

# Effects of Oropharyngeal Exercises on Snoring

## A Randomized Trial

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**BACKGROUND:** Snoring is extremely common in the general population and may indicate OSA. However, snoring is not objectively measured during polysomnography, and no standard treatment is available for primary snoring or when snoring is associated with mild forms of OSA. This study determined the effects of oropharyngeal exercises on snoring in minimally symptomatic patients with a primary complaint of snoring and diagnosis of primary snoring or mild to moderate OSA.

**METHODS:** Patients were randomized for 3 months of treatment with nasal dilator strips plus respiratory exercises (control) or daily oropharyngeal exercises (therapy). Patients were evaluated at study entry and end by sleep questionnaires (Epworth Sleepiness Scale, Pittsburgh Sleep Quality Index) and full polysomnography with objective measurements of snoring.

**RESULTS:** We studied 39 patients (age,  $46 \pm 13$  years; BMI,  $28.2 \pm 3.1$  kg/m<sup>2</sup>; apnea-hypopnea index (AHI),  $15.3 \pm 9.3$  events/h; Epworth Sleepiness Scale,  $9.2 \pm 4.9$ ; Pittsburgh Sleep Quality Index,  $6.4 \pm 3.3$ ). Control ( $n = 20$ ) and therapy ( $n = 19$ ) groups were similar at study entry. One patient from each group dropped out. Intention-to-treat analysis was used. No significant changes occurred in the control group. In contrast, patients randomized to therapy experienced a significant decrease in the snore index (snore index  $> 36$  dB/h),  $99.5$  (49.6-221.3) vs  $48.2$  (25.5-219.2);  $P = .017$  and total snore index (total power of snore/h),  $60.4$  (21.8-220.6) vs  $31.0$  (10.1-146.5);  $P = .033$ .

**CONCLUSIONS:** Oropharyngeal exercises are effective in reducing objectively measured snoring and are a possible treatment of a large population suffering from snoring.

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**ABBREVIATIONS:** AHI = apnea-hypopnea index

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OSA is a common condition characterized by recurrent upper airway obstruction during sleep.<sup>1,2</sup> Snoring is one of the most common symptoms associated with OSA and is caused by vibration of soft tissues obstructing the pharynx during sleep.<sup>3,4</sup> Among patients with OSA, snoring is common (70%-95%), and there is an association between snoring intensity and OSA.<sup>5,6</sup> On the other hand, subjects who suffer from snoring do not necessarily have OSA. The prevalence of snoring in the general population varies widely (from 15% to 54%), mainly because most studies rely on subjective reports.<sup>7-10</sup> Self-perception of snoring is imprecise<sup>11</sup> and is largely dependent on subjective reports from bed partners.<sup>12</sup> The social problems caused by snoring are most likely underestimated. Snoring is frequently denied, because it is a stigmatizing symptom that is poorly perceived by the beholder. In addition to the social problems caused by snoring, the vibration of the upper airway associated with snoring may contribute to pharyngeal neurogenic lesion,<sup>13</sup> progression of carotid artery atherosclerosis due to vibration transmitted locally,<sup>7</sup> as well as sleep disruption when associated with respiratory event-related arousal.<sup>14</sup> Despite the evidence that snoring is a major burden to our society, the management of

patients with primary snoring or patients with mild forms of OSA has been poorly investigated.

The treatment of primary snoring varies widely and includes general measurements, such as avoiding alcohol and sedatives, avoiding the supine position, weight reduction, treatment of nasal problems, palate and upper airway surgeries, and use of a mandibular advancement device.<sup>15,16</sup> However, the majority of the studies have not objectively measured snoring, and results are based on subjective questionnaires.<sup>17</sup> Therefore, new forms of treatment of snoring are necessary. Studies show that training the upper airway muscles either by playing a wind instrument (didgeridoo)<sup>18</sup> or oropharyngeal exercises<sup>19</sup> can ameliorate moderate OSA. A recent meta-analysis demonstrated that oropharyngeal exercises provides a reduction in apnea-hypopnea index (AHI) of 50% in adults and decreases snoring.<sup>20</sup> Oropharyngeal exercises are, therefore, an attractive possibility to treat patients suffering from snoring. In the present randomized controlled study, we tested the effects of oropharyngeal exercises on the snoring of minimally symptomatic patients with primary snoring and mild to moderate OSA. In contrast to most studies on this subject, snoring was measured objectively.

## Materials and Methods

### Patients

We considered eligible patients between 20 and 65 years of age referred to the Sleep Laboratory InCor-HCFMUSP, with a primary complaint of snoring and a recent diagnosis of primary snoring or mild to moderate OSA. Patients with BMI  $\geq 40$  kg/m<sup>2</sup>, smokers, history of alcohol abuse, edentulous, severe nasal obstruction, hypertrophic tonsils grade 3 or 4, craniofacial malformations, regular use of hypnotic medications, and severe comorbidities were excluded. The local ethics committee approved the study, and all patients gave written informed consent (CAPPESQ 0140/11).

### Polysomnography

All patients were evaluated by full polysomnography as previously described,<sup>21</sup> with the inclusion of a snore recording. Snore sound was captured by microphone, located at 1 m from the surface of the bed, of a digital audio recorder, ZoomH4n. The clocks of the snoring recorder and the polysomnography computer were synchronized. Since snoring is a predominantly low-frequency sound, a bandpass filter between 80 and 300 Hz was used. Snoring was automatically detected by using an algorithm with an intensity threshold cutoff of 36 dB. The World Health Organization guidelines indicate that indoor continuous sound pressure level above 30 dB should be avoided during sleep.<sup>22</sup> Our threshold was based on pilot studies in our sleep laboratory that evaluated the best threshold to discriminate between snoring and ambient sounds. In addition, all automatically detected snoring sounds were listened to and validated by one single researcher in a blinded fashion (V. I.). Results are expressed as snore index (total number of snores/total sleep time) and total snore index (sound intensity power generated by all snoring episodes/total sleep time, expressed in arbitrary units/10<sup>7</sup>). Primary snoring, mild OSA, and moderate OSA were defined as an AHI  $< 5$ ,  $\geq 5$  and  $< 15$ , and  $\geq 15$  and  $\leq 30$  events/h, respectively. The

investigator who scored the sleep study was blinded to the group allocation. Apnea was defined as the complete cessation of airflow for at least 10 s; hypopnea was defined as a significant reduction ( $> 30\%$ ) in respiratory signals for at least 10 s associated with an oxygen desaturation  $\geq 3\%$ .<sup>23</sup>

### Questionnaires

Snoring of the patient was evaluated by the patient as well as by the bed partner (whenever present) using questions derived from the Berlin questionnaire: snoring frequency (ranging from 0: never, to 4: every day) and snoring intensity (1: similar to breathing, to 4: very loud).<sup>24</sup> Subjective daytime sleepiness and quality of sleep were evaluated with the Epworth Sleepiness Scale questionnaire<sup>25</sup> and Pittsburgh Sleep Quality Index questionnaire,<sup>26</sup> respectively.

### Control Group and Therapy Group

Patients in the control group were instructed to use nasal dilator strips during sleep, to perform nasal lavage with saline solution tid, and to perform deep breathing exercises through the nose while sitting. Patients in the therapy group were instructed to perform nasal lavage three times a day followed by oropharyngeal exercises for approximately 8 min. The oropharyngeal exercises from our previous study<sup>19</sup> were simplified and included the following: (1) push the tip of the tongue against the hard palate and slide the tongue backward (20 times); (2) suck the tongue upward against the palate, pressing the entire tongue against the palate (20 times); (3) force the back of the tongue against the floor of the mouth while keeping the tip of the tongue in contact with the inferior incisor teeth (20 times); (4) elevation of the soft palate and uvula while intermittently saying the vowel "A" (20 times). After gaining control and coordination of movement (typically after 3-5 weeks), elevation of the soft palate and uvula was performed without vocalization for 5 s; (5) recruitment of the buccinator muscle against the finger that is introduced in the oral cavity, pressing the buccinator muscle outward

(10 times each side); and (6) alternate bilateral chewing and deglutition using the tongue in the palate, without perioral contraction, whenever feeding. The patients were instructed to incorporate this mastication pattern whenever they were eating.

### Experimental Design

After fulfilling entry criteria, patients were randomized for 3 months to either control or therapy group. The two groups attended weekly visits. The therapy group performed oropharyngeal exercises under supervision. The control group performed exercises of deep breathing through the nose under supervision. The control group received nasal dilators once a week, and the number of units used in the previous week was counted. All patients were also asked to keep a diary to record compliance to the 8-min set of exercises prescribed three times a day of either oropharyngeal exercises (therapy) or deep breathing exercises (control). The patient had to mark with a pen whether the assigned exercise section for that period of the day was performed ("yes") or not. The diary was returned to the investigator once a week and provided information about patient compliance in the previous week. Compliance was expressed as a percentage and calculated as the number of sections answered with "yes" divided by the total number of sections in the week. Anthropometric measures, questionnaires, and polysomnogra-

phy with recording of snoring were performed at the beginning and end of the study. The primary outcome was snoring analysis as expressed by the snore index and the total snore index.

### Statistical Analysis

Statistical analysis was performed using SPSS 20.0 (IBM Corporation) and R statistic software. Normality was assessed using the Kolmogorov-Smirnov test. We anticipated a 50% reduction in objective snoring in patients randomized to oropharyngeal exercises based on our previous research.<sup>19</sup> We included 38 patients ( $\beta = 80\%$ ,  $\alpha = 95\%$ ). Data are presented as mean and SD or median (25%-75%) percentile when appropriate. Baseline characteristics were compared using two-tailed unpaired *t* tests or Mann-Whitney test when appropriate. Paired *t* test or Wilcoxon test was performed to evaluate within-group changes over the study period. Repeated measures analysis of variance was used to compare the interaction between the two groups (control and therapy) and the two moments (baseline and after 3 months). In addition, we used the generalized estimation equation to determine the influence of the time in a supine position on the results. Comparisons were performed by intention-to-treat analysis. Missing data at study termination were repeated from baseline according to last observation carried forward methods.<sup>27</sup> A value of  $P < .05$  was considered significant.

## Results

We recruited 156 patients and 117 were excluded, leaving 39 patients in the final analysis. The reasons for exclusion were described in Figure 1. One patient in each group withdrew from the study after randomization. The demographic and sleep characteristics and symptoms of the population, according to the group assigned, are presented in Table 1. Patients assigned to control and therapy groups had similar baseline characteristics (Table 1). The demographic characteristics, questionnaires, and polysomnographic and snore characteristics of the patients assigned to control or oropharyngeal exercises at baseline and after 3 months are presented in Table 2. The percentage of adherence to the exercises according to the weekly diaries was  $> 75\%$  for all patients and was on average  $85\% \pm 8\%$ . No changes occurred in the control group in all variables during the study period, except on the subjective frequency of snoring reported by the patient. No changes in BMI or abdominal circumference during the study period were observed in patients randomized to oropharyngeal exercises (Table 2). In contrast, patients treated with oropharyngeal exercises had a small but significant decrease in neck circumference after 3 months (Table 2). Snoring

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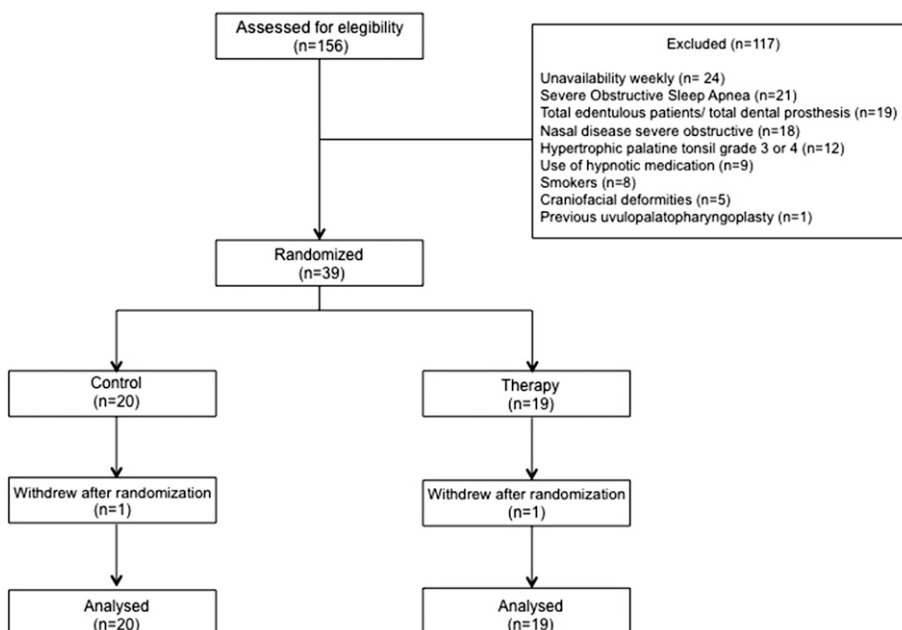


Figure 1 – Flow diagram of the progress through the phases.

**TABLE 1** ] Baseline Demographic Characteristics, Questionnaires, and Polysomnographic and Snore Characteristics of the Patients Assigned to Control or Oropharyngeal Therapy

| Measure   | Control Group (n = 20) | Therapy Group (n = 19) | P Value |
|---|------------------------|------------------------|---------|
| <b>Demographic characteristics</b>                                  |                        |                        |         |
| Male, No. (%)   | 11 (55)                | 11 (57.9)              | 1.000   |
| Age, y  | 45 ± 13                | 48 ± 14                | .458    |
| BMI, kg/m <sup>2</sup>  | 28.3 ± 2.5             | 28.1 ± 2.7             | .818    |
| Neck circumference, cm  | 38.0 ± 3.5             | 38.0 ± 2.6             | .994    |
| Abdominal circumference, cm   | 94.3 ± 10.2            | 93.9 ± 5.7             | .872    |
| <b>Polysomnography</b>  |                        |                        |         |
| TST, h  | 6.2 ± 0.6              | 6.1 ± 0.8              | .755    |
| Sleep efficiency, %   | 84.4 ± 7.5             | 86.0 ± 9.7             | .565    |
| Arousal index, events/h   | 15.3 ± 5.4             | 20.0 ± 10.2            | .080    |
| AHI, events/h   | 15.1 ± 9.5             | 15.6 ± 9.3             | .875    |
| Spo <sub>2</sub> min  | 85.1 ± 5.8             | 85.5 ± 7.5             | .844    |
| Desaturation index, events/h  | 12.3 ± 8.7             | 10.8 ± 8.8             | .600    |
| <b>Snoring measures</b>   |                        |                        |         |
| Snore index, events/h   | 180.6 ± 203.1          | 156.1 ± 164.4          | .682    |
| Total snore index, events/h   | 54.4 (3.5-386.6)       | 60.4 (21.8-220.6)      | .613    |
| <b>Questionnaires</b>   |                        |                        |         |
| <b>Patient</b>  |                        |                        |         |
| Pittsburgh Sleep Quality Index                                      | 6.9 ± 3.4              | 6.0 ± 3.2              | .427    |
| Epworth Sleepiness Scale  | 9.0 (7.0-13.5)         | 7.0 (3.0-11.0)         | .154    |
| Subjective snore intensity  | 3.0 ± 1.0              | 2.3 ± 1.1              | .037    |
| Subjective snore frequency  | 4.0 (3.0-4.0)          | 3.0 (2.0-4.0)          | .070    |
| <b>Bed partner (control group [n = 12], therapy group [n = 13])</b> |                        |                        |         |
| Subjective snore intensity  | 3.5 (2.3-4.0)          | 4.0 (2.5-4.0)          | .858    |
| Subjective snore frequency  | 4.0 (3.0-4.0)          | 4.0 (3.0-4.0)          | .698    |

Data are presented as mean ± SD. Epworth Sleepiness Scale, subjective snore frequency, subjective snore intensity, and total snore index are presented as median (25%-75%) because of skewed distribution. AHI = apnea-hypopnea index; Spo<sub>2</sub> min = lowest oxygen saturation; TST = total sleep time.

perception as reported by the bed partner also decreased (Table 2). Objectively measured snore index (Fig 2) and total snore index (Fig 3) did not change in the control group and decreased significantly in the patients assigned to oropharyngeal exercises. The mean AHI of the population studied was relatively low at study entry (15.3 ± 9.3 events/h) and did not change significantly in either group. However, in the subgroup of patients with moderate OSA at study entry, AHI decreased significantly in the patients assigned to oropharyngeal exercises (n = 8; AHI, 25.4 [22.1-28.7] vs 18.1 [15.4-24.1], P = .017, baseline and study termination, respectively) (Fig 4).

## Discussion

This randomized controlled study was designed to objectively measure the effects of oropharyngeal exercises on

snoring in patients with primary snoring and mild to moderate OSA. We showed that 3 months of oropharyngeal exercises significantly reduced both the frequency of snoring by 36% and the total power of snoring by 59%. The objective decrease in snoring was associated with a decrease in the perception of snoring by the bed partner but not by the patient.

This study shows the beneficial effects of oropharyngeal exercises in a population that is poorly evaluated by the scientific community. The population studied was composed of middle-aged and overweight patients who were disturbed by snoring, were on average not sleepy (Epworth Sleepiness Scale = 9.2 ± 4.9), and did not present severe OSA (AHI = 15.3 ± 9.3 events/h). This group of patients benefit from a sleep study because severe OSA is ruled out. However, they typically do not receive

**TABLE 2 | Demographic Characteristics, Questionnaires, and Polysomnographic and Snore Characteristics of the Patients Assigned to Control or Oropharyngeal Therapy on Basal and After 3 Mo**

| Measure   | Control Group (n = 20) |                |                   | Therapy Group (n = 19) |                |         | P Value           |
|---|------------------------|----------------|-------------------|------------------------|----------------|---------|-------------------|
|   | Baseline               | 3 mo           | P Value           | Baseline               | 3 mo           | P Value |                   |
| <b>Demographic characteristics</b>                                  |                        |                |                   |                        |                |         |                   |
| BMI, kg/m <sup>2</sup>  | 28.3 ± 2.5             | 28.2 ± 3.5     | .453              | 28.1 ± 2.7             | 28.2 ± 2.8     |         | .469              |
| Neck circumference, cm  | 38.0 ± 3.5             | 37.9 ± 3.4     | .628              | 37.9 ± 2.5             | 37.5 ± 2.4     |         | .000 <sup>a</sup> |
| Abdominal circumference, cm   | 94.3 ± 10.2            | 94.6 ± 10.4    | .673              | 93.9 ± 5.7             | 93.7 ± 4.5     |         | .687              |
| <b>Polysomnography</b>  |                        |                |                   |                        |                |         |                   |
| TST, h  | 6.2 ± 0.6              | 6.2 ± 1.1      | .894              | 6.1 ± 0.8              | 6.5 ± 0.9      |         | .079              |
| Sleep efficiency, %   | 84.4 ± 7.5             | 85.0 ± 11.1    | .776              | 86.0 ± 9.7             | 86.3 ± 8.6     |         | .825              |
| Arousal index   | 15.3 ± 5.4             | 16.9 ± 5.2     | .239              | 20.0 ± 10.2            | 6.2 ± 1.4      |         | .077              |
| Spo <sub>2</sub> min  | 85.1 ± 5.8             | 84.0 ± 7.6     | .325              | 85.5 ± 7.5             | 83.8 ± 8.9     |         | .120              |
| Desaturation index  | 12.3 ± 8.7             | 12.1 ± 6.9     | .881              | 10.8 ± 8.8             | 9.7 ± 6.0      |         | .437              |
| <b>Questionnaires</b>   |                        |                |                   |                        |                |         |                   |
| <b>Patient</b>  |                        |                |                   |                        |                |         |                   |
| Pittsburgh Sleep Quality Index                                      | 6.9 ± 3.4              | 6.4 ± 3.9      | .459              | 6.0 ± 3.2              | 4.0 ± 2.6      |         | .004              |
| Epworth Sleepiness Scale  | 9.0 (7.0-13.5)         | 8.0 (3.5-12.5) | .190              | 7.0 (3.0-11.0)         | 7.0 (4.0-10.0) |         | .084              |
| Subjective snore intensity  | 3.0 (2.0-4.0)          | 3.0 (2.0-3.0)  | .083              | 2.0 (2.0-3.0)          | 2.0 (1.0-2.0)  |         | .155              |
| Subjective snore frequency  | 4.0 (3.0-4.0)          | 3.5 (2.0-4.0)  | .010 <sup>a</sup> | 3.0 (2.0-4.0)          | 2.0 (1.0-4.0)  |         | .030              |
| <b>Bed partner (control group [n = 12], therapy group [n = 13])</b> |                        |                |                   |                        |                |         |                   |
| Subjective snore intensity  | 3.5 (2.3-4.0)          | 3.0 (2.0-4.0)  | .194              | 4.0 (2.5-4.0)          | 1.0 (1.0-2.0)  |         | .003 <sup>a</sup> |
| Subjective snore frequency  | 4.0 (3.0-4.0)          | 3.5 (3.0-4.0)  | .180              | 4.0 (3.0-4.0)          | 2.0 (1.5-3.0)  |         | .004 <sup>a</sup> |

Data are presented as mean ± SD or median (25%-75%). See Table 1 legend for expansion of abbreviations.

<sup>a</sup>P < .05 for the comparisons using repeated measures analysis of variance: compare the interaction between the two groups (control and therapy) and the two moments (baseline and after 3 mo).

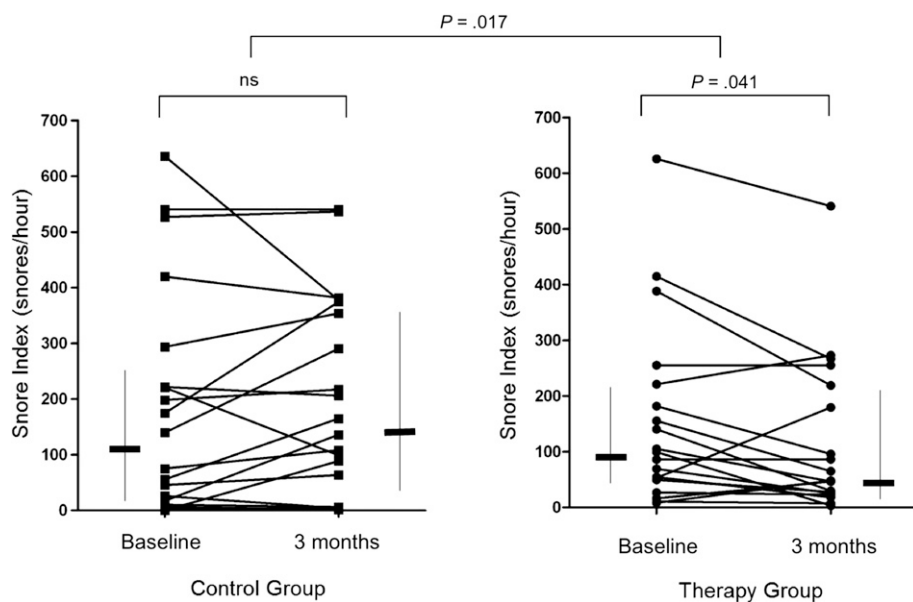


Figure 2 – Individual values for snore index. In the control group, the snore index from baseline to 3 mo was similar. In contrast, the snore index significantly declined in the group randomized to oropharyngeal exercises. There were group  $\times$  time interaction effects ( $P = .017$ ). Short horizontal lines and bars are mean  $\pm$  SD. ns = not significant.

standardized medical follow-up. The prevalence and significance of snoring in the general population varies widely in epidemiologic studies (from 15% to 54%).<sup>7-10</sup> It is plausible, although not proven, that every night vibration of the palate caused by snoring may contribute to upper airway neurogenic lesion<sup>13</sup> and progression of mild forms of OSA.<sup>28</sup> In addition, primary snoring (ie, AHI < 5 events/h) may be associated with disrupted sleep due to respiratory events, related arousals,<sup>14,29</sup> or progression of carotid atherosclerosis due to vibration.<sup>7</sup> Independent of the possible health problems aggravated by snoring, most patients with mild forms of OSA must

have some degree of social burden generated by snoring.<sup>30,31</sup> For instance, a Google search using the key words “snoring” and “treatment” showed > 5 million results, indicating that snoring is a major burden to the society.

In contrast to well-established metrics like the AHI, snoring is not a standard measurement during full polysomnography.<sup>32</sup> In a previous study, our group proposed a simple and accurate method to identify OSA based on time intervals between snoring events.<sup>33</sup> In this study, we objectively quantified the frequency and

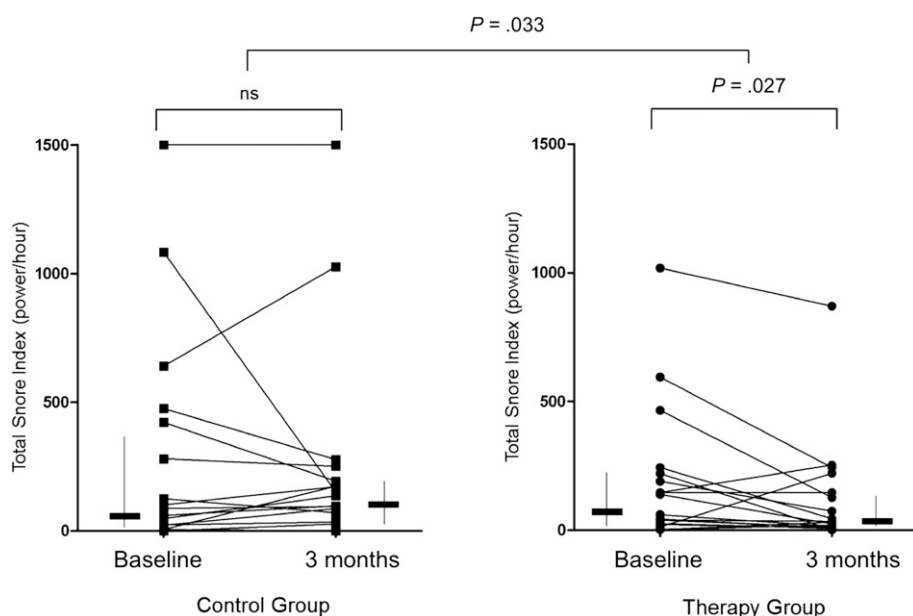


Figure 3 – Individual values for total snore index. In the control group, the total snore index from baseline to 3 mo was similar. In contrast, the total snore index significantly declined in the group randomized to oropharyngeal exercises. There were group  $\times$  time interaction effects ( $P = .033$ ). Short horizontal lines and bars are mean  $\pm$  SD. See Figure 2 legend for expansion of abbreviation.

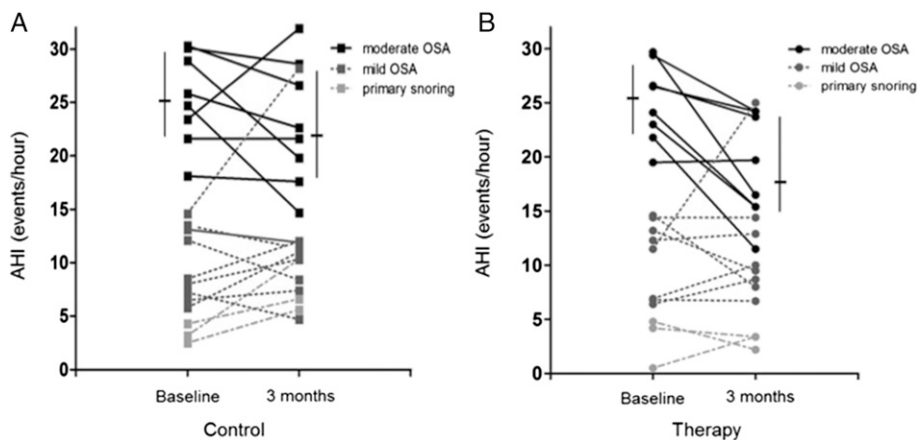


Figure 4 – A, B, Individual values for AHI at baseline and after 3 mo. There were no statistical differences on AHI in either group. However, the subgroup of patients with moderate OSA ( $15 \leq \text{AHI} \leq 30$ ) randomized to oropharyngeal exercises had a significantly decreased AHI. In the control group, the AHI from baseline to 3 mo (from 25.3 [22.1–29.8] to 22.1 [18.2–28.1] events/h) was similar. In contrast, the AHI significantly declined in the group randomized to oropharyngeal exercises (from 25.4 [22.1–28.7] to 18.1 [15.4–24.1] events/h;  $P = .017$ ). Short horizontal lines and bars are medians (25%–75%), because of skewed distribution. AHI = apnea-hypopnea index.

intensity of snoring. We used a similar distance of the microphone to the patient (1 m)<sup>34,35</sup> and adopted the snore index to express our results as previously reported.<sup>34,36–38</sup> In addition, we used the total snore index to represent the total snore intensity power generated during sleep. The objective reduction in snore indexes among patients randomized to oropharyngeal exercises occurred in conjunction with an improvement in the perceived snoring evaluated by the bed partner. Our study is in-line with previous studies that showed the beneficial effects of different forms of oropharyngeal exercises, such as didgeridoo playing,<sup>18</sup> singing,<sup>39,40</sup> and specific oropharyngeal exercises,<sup>19</sup> on upper airway physiology during sleep. Upper airway exercises have been also used to treat children and teenagers with promising results.<sup>41–43</sup> Our study was based on exercises previously reported by our group. We extended our previous study by reducing the number of exercises by 50% that were applied for 3 months.

Our study has strengths and limitations. First, the oropharyngeal exercises are based on an integrative approach and, therefore, do not allow determining the effects of each specific exercise on the overall result. Moreover, these exercises are derived from oral motor techniques to improve speech activity, swallowing activity, or both, an area that lacks the empirical support necessary for evidence-based practices.<sup>44</sup> As compared with our previous study that evaluated the effects of oropharyngeal exercises on moderate OSA, the number of exercises proposed in the present study was reduced

by 50%. In contrast to the original study, we found no overall significant reduction in AHI after oropharyngeal exercises, which could be due to a reduction in the exercises protocol.<sup>19</sup> However, our clinical experience accumulated over the last 5 years has shown that reducing the number of exercises does not affect the effectiveness of therapy. Moreover, there was a significant reduction in AHI of patients with moderate OSA at study entry randomized to oropharyngeal exercises. The most likely explanation is that a “floor effect” in the AHI prevented the observation of any effect on this metric among patients with mild or no OSA at study entry. Our results point out that snoring rather than AHI is probably the best metric to follow patients with mild forms of OSA in whom the most significant complaint is snoring. On the other hand, we acknowledge that there are no standard methods to measure snore, and the field needs to be developed. Finally, there is a perceived concept that exercises are difficult to incorporate. To this end, the simplified protocol is a feasible series of 8 min (tid) that could be more easily incorporated into daily activities, such as immediately after tooth brushing or commuting to work.

In conclusion, oropharyngeal exercises can reduce the objective measurements of frequency and intensity of snoring. This set of oropharyngeal exercises is a promising treatment of large populations suffering from snoring who are currently largely ignored by the medical community.

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**Author contributions:** V. I. takes responsibility for the integrity of the work as a whole, from inception to published article. V. I. contributed to data collection and analysis; F. K. contributed to data collection; M. I. M. contributed to analysis and interpretation; R. P. H. and M. G. G. contributed to data collection, analysis, and interpretation; and V. I., F. K., M. I. M., R. P. H., M. G. G., A. M. A., L. F. D., P. R. G., and G. L. F. contributed to study design and manuscript draft.

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