS compared to control patients in our study, with a probability of a type-I error of 5% and a power of 80%, at least 60 patients would be required for our analysis. In order to compensate for possible study dropouts, we finally enrolled a slightly larger population (67 patients). This is clearly stated in the “Methods” section now (page 6, paragraph 2, lines 6-12).

- PLEASE INCLUDE THE NAME OF THE ETHICS COMMITTEE PLUS THE APPROVAL NO. IF AVAILABLE.

Reply: The study was approved by the Internal Review Board and Ethics Committee of the University of Thessaly (UT) – ID: 455629/October/2011. This is stated in the text now (page 6, paragraph 3, lines 15-16)

- IN THE METHODS, PLEASE SPECIFY THAT CONSENT WAS FOR INCLUSION IN THE PRESENT STUDY (RATHER THAN JUST E.G. TREATMENT).

Reply: Done as recommended (page 6, paragraph 3, line 13)

- PLEASE CHANGE “WORLD MEDICAL ASSOCIATION OF HELSINKI” TO “WORLD MEDICAL ASSOCIATION DECLARATION OF HELSINKI”

Reply: Done as recommended (page 6, paragraph 3, line 14-15)

- PLEASE PROVIDE DETAIL IN THE METHODS OF THE ANALYSES FOR THE CORRELATION COEFFICIENTS REPORTED IN THE RESULTS SECTION.

Reply: Done as recommended ("Statistical Analysis" section; page 10, paragraph 3, lines 18-19)

PLEASE DELETE THE INFORMED CONSENT SECTION FROM BEFORE THE REFERENCE LIST, AS THIS INFORMATION IS INCLUDED IN THE METHODS.

Reply: Done as recommended. In addition, a type error ("… criteria 2-9" instead of "… criteria 2-8") was also corrected (page 6, paragraph 1, lines 3-4)

Thank you for the thorough and helpful reviewing you made. We hope that we have adequately responded to your excellent comments.

J. Papanikolaou

Answer to Reviewer 1

Dear Madam/Sir,

Thank you so much for your valuable comments that helped us improve by far our manuscript: "Diastolic dysfunction in men with severe obstructive sleep apnea syndrome but without cardiovascular or oxidative stress-related comorbidities" by J. Papanikolaou et al. Please find a point-by-point response to your comments.

REVIEWER COMMENTS (CAPITAL LETTERS)

Authors reply (lower case letters)

NICE RESEARCH EXPLORING POTENTIAL PREDICTORS OF DIASTOLIC DISFUNCTION IN OSA PATIENTS.

Reply: Thank you for your thorough review and your helpful comments.

MAIN LIMITATION IS RELATED TO SAMPLE SIZE.

Reply: We certainly acknowledge that our population (n=67) could be considered as quite small. This limitation of our study is clearly stated in the "Discussion section" (page 18, paragraph 1, lines 1-7). However, our sample selection was based on previously reported data (Arias MA. Circulation. 2005;112(3):375-383) on the prevalence of diastolic dysfunction in an OSAS population quite similar to ours (prospective power calculation was performed); this is stated in the "Methods section" (page 6, paragraph 2, lines 6-12). In addition, the utmost effort was made in order only OSAS patients without cardiovascular or oxidative stress-associated diseases known to be related with LV diastolic dysfunction to be included in the study. It is well established that the more homogenous a

population is, the smaller the sample size is required to obtain a given level of precision [Israel G.D. Determining Sample Size. University of Florida Cooperative Extension Service, Institute of Food and Agriculture Sciences, EDIS, Florida 1992]. In such a highly-selected homogenous population, we aimed at testing the role of OSAS per se as an independent determinant of diastolic dysfunction; this is also highlighted in the "Discussion" section (page 13, paragraph 3, lines 16-18 and page 14, paragraph 1, lines 4-14).

IN RESULT SECTION, SEVERAL POLYSOMNOGRAPHIC MEASURES SHOWED STATISTICALLY TENDENCY THAT AUTHORS SHOULD INCLUDE AS STUDY LIMITATIONS/ FUTURE RESEARCH IN THIS FIELD.

Reply: You are right that apart from increased AHI values, several polysomnographic (i.e. apnea, desaturation and arousal) indices also tended to be associated with LV diastolic dysfunction in our limited OSAS population. We certainly agree that the role of these indices on LV diastolic properties warrant further investigation in the future. In light of your useful comment, this is clearly highlighted in the "Discussion section" now (page 15, paragraph 1, lines 2-5).

FINALLY, IT WOULD BE GRATEFUL IF AUTHORS CAN EVALUATED THE RISK OF SEVERE HYPOXEMIA DURING PSG USING A CUT OFF OF TST-90 >20% AND NADIR SAO2 < 75% IN UNIVARIATE ANALYSIS. THESE PARAMETERS SHOULD BE LINKED TO POOR DIAGNOSIS IN MODERATE/SEVERE OSAS (SLEEP BREATH. 2019 MAY 12. DOI: 10.1007/S11325-019-01860-0.)

Reply: Thank you for your valuable comment. The role of SaO2 nadir <75% and T90 (total sleep time spent with SaO2 < 90%) >20% in diagnosing and/or predicting the development of comorbidities in patients with severe OSAS has been recently highlighted [Labarca G et al. Sleep Breath. 2019]. In our population with severe OSAS but without comorbidities, however, no association was found between these indices and LV diastolic dysfunction (reviewed Table 3). This may indicate that the role of the mentioned indices is likely to be minor in patients free of cardiovascular and/or oxidative stress-related comorbitidies. In light of your recommendation, a relative comment was added in the "Discussion section" (page 15, paragraph 1, lines 5-9).

Thank you for the thorough and helpful reviewing you made. We hope that we have adequately responded to your excellent comments.

J. Papanikolaou

Answer to Reviewer 2

Dear Madam/Sir,

Thank you so much for your valuable comments that helped us improve by far our manuscript: "Diastolic dysfunction in men with severe obstructive sleep apnea syndrome but without

cardiovascular or oxidative stress-related comorbidities" by J. Papanikolaou et al. Please find a point-by-point response to your comments.

On behalf of the authors, I would like to ask you to review this manuscript and to consider the possibility of acceptance for publication in the Therapeutic Advances in Respiratory Disease.

REVIEWER COMMENTS (CAPITAL LETTERS)

Authors reply (lower case letters)

THIS IS AN INTERESTING MANUSCRIPT. WHILE MANY OTHER PAPERS HAVE EXAMINED THIS TOPIC, MANY HAVE INCLUDED AND CONTROLLED FOR COVARIATES SUCH AS BMI, AND OTHER CV RISK FACTORS. THIS STUDY IS DIFFERENT BECAUSE OF THE INCLUSION OF PARTICIPANTS WHO DID NOT HAVE ANY OF THOSE RISK FACTORS – JUST SEVERE OSAS - SO AS TO TEST IF OSAS WAS AN INDEPENDENT RISK FACTOR FOR DIASTOLIC DYSFUNCTION. OVERALL, IT IS WELL DONE.

Reply: Thank you for your thorough review and your helpful comments.

THE INTRODUCTION AND METHODS SECTIONS INCLUDING STATISTICS SEEM APPROPRIATE.

Reply: Thank you again for your valuable comments.

THE DISCUSSION SECTION NEEDS ATTENTION TO SOME POSSIBLE LIMITATIONS.

Reply: A relative paragraph has been added in the text ("Discussion" section) according to your useful recommendation (page 18, paragraph 1, lines 1-19).

THE MAJORITY OF PARTICIPANTS WITH EVEN VERY SEVERE OSAS HAD MILD AND NOT MODERATE OR SEVERE DIASTOLIC DYSFUNCTION WHICH IS A DISTINCTION THAT SHOULD BE HIGHLIGHTED/DISCUSSED.

Reply: We certainly agree that the majority of our patients with severe OSAS demonstrated mild rather than more severe degrees of diastolic dysfunction, as the latter was diagnosed and graded according to the stepwise approach proposed by recent 2016 ASE/EACVI recommendations [Nagueh SF. J Am Soc Echocardiogr. 2016;29(4):277-314.]. More severe degrees of diastolic dysfunction have been previously reported in patients with other comorbidities/risk factors for the development of diastolic dysfunction (i.e. Wachter R et al. Eur Respir J. 2013;41(2):376-383); however, such patients were meticulously excluded in our series. Interestingly, in other highly-selected OSAS populations without cardiovascular comorbidities (Arias MA. Circulation. 2005;112(3):375-383), impaired relaxation was the dominant pattern of diastolic dysfunction, in line with our report. Our findings may also highlight that diastolic dysfunction secondary to OSAS per se is possibly modifiable in nature and reversible if treated in early stages. In light of your useful comment, the issue is discussed in the text now ("Discussion" section, page 14, paragraph 2, lines 20-25, page 15, paragraph 2, lines 22-25, page 16, paragraph 1, line 1, and page 18, paragraph 2, lines 22-25).

PATIENTS WITH SEVERE OSAS AND NO CV RISK FACTORS ARE NOT COMMON. DISCUSS IF THIS IS RELEVANT TO THE FINDINGS OR RELEVANT TO THE DISTINCTION BETWEEN MILD AND SEVERE DIASTOLIC DYSFUNCTION.

Reply: You are right that only patients with severe OSAS but with no additional risk factors predisposing to LV diastolic dysfunction were included in our study; hence, we agree that our population could be considered as quite uncommon. One could argue that the synthesis of our population may have interfered with our findings, including the high prevalence of mild diastolic dysfunction in this specific population. We certainly acknowledge this skepticism. Thus, it is imperative that our findings be validated in similar comorbidity-free populations in larger scale studies in the future. This is clearly stated in the text now ("Discussion" section, page 18, paragraph 1, lines 7-8 and 10-12).

STUDY WAS IN ALL MEN, MEAN AGE IN THEIR 40'S. IT WAS UNKNOWN HOW LONG THEY HAD OSAS PRIOR TO DIAGNOSIS WHICH COULD AFFECT OSA SEVERITY - AND IF IT HAD GONE ON LONG ENOUGH TO DEVELOP LV DYSFUNCTION. THE TITLE OF THIS PAPER SHOULD BE "DIASTOLIC DYSFUNCTION IN MEN WITH SEVERE…" THE FINDINGS ARE NOT GENERALIZABLE TO WOMEN WITH SEVERE OSAS AS NONE WERE INCLUDED IN THE SAMPLE. WHILE NOT ABUNDANT, THERE IS EVIDENCE THAT WOMEN'S OSAS CV OUTCOMES ARE DIFFERENT THAN MEN'S OUTCOMES.

Reply: You are right that all patients with severe OSAS included in the present study were middle-aged men. However, in previous reports having enrolled OSAS patients without cardiovascular risk factors, diastolic dysfunction was also prominent in male sex (Arias MA. Circulation. 2005;112(3):375-383). Nevertheless, we certainly agree that our findings should not be generalized in women, as none were included in the sample. Of course, genetic differences may exert diverse influence on the clinical phenotypes of special diseases in different populations; thus, it is imperative that our findings be re-evaluated in similar comorbidity-free populations, yet in larger-scale studies in the future. A relative comment has been added in the text accordingly ("Discussion" section, page 18, paragraph 1, lines 7-19). We also agree that the natural history of OSAS prior to the development of clinically severe syndrome is largely undetermined in our patients, while our OSAS subset also lacks information regarding the clinical course of LV diastolic dysfunction. We certainly acknowledge that the topic requires further investigation in the future, and this is underlined in the text now ("Discussion" section, page 18, paragraph 1, lines 13-19). Finally, in light of your useful comment, the title of this paper was now modified accordingly (from "Diastolic dysfunction in severe obstructive sleep apnea syndrome patients without cardiovascular or oxidative stress-related comorbidities" to "Diastolic dysfunction in men with severe obstructive sleep apnea syndrome but without cardiovascular or oxidative stress-related comorbidities").

Thank you for the thorough and helpful reviewing you made. We hope that we have adequately responded to your excellent comments.

J. Papanikolaou

We thank you again for the opportunity to improve the manuscript. Having made this revision, we hope that the manuscript would be acceptable for publication at the Therapeutic Advances in Respiratory Disease in its current version.