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Effects of obesity on the association between long term sleep apnea treatment and changes in interleukin-6 levels: the Icelandic Sleep Apnea Cohort

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SUMMARY

The aim of this study was to evaluate changes in interleukin-6 and soluble interleukin-6 receptor levels in obstructive sleep apnea patients and assess the role of positive airway pressure treatment and obesity on these changes. A total of 309 newly diagnosed subjects with sleep apnea from The Icelandic Sleep Apnea Cohort, referred for treatment and re-assessed at a 2-year follow-up. Full treatment was defined objectively as use 4 hours/day and 20 days/month. At the 2-year follow-up, there were 177 full users, 44 partial users and 88 non-users. The mean change in biomarker levels from baseline to the 2-year follow-up was assessed in a primary model that included adjustment for baseline biomarker levels, baseline body mass index and change in body mass index, as well as after adjustment for numerous relevant covariates. No significant overall difference in interleukin-6 level change was found among full, partial and non-users. However, in severely obese patients (body mass index ≥ 35), a significant increase in interleukin-6 levels over the 2 year period was found in partial and non-users, compared to no change in full users. Results were attenuated in a smaller propensity score matched subsample, although similar trends were observed. No differences were found in soluble interleukin-6 receptor levels between full users and non-users, after adjustment for confounders. In conclusion, among untreated obese sleep apnea patients, interleukin-6 levels increase substantially over 2 years, while adherence to positive airway pressure treatment may prevent further increases in this inflammatory biomarker.

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Keywords

sleep apnea; obstructive; obesity; continuous positive airway pressure; inflammation; IL-6; soluble IL-6 receptor levels

INTRODUCTION

Untreated obstructive sleep apnea (OSA) is associated with an increased risk of cardiovascular events and mortality that is reduced with positive airway pressure (PAP) treatment (Marin et al., 2005, Campos-Rodriguez et al., 2012, Yaggi et al., 2005). However, some untreated OSA patients appear to be protected against cardiovascular consequences (Arnardottir et al., 2009, Pack and Gislason, 2009). To understand this inter-individual difference, it is important to study the common pathophysiological mechanisms of OSA that are also implicated in cardiovascular disease, for example, chronic inflammation (Heymans et al., 2009, Goldstein et al., 2011). Furthermore, the role of obesity, which often co-exists with OSA (Arnardottir et al., 2009) and is associated with chronic inflammation, deserves specific attention as a risk factor (Van Gaal et al., 2006).

Interleukin-6 (IL-6) is a cytokine associated with inflammation (Ikonomidis et al., 2008), cardiovascular disease (Ikonomidis et al., 2008), OSA (Arnardottir et al., 2009) and obesity (Bastard et al., 2006). IL-6 is one measure of systemic inflammation and is produced by many types of cells, with 15-30% of circulating IL-6 levels coming from adipose tissue (Bastard et al., 2006). When produced by T cells and macrophages, IL-6 is involved in the regulation of the acute phase response, the immune response, hematopoiesis, and inflammation (Mihara et al., 2012). IL-6 release has been found to be higher in visceral than subcutaneous fat (Fried et al., 1998, Fain et al., 2004) and its effect both locally and systemically is the subject of active investigation (Trayhurn et al., 2008). IL-6 becomes biologically active by binding to the IL-6 receptor. IL-6R has two forms: (1) membrane bound (mIL-6R), on the membranes of hepatocytes and leukocytes, including macrophages (Mihara et al., 2012), and (2) soluble (sIL-6R), secreted by monocytes, hepatocytes and endothelial cells (Naka et al., 2002).

The literature on the role of OSA and PAP therapy on IL-6 levels is mostly based on small non-randomized studies with short follow-up times. These studies show conflicting results, as indicated in two recent meta-analyses (Baessler et al., 2013, Xie et al., 2013). Our previous study (Arnardottir et al., 2012) showed an association between OSA severity and increased IL-6 levels in the most obese patients. The association was not seen in non-obese patients, suggesting OSA has a more pronounced contribution to systemic inflammation (as measured by IL-6) and associated cardiovascular risk in obese patients. Given this interaction between obesity and OSA, we hypothesized that PAP therapy for OSA would reduce IL-6 levels in obese subjects, but not among the non-obese. There are no available studies assessing the effect of PAP therapy on sIL-6R levels.

Thus, the aim of this study was to evaluate changes in IL-6 and sIL-6R levels in OSA patients over 2 years in a large cohort of “real life” OSA patients. Included patients had various comorbidities and a wide range of obesity levels, allowing us to assess the

interaction of OSA and obesity on the effect of long term PAP treatment on inflammatory biomarkers.

METHODS

OSA cohort

OSA patients were those from the Icelandic Sleep Apnea Cohort (ISAC) who completed two-year follow-up as of June 2011 (Figure 1). Additional information about ISAC has been presented recently (Arnardottir et al., 2012, Arnardottir et al., 2013). For this study, we selected subjects with: (1) a sleep study before starting PAP with a recorded apnea-hypopnea index (AHI) and oxygen desaturation index (ODI, number of desaturations/hour 4%); (2) baseline abdominal magnetic resonance imaging (MRI); (3) fasting morning blood samples while untreated and at follow-up; (4) objective PAP compliance data at follow-up or no PAP use. Participants were stratified by baseline BMI into three groups: <30, 30–<35, 35 kg/m². This study was approved by the National Bioethics Committee, the Data Protection Authority of Iceland and the Institutional Review Board of the University of Pennsylvania. Written consent was obtained from all subjects.

Questionnaires and measurements

A core questionnaire was answered by subjects at baseline and two-year follow-up. Height and weight were measured in the same manner for all participants. Blood was drawn in the morning after sleep from the antecubital vein of fasting untreated participants. All subjects underwent MRI of the whole abdomen using a 1.5T scanner (Siemens Avanto, Germany) at baseline. Prior to referral for PAP treatment, all ISAC subjects had a type 3 sleep study with an Embletta type 3 portable monitor, an Embla 12 channel system (Natus Medical Inc., Ontario, Canada) or a T3 device (Nox Medical, Reykjavik, Iceland). The same signals were recorded on all studies and subjects with moderate-to-severe OSA (defined as AHI ≥ 15) were referred to PAP treatment. For further details, see supplement and previous publications (Arnardottir et al., 2012, Arnardottir et al., 2013).

PAP use

PAP adherence at follow-up was objectively measured by downloading the mask-on time stored by the PAP unit in the previous 4 weeks (ResMed S8 machines). Participants who used PAP for ≥ 20 days and ≥ 4 hrs/day on average for the previous four weeks, were considered full users. PAP users not meeting these criteria were classified as partial users. Non-users were defined as those who had returned their PAP device within one year of therapy initiation and did not undergo upper airway surgery. For further details, see supplement.

Biomarker assessment

Blood was collected in SST vacutainers (Greiner, Kremsmünster, Austria) and allowed to clot at room temperature for 20 minutes before centrifugation for 10 minutes at 3,000 rpm. After separation, the serum samples were stored at -20°C. A high sensitivity enzyme-linked immunosorbent assay was used to determine serum IL-6 levels (R&D Systems, Minneapolis, MN) and sIL-6R levels (R&D Systems, Minneapolis, MN). Assays were

performed according to the recommendations of the manufacturer. CVs were all within 10%. One subject with an sIL-6R increase of 132.454 ng/mL from baseline to the 2 year follow-up (27% larger than the next largest change) was excluded as an outlier from the sIL-6R assessment.

Statistical analysis

For bivariate analyses, the chi-square or Fisher's exact test and t-test were used for nominal and continuous variables, respectively. Continuous variables were summarized using means $\pm$ standard deviations and nominal variables using frequencies and percentages. The primary models assessed within subject two-year changes in IL-6 and sIL-6R, adjusted for baseline BMI, change in BMI and baseline biomarker level. Differences in change scores among PAP usage groups were assessed using an analysis of covariance (ANCOVA). In addition to the primary models, we assessed the impact of various covariates at baseline and follow-up by adding them individually to the models and examining the changes in results. Specifically, the following covariates were controlled for by including baseline levels and change scores (for continuous variables) or dummy variables representing status at baseline and follow-up (i.e., 0/0, 0/1, 1/0 and 1/1) for binary variables: current smoking status, pack-years of smoking, cardiovascular disease (doctor diagnosis of coronary artery occlusion [myocardial infarction or heart attack], heart failure and/or stroke), use of lipid-lowering medication, medically treated hypertension, medically treated diabetes, excessive alcohol use and exercise participation. For a participant to meet the criteria of medically treated hypertension or diabetes, they must 1) have a self-reported previous diagnosis of the disease and 2) be currently taking medication for the condition. Given this, any change in the prevalence of both medically treated hypertension and diabetes between baseline and follow-up is a result of changes in reported conditions/medications at follow-up. In addition, we examined the impact of the following variables, which were available or appropriate to control for at baseline only: age, gender, AHI, ODI, minimum oxygen saturation, hypoxia time, visceral abdominal fat volume and visceral to subcutaneous fat volume ratio. Finally, we examined complete models for within subject two-year changes in IL-6 and sIL-6R that included all of covariates assessed individually above. Results are presented as predicted least square mean change $\pm$ standard error. A $p < 0.05$ was considered statistically significant. If a difference was observed among PAP groups, pairwise comparisons were performed with Tukey-Kramer adjustment for multiple comparisons. As a complementary analysis, we tested whether there is a linear trend, i.e. an association between biomarker change and PAP usage as an ordinal variable (1=full user, 2=partial user, 3=non-user). A significant trend implies the difference between full and partial users is similar in magnitude and direction to that between partial and non-users. Statistical analyses were performed using STATA, Version 12.0 (StataCorp, College Station, TX) or SAS Software, Version 9.3 (SAS Institute, Cary, NC).

Propensity Score Analysis

To further control for potential bias due to covariate imbalance in our observational study, we performed a complementary analysis using sub-classification by propensity score (PS) quintiles. We note that the importance of such methodologies within the context of

observational studies has been recently highlighted by a working group from the National Heart Lung and Blood Institute (Lieu et al., 2011). Specifically, using a sequential heuristic described previously (Maislin and Rubin, 2010), a subset of full and non-users were identified such that there was within subclass balance with respect to relevant measured covariates at baseline including: age, gender, BMI, visceral abdominal fat volume, visceral to subcutaneous fat volume ratio, smoking status (never, past or current), pack-years of smoking, medically treated hypertension, cardiovascular disease, statin medication use, medically treated diabetes, obstructive lung disease, exercise participation, excessive alcohol use, Epworth Sleepiness Scale (ESS), sleep medication use, OSA severity (AHI, ODI, minimum O₂ saturation, hypoxia time [percentage of sleep time with SaO₂<90]) and baseline IL-6 and sIL-6R levels. See online supplement and previous publications (D'Agostino, 2007, Maislin and Rubin, 2010, Rosenbaum and Rubin, 1983, Rubin, 2001, Ahmed et al., 2006) for additional details.

RESULTS

Characteristics of the study population

The study cohort included 309 subjects, 245 (79.3%) were male (Table 1 and Figure 1). The cohort was divided into BMI strata for analysis. Obese subjects had a higher prevalence of medically treated hypertension, more severe OSA, and were less likely to be current smokers. They had higher baseline IL-6 levels on average compared to non-obese subjects (Table 1).

When the study cohort was divided based on PAP usage at follow-up, there were 177 full users with a mean $\pm$ SD use of 26.5 ± 2.1 days in the previous four weeks and 7.0 ± 1.2 hours/day (across all days), 44 partial users with an average use of 15.6 ± 6.8 days and 4.3 ± 2.1 hours/day, and 88 non-users. On average, PAP users had a higher BMI and higher OSA severity at baseline compared to non-users (Table 2). They were also more likely to have medically treated hypertension and be sleepier. For further details, see supplement.

Change in IL-6 levels with PAP treatment

The predicted mean changes in IL-6 from baseline to the 2-year follow-up in the OSA patients are shown in Table 3 and Figure 2A (adjusted for baseline IL-6 levels, baseline BMI and BMI change). Unadjusted data are shown in the supplement (Table S1). Overall, no significant difference in IL-6 change was found among full, partial and non-users ($p=0.10$). However, there was a significant difference in IL-6 change when looking in BMI strata. Specifically, there is a difference in IL-6 changes in subjects with BMI 30-<35 ($p=0.047$) and BMI ≥ 35 ($p=0.02$), not in the BMI<30 group.

Interestingly, patients with moderate obesity (BMI 30-<35) that were partial users had a significant decrease in IL-6 levels ($p=0.04$), but there was no significant change in full or non-users with a non-significant linear trend between the PAP usage groups ($p=0.79$). Within the most obese group (BMI ≥ 35), there was a significant difference in IL-6 change between full and non-users ($p=0.047$) and a significant linear trend between PAP usage groups ($p=0.008$). Specifically, the most obese full users had no increase in IL-6 after 2

years of PAP treatment (+0.13 pg/ml, $p=0.61$) compared to an increase in IL-6 in partial users (+1.29 pg/ml, $p=0.02$) and an even larger IL-6 increase in non-users (+1.54 pg/ml, $p=0.004$). Therefore, the relationship between IL-6 change and PAP usage was moderated by obesity, with the largest difference found among the most obese patients.

Potential confounders were assessed by adding variables individually to the model (see detailed list in methods) and did not change the findings. did not change our findings. Finally, a model fully adjusted for the same potential confounders, is shown in Table 4. The results found were similar to the primary model, with more statistically significant results for the BMI 35 subgroup, i.e. a $p=0.005$ for among group comparisons ($p=0.02$ in our primary model) and a $p=0.001$ for a linear trend ($p=0.008$ in our primary model). This increased statistical significance was driven by a larger magnitude difference in IL-6 change between the full and non-users ($p=0.008$).

Given the observed relationship between PAP usage and IL-6 changes in patients with BMI 35, we assessed whether there was a relationship between continuously measured PAP usage and IL-6 change in PAP users only. Within this obesity group, each additional hour of mean PAP use was associated with a 0.4 pg/mL decrease in IL-6 changes ($p=0.002$), after adjustment for baseline IL-6, baseline BMI and BMI change.

Lastly, associations were made between baseline OSA severity, BMI and IL-6 level changes among PAP non-users. No associations were found overall after adjusting for baseline IL-6, BMI and BMI change. However, within the most obese group, IL-6 change was associated with AHI (+0.058 pg/mL, $p=0.03$) and ODI (+0.064 pg/mL, $p=0.006$), such that more severe baseline OSA demonstrated a larger IL-6 increase over the two-year follow-up period.

Change in sIL-6R levels with PAP treatment

A significant difference in sIL-6R between PAP usage groups was found in unadjusted data, with a larger increase in sIL-6R values over the 2 year period in partial and full users than nonusers, within all BMI groups (Table S1). After adjustment for baseline sIL-6R levels, baseline BMI and BMI change, a significant difference among PAP usage groups remained overall ($p=0.04$), but not within the BMI groups (Table 3 and Figure 2B). The sIL-6R differences between PAP groups appear to be driven by partial users; as there was no significant differences found between full and non-users ($p=0.97$). We observed no significant interaction between PAP usage and BMI group ($p=0.46$). The effect of confounders was assessed similarly as for IL-6 and did not change the findings. A model fully adjusted for the individual covariates showed similar results (Table 4).

Analyses Based on Sub-Classification using Propensity Scores (PS)

As described in the *Methods*, sub-classification by PS quintiles was used to minimize bias due to imbalance in measured covariates in our non-randomized group comparison. We note that the PS analyses were limited to full and non-users since (1) the partial user group was too small to obtain an adequate PS overlap and (2) this was our key comparison of interest to estimate a true treatment effect. In order to obtain an adequate PS designed sample, a total of 97 full and 24 non-users were excluded from the analysis, resulting in a final PS sample of

80 full users and 64 non-users. Differences in included and excluded patients are important to consider when generalizing the results in the PS sample (see Table S2). The excluded full users had more severe obstructive sleep apnea, higher levels of obesity, and were more likely to have diabetes and hypertension compared to full users included in the PS sample (Table S2). In addition, full users that were excluded had higher IL-6 and lower sIL-6R levels at baseline than those included. Excluded non-users had less severe OSA on average compared to non-users retained in the final PS sample. Figure 3, a modified *Love Plot* (Ahmed et al., 2006), illustrates the covariate balance resulting from the PS methodology. In the original sample, there were significant differences between full and non-users for 9 covariates, including obesity measures, OSA severity, and sIL-6R. In the PS designed sample, after adjusting for PS sub-class, there were no differences in any of the baseline covariates. Balance in these measured covariates was therefore similar to that expected through randomization, minimizing selection bias and allowing for causal inferences from our non-randomized group comparisons (D'Agostino, 2007, Maislin and Rubin, 2010, Rosenbaum and Rubin, 1983, Rubin, 2001, Ahmed et al., 2006, particularly Maislin & Rubin, 2010).

IL-6

Overall, there was no significant IL-6 change between full and non-users in either the observational or PS designed analysis (Table S3). The most obese patients (BMI ≥ 35), had an IL-6 change between full and non-users that was significant in the observational analysis. Within this group, we see that the magnitude of the difference in IL-6 change between full and non-users was reduced in the PS subsample (-0.65 pg/ml; $p=0.48$) compared to the original result (-1.41 pg/ml; $p=0.047$) and the result was no longer significant (Table S3). However, although absolute differences were not significant, both the observational and PS analysis demonstrated more than a three-fold increase in IL-6 change between full and non-users in the most obese patients compared to other BMI groups (In PS designed analysis: -0.65 pg/ml for BMI ≥ 35 group vs. -0.20 pg/ml for all BMI groups). Thus, while the result were no longer significant in the PS sample, the relationship seen in the observational sample is unlikely to be driven solely by covariate imbalance.

sIL-6R

For sIL-6R, we did not observe significant differences in the level of change between full and non-users in either the observational analysis or PS analysis, overall or within BMI subgroups (Table S3).

DISCUSSION

We report that severely obese (BMI ≥ 35) OSA subjects who are non-compliant with PAP treatment demonstrate a significant increase in IL-6 levels over 2 years, whereas those that were compliant with PAP treatment maintain their IL-6 levels. We see no clear relationship between use of PAP therapy and changes in sIL-6R levels.

Increases in IL-6 levels without PAP treatment in the most obese

Since obesity is the most important risk factor for OSA (reviewed in (Arnardottir et al., 2009)), studying their independent effects on other chronic diseases like cardiovascular disease is not only critical, but also challenging. We have previously shown in a cross-sectional analysis that OSA severity is an independent predictor of IL-6 and CRP levels, but this association is found only in obese patients (Arnardottir et al., 2012). Now we show that PAP treatment may halt the progression of IL-6 increases in patients with BMI ≥ 35 . That is, if obesity is held constant over 2 years, untreated OSA patients (e.g. noncompliant with PAP) will have an additional increase in IL-6 levels, and those where breathing during sleep is normalized (e.g. compliant with PAP) will have no further increases. Similar results were observed in our recent publication examining changes in intercellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule 1 (VCAM-1) levels (Pak et al., 2014). For ICAM-1, the largest effect of PAP was observed in the patients with BMI ≥ 35 kg/m², with non-users showing increased levels after 2 years, compared to a small decrease in full users. For VCAM-1, full PAP usage limited increases overall, with non-users showing a more than three-fold larger VCAM-1 increase after 2-years compared to full-users.

The primary result in our observational study was likely partially driven by selection bias due to baseline covariate imbalance. Our smaller, purposefully designed observational study using propensity scores, showed attenuated results, although similar trends were observed. However, we note that a model adjusted for a large number of potential confounders in the observational sample confirmed the findings of the primary model. This suggests that covariate imbalance alone does not drive the results in the most obese group. Larger randomized trials focusing on this obesity group could detect a significant relationship at the levels observed in our PS sample. Regardless, results from both analyses suggest that obese OSA patients compliant with PAP have the greatest treatment effect, e.g. inflammatory response as measured by IL-6 levels, which may translate to a reduction in OSA related cardiovascular events compared to nonusers with increased inflammatory levels.

This 2 year follow up observational study can be compared to two recent meta-analyses, one showing borderline significant improvement in IL-6 levels with PAP treatment (Baessler et al., 2013) and the other non-robust significant, improvement in IL-6 levels with PAP treatment ($p=0.049$) (Xie et al., 2013). Most of the studies in the meta-analyses were low-level evidence, with less than 50 subjects per study short follow-up period of 4-6 weeks. Only one study was a randomized controlled trial (RCT) showing no IL-6 changes after 4 weeks of PAP treatment (Kohler et al., 2009). The Xie et al. subgroup analysis showed a larger benefit within the Asian population. Unlike our study, the Xie et al. meta-analysis did not observe a significant heterogeneity due to BMI despite inclusion of obese subjects. A subgroup analysis within different BMI strata in Asian vs. non-Asian populations would be of considerable interest. This lack of change in inflammatory markers is also consistent with the results of the recent Chirinos et al., paper which found no reduction in C-reactive protein with CPAP treatment, but did with weight loss (Chirinos et al., 2014). Since the Chirinos study a) did not include a group of non-users who did not undergo a weight-loss protocol and b) did not directly examine patients with BMI ≥ 35 (where we see the strongest effect of

PAP), it is unclear whether a similar result to that in our study would have been found for inflammatory markers as in our study.

It is important to consider why we see the largest increase in IL-6 levels in obese untreated OSA patients. The expanding adipose tissue in the obese, already hypoxic during normal breathing (Trayhurn, 2013), most likely becomes more hypoxic when exposed to cycling oxygen levels during OSA, possibly leading to a chronic increased production of IL-6. Thus, treatment with PAP blunts this response, which is consistent with no further increases in IL-6 in obese OSA compliant PAP patients at 2 years. This likely results from the effects of cyclical intermittent hypoxia (CIH) produced by OSA on the increased number of adipocytes and resident macrophages in obese individuals (Fain, 2006, Hajer et al., 2008). The mechanism of how CIH results in elevated IL-6 in the obese can be in part answered by studies performed in mice. PO₂ measurements in adipose tissue of mice exposed to CIH demonstrate very small oscillations of PO₂, such that adipose tissue remains continuously hypoxic during the period of CIH (Reinke et al., 2011, Almendros et al., 2011). This sustained hypoxia is more pronounced in obese mice than in lean and is likely the result of adipose tissue having limited blood flow (Reinke et al., 2011, Almendros et al., 2011). When adipose tissue is exposed to hypoxia, there is an increase in stabilization of hypoxia inducible factor 1 α (Hif1 α) (Wang et al., 2007, Sun et al., 2013) leading downstream to an increase in interleukin-6 expression (Kim et al., 2010).

Unclear relationship between sIL-6R levels and PAP treatment

A previous study (Mehra et al., 2006) found that sIL-6R levels increased after one night of sleep disordered breathing, with the degree of increase being associated with increased OSA severity. There was no association with IL-6. We found sIL-6R levels increased in full and partial users after two years when using unadjusted values (Table S1). This difference in sIL-6R changes remains significant after adjustment for baseline sIL-6R levels, BMI and BMI change ($p=0.04$). However, the association is unclear in that this sIL-6R change is largely driven by the partial users. Adjusted sIL-6R levels demonstrated no differences between full and non-users. Reasons for the large increase in sIL-6R in partial users are unclear; additional studies are needed to better understand pathways and regulation. Therefore, while our findings are not consistent with the Mehra et al. study, more research is needed to determine the relationship between PAP and sIL-6R including the potential for differences in acute vs. chronic effects.

Strengths and limitations of the study design

ISAC is an observational study, not an RCT, which may be considered a limitation. However, an RCT study of PAP treatment with long-term follow-up of severely affected OSA patients, such as the group we studied, would be difficult to perform for ethical reasons. Moreover, potential bias was addressed through a complementary analysis involving sub classification using propensity scores, which creates balance for measured covariates similar to that expected in an RCT. A limitation of propensity score methods is the inability to directly control for unmeasured or unknown confounders, which are theoretically controlled for in an RCT. However, unmeasured variables are indirectly controlled for in the PS analysis to the extent that they are correlated with included

covariates; we added as many relevant variables as possible to the design to limit the potentially for missing important independent confounders. In addition, the scope of the inference for the unbiased comparison in the propensity score analysis is smaller than for the observational cohort, as more obese full users were excluded.

ISAC includes a smaller number of females compared to males, a consequence of the Icelandic patients in whom OSA was diagnosed. However, our study does include a higher proportion of females than 16 studies in a recent meta-analysis (Xie et al., 2013) on the effects of PAP treatment on IL-6 levels (20.7% in our study vs. 7.1%). The ISAC cohort did not include mild OSA patients because, generally, these patients are not prescribed PAP treatment in Iceland. Therefore, we can only assess the role of PAP treatment for patients with moderate-to-severe OSA.

Another potential source of bias in the PAP effect is the possibility that patients adherent to PAP therapy are also more adherent to other medications. While we recognize this possibility, we note that the relationship between PAP adherence and medication compliance is unclear. One previous study found no difference in adherence to a number of cardiovascular medications and persistence over 2 years between CPAP adherent users and non-users (Villar et al., 2009), while another observed a relationship between more adherence to lipid-lowering medications and higher probability of CPAP compliance ($p=0.04$), although this relationship became non-significant after adjusting for marital status ($p=0.12$) (Platt et al., 2010). Although patients were treated with PAP over two years, adherence information was obtained over the period closest to follow-up. This was part of the cohort design, and chosen since adherence information most proximal to follow-up is believed to be the most relevant and the fact that several studies have shown a significant association between early and long-term PAP compliance suggests levels of compliance near the two-year follow-up are likely representative of overall adherence (Weaver et al., 1997, Weaver and Grunstein, 2008, McArdle et al., 1999, Budhiraja et al., 2007).

Finally, our study included assessment of OSA severity at baseline without EEG recording; therefore arousal-related events were not assessed. OSA severity was reassessed only in a portion of the untreated patients at follow-up; no significant decrease in OSA severity was found. This is not surprising given what we know about the very small changes in OSA severity over a period of a few years if weight remains constant (Kuna et al., 2013, Peppard et al., 2000).

The strengths of the ISAC study include the relatively large number of participants, the broad distribution of obesity, and the detailed analysis performed in each subject, which allows for assessment of many potential confounders. This allows us to examine PAP treatment effects separately in overweight, obese, and severely obese patients. The ISAC cohort is a clinical cohort with “real life” patients and no exclusion of comorbidities. We consider this a major strength because investigation of only “healthy” patients with OSA, as often is done in RCTs, could result in study bias. Finally, a rigorous statistical technique employing sub-classification by propensity scores was used to assess the impact of selection bias on the findings from the observational cohort, allowing for stronger causal inferences to be made from our results.

CONCLUSIONS

PAP treatment for sleep apnea may prevent increases in IL-6 in the most obese (BMI 35) OSA patients over two years, which would otherwise occur if left untreated or patients are noncompliant. Our study adds support to the concept that inflammatory consequences, as measured by IL-6 levels, occur in obese OSA subjects, but not in those less obese. This has important implications for future clinical trials with cardiovascular end-points, suggesting such trials should be stratified by the degree of obesity.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.
Flow chart of study cohort.

Figure 2. Adjusted mean change in A) IL-6 and B) sIL-6R levels from baseline in entire population and within BMI groups

The predicted least squares mean change in IL-6 and sIL-6R levels for each PAP usage group (full, partial and non-PAP users) are presented in the overall population, and in the three BMI groups, along with the associated 95% confidence intervals. Results are adjusted for baseline IL-6 and sIL-6R levels, respectively as well as BMI and change in BMI. Estimates with 95% confidence intervals not crossing 0 represent significant changes within PAP group: ★ $p < 0.05$.

Abbreviations: LS; least squares, IL-6; Interleukin-6, sIL-6R; soluble IL-6 receptor, BMI; body mass index, PAP; positive airway pressure.

Figure 3. Modified ‘Love Plot’ illustrating the bias reduction for each covariate in propensity score (PS) matched sample

The test statistics (T-statistics for continuous variables and signed square root of the chi-square statistics for categorical variables) comparing covariates between full and non PAP users are shown both in the original sample and after PS matching and quintile adjustment. While there were significant differences in covariates in the original sample, after PS matching all statistics are close to zero, indicating balance between full and non-users for all relevant covariates.

Abbreviations: IL-6; Interleukin-6 levels, sIL-6R; soluble IL-6 receptor levels, SaO2; oxygen saturation; ODI; Oxygen Desaturation Index, AHI; Apnea-Hypopnea Index, ESS; Epworth Sleepiness Scale, BMI; body mass index, Obstructive Lung Dx; presence of obstructive lung disease, CVD; presence of cardiovascular disease.

Table 1

Characteristics of the whole study cohort at baseline and by a comparison of the three BMI groups.

Characteristic	Overall (n=309)	BMI <30 kg/m ² (n=111)	BMI 30-35 kg/m ² (n=110)	BMI ≥35 kg/m ² (n=88)	p-value*
Male gender	79.3%	82.9%	81.8%	71.6%	0.11
Age (years)	55.4 ± 9.6	54.9 ± 8.9	57.3 ± 10.4	53.7 ± 9.1	0.03
Body mass index (kg/m ²)	32.4 ± 5.1	27.3 ± 2.1	32.5 ± 1.5	38.7 ± 3.0	< 0.0001
Total abdominal fat (cm ³)	13,030 ± 4,554	8,917 ± 2,454	13,296 ± 2,566	17,886 ± 3,470	< 0.0001
Subcutaneous abdominal fat (cm ³)	7,826 ± 3,298	5,087 ± 1,454	7,650 ± 1,899	11,502 ± 2,833	< 0.0001
Visceral abdominal fat (cm ³)	5,204 ± 2,173	3,830 ± 1,589	5,646 ± 1,916	6,384 ± 2,203	< 0.0001
Medically treated hypertension	44.0%	30.6%	47.3%	56.8%	0.0007
Medically treated diabetes	5.2%	3.6%	3.6%	9.2%	0.14
Cardiovascular disease	14.6%	11.7%	15.5%	17.2%	0.52
Current smoker	17.9%	26.1%	15.5%	10.3%	0.01
Apnea-hypopnea index (events/hr)	45.0 ± 20.2	39.1 ± 17.3	47.0 ± 19.5	49.8 ± 22.9	0.0004
Oxygen desaturation index (events/hr)	35.8 ± 20.2	28.6 ± 14.6	37.2 ± 18.9	43.3 ± 24.5	< 0.0001
Minimum SaO ₂ (%)	76.7 ± 7.8	79.2 ± 5.6	77.2 ± 7.0	73.0 ± 9.5	< 0.0001
Hypoxia time % (% time SaO ₂ <90%)	12.6 ± 17.1	7.0 ± 10.2	13.1 ± 16.3	19.0 ± 22.1	< 0.0001
Epworth Sleepiness Scale score	12.0 ± 4.9	12.1 ± 4.7	11.5 ± 4.9	12.5 ± 5.1	0.33
IL-6 levels (pg/ml) [†]	2.4 ± 1.8	1.8 ± 1.1	2.7 ± 2.2	2.9 ± 1.8	< 0.0001
sIL-6R (ng/ml)	42.9 ± 18.0	42.9 ± 18.9	41.6 ± 17.7	44.7 ± 17.4	0.57

Data are presented as mean ± standard deviation or % as indicated.

Abbreviations: BMI; body mass index; IL-6; interleukin-6, sIL-6R; soluble interleukin-6 receptor.

* p-value from ANOVA (continuous variables) or chi-square or exact test (categorical variables) testing whether there were differences between BMI groups, significant findings, (p < 0.05) are shown in bold.

[†] p-value is for natural log transformed data.

Table 2

Characteristics of the study cohort at baseline and follow-up, stratified by PAP usage.

Characteristic	Baseline				2-year follow-up			
	Full User (N=177)	Partial User (N=44)	Non User (N=88)	* p	Full User (N=177)	Partial User (N=44)	Non User (N=88)	* p
Male gender	80.2%	81.8%	76.1%	0.67	--	--	--	--
Age (years)	55.2 ± 10.1	56.5 ± 9.0	55.4 ± 8.9	0.74	57.2 ± 10.1	58.5 ± 9.1	57.4 ± 8.9	0.73
Body mass index (kg/m ²)	33.2 ± 4.9	32.8 ± 5.1	30.5 ± 4.8	0.0001	34.0 ± 5.0	32.8 ± 5.3	30.8 ± 4.9	<0.0001
Total abdominal fat (cm ³)	13,586 ± 4,415	14,238 ± 4,621	11,308 ± 4,368	0.0001	--	--	--	--
Subcutaneous abdominal fat (cm ³)	8,168 ± 3,343	8,358 ± 3,240	6,873 ± 3,065	0.005	--	--	--	--
Visceral abdominal fat (cm ³)	5,418 ± 2,113	5,880 ± 2,433	4,435 ± 1,956	0.0002	--	--	--	--
Medically treated hypertension	48.6%	59.1%	27.3%	0.0004	47.2%	54.0%	31.8%	0.02
Medically treated diabetes	6.3%	4.5%	3.4%	0.62	6.9%	4.5%	2.3%	0.29
Cardiovascular disease	13.1%	15.9%	17.0%	0.67	13.6%	15.9%	17.0%	0.75
Current smoker	17.6%	13.6%	20.5%	0.62	14.7%	18.2%	21.6%	0.37
Apnea-hypopnea index (events/hr)	50.8 ± 20.3	44.4 ± 19.4	33.6 ± 15.3	<0.0001	--	--	--	--
Oxygen desaturation index (events/hr)	41.2 ± 20.7	35.5 ± 19.9	25.2 ± 14.5	<0.0001	--	--	--	--
Minimum SaO ₂ (%)	75.9 ± 7.8	75.1 ± 8.4	79.1 ± 7.0	0.002	--	--	--	--
Hypoxia time % (% time SaO ₂ <90%)	15.4 ± 18.6	13.8 ± 17.6	6.2 ± 11.1	0.0001	--	--	--	--
Epworth Sleepiness Scale score	12.7 ± 4.9	12.4 ± 4.8	10.5 ± 4.7	0.002	8.1 ± 4.5	9.4 ± 5.2	9.0 ± 4.6	0.16
IL-6 levels (pg/ml) [†]	2.4 ± 1.7	2.6 ± 1.9	2.4 ± 2.0	0.53	2.8 ± 2.0	2.5 ± 2.2	3.0 ± 2.2	0.24
sIL-6R (ng/ml)	40.2 ± 18.2	42.6 ± 20.5	48.6 ± 14.7	0.002	48.1 ± 18.0	55.9 ± 20.1	47.7 ± 12.3	0.10

Data are presented as mean ± standard deviation or % as indicated.

Abbreviations: IL-6; interleukin-6, sIL-6R; soluble interleukin-6 receptor, PAP; positive airway pressure.

* p-value from ANOVA (continuous variables) or chi-square or exact test (categorical variables) testing whether there were differences between BMI groups, significant findings (p < 0.05) are shown in bold.

[†] p-value is for natural log transformed data.

Table 3

Effect of PAP adherence on changes in IL-6 and sIL-6R within the observational sample, adjusted for baseline biomarker levels, baseline BMI and change in BMI.

BMI Group	Full Users	Partial Users	Non-Users	[*] p	[†] PTREND
IL-6 change (pg/ml)					
Overall (n=309)	0.27 ± 0.14	0.02 ± 0.28	0.71 ± 0.2 [#]	0.10	0.12
BMI<30 (n=111)	0.58 ± 0.23 [#]	0.02 ± 0.42	0.57 ± 0.24 [#]	0.48	0.96
BMI 30-35 (n=110)	0.16 ± 0.23	-0.99 ± 0.47 [#]	0.43 ± 0.36	0.047	0.79
BMI ≥ 35 (n=88)	0.13 ± 0.26	1.29 ± 0.54 [#]	1.54 ± 0.52 [#]	0.02	0.008
sIL-6R change (ng/ml)					
Overall (n=309)	5.97 ± 1.25 [#]	10.89 ± 2.48 [#]	3.16 ± 1.81	0.04	0.35
BMI<30 (n=111)	6.89 ± 2.21 [#]	11.04 ± 4.12 [#]	4.20 ± 2.35	0.34	0.44
BMI 30-35 (n=110)	7.80 ± 2.15 [#]	8.37 ± 4.42	3.44 ± 3.45	0.54	0.33
BMI ≥ 35 (n=87)	2.47 ± 1.97	13.47 ± 4.20 [#]	2.29 ± 3.91	0.06	0.60

Abbreviations: BMI; body mass index, IL-6; interleukin-6, sIL-6R; soluble interleukin-6 receptor, PAP; positive airway pressure.

^{*} p-value from ANCOVA testing the global null hypothesis of no differences between PAP usage groups and biomarker level change, adjusted for baseline IL-6 or sIL-6r levels, BMI and change in BMI. Results are shown as group specific predicted least square mean change ± standard errors

[†] p-value testing whether there is a linear trend, i.e. an association between biomarker change and PAP usage as an ordinal variable (1=full user, 2=partial user, 3=non-user). Significant findings (p < 0.05) are shown in bold.

[#] within subgroup change score significantly different from zero (p<0.05). Significant findings (p < 0.05) are shown in bold.

Table 4

Effect of PAP adherence on changes in IL-6 and sIL-6R in a fully adjusted model within the observational sample.

BMI Group	Full Users	Partial Users	Non-Users	* p	† PTREND
IL-6 change (pg/ml)					
Overall (n=309)	0.26 ± 0.17	-0.13 ± 0.32	0.79 ± 0.25 [#]	0.08	0.17
BMI<30 (n=111)	0.58 ± 0.26 [#]	0.04 ± 0.48	0.63 ± 0.27 [#]	0.56	0.90
BMI 30-35 (n=110)	0.15 ± 0.30	-1.10 ± 0.60	0.32 ± 0.50	0.15	0.83
BMI 35 (n=88)	-0.05 ± 0.29	1.28 ± 0.66	2.48 ± 0.68 [#]	0.005	0.001
sIL-6R change (ng/ml)					
Overall (n=309)	6.49 ± 1.42 [#]	10.99 ± 2.77 [#]	3.93 ± 2.12	0.14	0.53
BMI<30 (n=111)	9.76 ± 2.90 [#]	12.50 ± 5.22 [#]	3.79 ± 3.07	0.29	0.22
BMI 30-35 (n=110)	6.96 ± 2.72 [#]	8.67 ± 5.53	6.35 ± 4.70	0.95	0.98
BMI 35 (n=87)	3.44 ± 2.35	14.08 ± 5.94 [#]	-2.26 ± 5.36	0.16	0.79

Abbreviations: BMI; body mass index, IL-6; interleukin-6, sIL-6R; soluble interleukin-6 receptor, PAP; positive airway pressure.

* p-value from ANCOVA testing the global null hypothesis of no differences between PAP usage groups and biomarker level change, adjusted for baseline IL-6 or sIL-6r levels, age, gender, BMI and change in BMI, current smoking status at baseline and follow-up, pack-years of smoking and change in pack-years of smoking, medically treated hypertension at baseline and follow-up, exercise participation at baseline and follow-up, visceral abdominal fat volume at baseline, visceral to subcutaneous fat ratio at baseline, baseline apnea-hypopnea index, baseline oxygen desaturation index, baseline minimum oxygen saturation, baseline hypoxia time, lipid-lowering medication use at baseline and follow-up, cardiovascular disease at baseline and follow-up, medically treated diabetes at baseline and follow-up and excessive alcohol use at baseline and follow-up. Results are shown as group specific predicted least square mean change ± standard errors

† p-value testing whether there is a linear trend, i.e. an association between biomarker change and PAP usage as an ordinal variable (1=full user, 2=partial user, 3=non-user)

[#] within subgroup change score significantly different from zero (p<0.05); Significant findings (p < 0.05) are shown in bold.