TITLE PAGE

Prevalence of laryngopharyngeal reflux symptoms, dysphonia and vocal tract discomfort in amateur choir singers

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ABSTRACT

Introduction: vocal tract discomfort (VTD), dysphonia and laryngopharyngeal reflux (LPR) symptoms are complaints frequently reported by amateur singers. There are two aims of this study. The first is to evaluate the prevalence of these symptoms using validated questionnaires. The second is to correlate singing-related variables with the questionnaire responses.

Methods: a total of 392 amateur choir singers (ACS) and 514 control subjects completed an online survey divided into four parts: (1) clinical and demographic characteristics; (2) training in singing and singing experience; (3) history of gastroesophageal reflux (GERD) and LPR symptoms; (4) validated questionnaires. Specifically, the Reflux symptom index (RSI), the Vocal Tract Discomfort Scale (VTDS) and the Voice Symptom Scale (VoiSS) were included to analyze the actual burden related to LPR symptoms, VTD and dysphonia.

Results: ACS demonstrated a healthier lifestyle and a lower prevalence of GERD symptoms in comparison with control subjects. ACS scored significantly higher in VTDS and VoiSS than control subjects, while no differences in the RSI results were found. Significant correlations among the questionnaires' results were demonstrated. Occasional professional singing (OPS) was the variable influencing VTDS and VoiSS results the most.

Conclusion: ACS do not evidently manifest a higher impairment connected to LPR (RSI score), while they do report higher levels of voice (VoiSS score) and vocal tract (VTDS score) impairments, in comparison with control subjects. The relevant correlations among the PRO measures suggest that LPR symptoms, VTD and dysphonia are related to each other. Given the relevant repercussion on the severity of VTD and dysphonia, providers should specifically ask about OPS when treating amateur singers.

Keywords: quality of life, dysphonia, vocal tract, laryngopharyngeal reflux

1. Introduction

Singers may be considered elite professional voice users (PVU) because they carry out complex phonatory tasks requiring endurance, flexibility and vocal tract control exceeding the needs of the speaking voice. In singers, even a slight voice disorder may represent a significant, functional, and occupational impairment, with a negative effect on their quality of life [1-5]. Previous studies demonstrated that singers more commonly complain of vocal tract discomfort (VTD), dysphonia and laryngopharyngeal reflux (LPR) symptoms [2]. VTD is considered as the consequence of an impairment of the pain system as a whole and it is frequently reported as a variable combination of symptoms of inflammation (soreness, burning, tickling, irritability), musculoskeletal symptoms (aching, constriction, tightness) and feeling of increased secretions [5-8]. Dysphonia is also commonly reported by singers. In a recent systematic review, Pestana *et al*. [9] found an overall prevalence of self-reported dysphonia of 46.01% in this population compared to 18.8% [10] in the general population. It is possible that the increased vocal demands which characterize singing activities may facilitate the development of laryngeal lesions that may finally lead to dysphonia [11]. Finally, LPR symptoms are frequently complained by singers [2]. These symptoms are related to the backflow of gastric or gastroduodenal contents into the upper aerodigestive tract which determines a chronic state of irritation and inflammation [12-16]. Hoarseness represents one of the key symptoms of LPR. It is related to modification of the biomechanical properties of the superficial layer of the vocal folds [17, 18] and may facilitate the adoption of inappropriate vocal behaviors [19].

Even if significant correlations between LPR symptoms, dysphonia and VTD have been demonstrated [20-22], no previous study investigated these symptoms simultaneously in singers. Furthermore, even if LPR may constitute a potentially harmful threat for singers [23], the majority of previous reports analyzed the influence of GERD symptoms rather than LPR complaints in this population [24-28]. In addition, previous studies focused almost exclusively on professional singers [8,9,24,27], while scarce information regarding amateur singers (i.e., choristers, amateur soloists, singing students) are available despite their numerousness. For instance, the Italian National Federation of the Regional

Choral Associations (Feniarco) includes more than 2800 choirs, most of them being amateur or semi-professional ensembles [29]. Finally, many studies were conducted administrating generic questionnaires or non-validated patient-reported outcome (PRO) instruments for VTD, dysphonia and LPR symptoms evaluation, therefore yielding non-reproducible outcomes and limiting the chance to share and compare results between different countries and cultures.

The aims of this study were: (1) investigate the actual burden of VTD, dysphonia and LPR symptoms simultaneously in amateur (non-professional) choir singers (ACS) using validated PRO measures; (2) analyze the correlations between the results of the employed PRO measures; (3) evaluate the influence of singing-related variables on these results. The underlying hypotheses were: (1) VTD, dysphonia and LPR symptoms are frequent in ACS; (2) a significant correlation exists among the PRO measures results even if these questionnaires assess different constructs; (3) some singing-related variables (such as singing training, singing experience, etc.) may influence the PRO measures results.

The relevance of this study lies in the fact that a deeper knowledge of the prevalence and the characteristics of VTD, dysphonia and LPR symptoms in ACS could be particularly helpful in the management of these patients, providing additional useful information for both the decision-making process and for outcome evaluation. Moreover, the use of validated PRO measures might facilitate comparisons between studies conducted in different cultural settings, allowing pooling of data and international studies.

2. Materials and Methods

This cross-sectional survey study with controls was designed and carried out following the principles stated in the Declaration of Helsinki, after being approved by the Institutional Review Board of our Institution.

2.1 Study population

Two different groups of subjects were recruited for the present research: (a) ACS as the main study group (group A); (b) healthy subjects as the control group (group B). For the recruitment of the former group, directors of amateur choirs were contacted by phone to assess the choristers' availability for enrollment, providing a thorough description of the study aims and their potential clinical implications. Regarding the latter group, healthy subjects were consecutively recruited among hospital staff members, as well as members of youth organizations, sport clubs, and universities of the third age. To reduce geographical biases, subjects of group B were recruited within the same areas of the enrolled choirs. An invitational email containing a thorough description of the research purposes, the link to the online form, and detailed access instructions was only sent to those who agreed to participate. All study participants were able to provide their informed consent through a dedicated section of the online form. For each underage participant, written parental consent and the minor's online assent were documented. Inclusion criteria for both groups were as follows: age between 15 and 75 years; good understanding of written Italian; preserved reading skills; absence of neurological and psychiatric pathologies; no history of major head and neck traumas, head and neck cancer, or lung cancer. Specifically, the lower limit of the abovementioned age range was selected to reduce the risk of recruiting children in the middle of the voice change (which is completed approximately by the age of 15 years in both sexes) [30]. Exclusion criteria were as follows: for group A, full-time professional singing activity (singing as the primary source of income); for group B, training in singing and current or prior singing experiences (both as soloists and choristers).

2.2 Data collection

The online form for data collection was anonymous. Items were intentionally set as mandatory, so that all questions required a response before the form could be submitted. The survey was structured into four sections: (1) clinical and demographic characteristics; (2) training in singing and singing experience; (3) history of GERD and LPR; (4) PRO measures. ACS were asked to fill in all four sections, while section 2 was omitted from the form sent to control subjects. The relevance of items and PRO measures was discussed together with a team of national and international clinicians experienced in professional voice care.

(1) Clinical and demographic characteristics. Included: age, gender, weight, height, smoking, drinking habits, recent (last 12 months) upper and lower respiratory tract infections (URTI and LRTI, respectively), allergies, physical exercise, diet, eating habits (including late-night dinner), previous laryngeal pathologies and their treatments.

(2) Training in singing and singing experience. Participants of group A were asked about: overall singing experience (years); frequency of singing (daily, weekly or monthly); average duration of singing sessions; habit of performing with amplification (microphone); vocal type (soprano, mezzo-soprano, alto, tenor, baritone, bass); training in singing (total duration, type of training); involvement in professional singing activities (singing as a secondary source of income).

(3) History of reflux. Data collected within this section included: previous diagnoses of GERD or LPR; GERD major symptoms (heartburn, acidic regurgitation); previous instrumental examinations to investigate GERD or LPR; commonly prescribed medications to treat GERD and LPR.

(4) PRO instruments (questionnaires). Within the last section of the survey, three cross-culturally adapted and validated PRO measurements were administered. Specifically, the Italian version of the following questionnaires were used.

- Reflux Symptom Index (RSI) [31,32], a nine-item questionnaire developed to investigate LPR symptoms and their response to therapy. The total RSI scores may range from 0 to 45, with a

higher score representing a worse impact of LPR on QOL. A total RSI score higher than 13 was considered to be suggestive for LPR [31];

- Vocal Tract Discomfort Scale (VTDS) [5,6,33], a self-rating questionnaire aimed at investigating eight symptoms or sensations commonly referred by patients to the vocal tract. Each subscale score may range from 0 to 48, while total score ranges from 0 to 96. This questionnaire was included in the panel of PRO measurements because it explores symptoms and sensations commonly reported by singers [7,8].
- Voice Symptom Scale (VoiSS) [34,35], a thirty-item patient-derived outcome instrument developed for the self-assessment of voice problems and their impact on QOL. It comprises three subscales (Impairment, Emotional, Physical). Its total score ranges from 0 to 120. A total score higher than 15 might discriminate between dysphonic and non-dysphonic patients [35]. The VoiSS was selected because its Physical subscale includes pharyngeal symptoms, which are not explicitly screened by the most widespread Voice Handicap Index (VHI) [36-38].

2.3 Statistical analysis

All statistical tests were performed using the SPSS Statistics 24.0 software (SPSS Inc, Chicago, IL). The normality of the distribution and the equality of variances were preliminarily tested using Kolmogorov-Smirnov's test and Levene's test, respectively. Since a normal distribution was found, parametric tests were used. The differences between the two study groups were investigated using Student's t-test and Chi-Squared test for continuous and categorical variables, respectively. The correlations between the adopted outcome instruments (RSI, VTDS, VoiSS) were assessed for both study groups using Pearson's Correlation Coefficient (PCC). According to Evan's classification, a value below 0.20 should be interpreted as a very weak correlation, 0.20 to 0.39 as weak, 0.40 to 0.59 as moderate, 0.60 to 0.79 as strong, and 0.80 or greater as a very strong correlation [39]. A simple linear regression analysis (Cox model) was finally conducted to assess the influence of singing-related

variables on the results of the PRO-measures in the group of ACS. A significance level of $p<0.05$ was adopted for all comparisons.

3. Results

Out of the 453 ACS invited to participate in the study, 392 ACS completed the online form (response rate 86.53%). There were 129 males and 263 females. The mean age was 39.53 ± 15.35 years (range 15-75 years). From the 514 healthy subjects who were asked to participate, 456 participants completed the evaluation (response rate 88.72%). There were 179 males and 277 females. The mean age was 41.83 ± 13.64 years (range 15-75 years).

3.1 Clinical and demographic characteristics

Clinical and demographic characteristics of the two study groups are depicted in Tables 1 to 4. No significant differences were found between the two groups regarding gender, age, BMI, URTI, LRTI, allergies, and physical activity. Relevant differences were demonstrated instead for smoking and drinking habits. In particular, routine alcohol consumption and smoking were less frequent in ACS (p=0.038 and p=0.032 at Chi-Squared test respectively). As far as diet and eating habits are concerned, the only significant difference between the two groups was found to be the habit of late-night dinner, which was less frequent among ACS (p=0.014 at Chi-Squared test). Regarding previous laryngeal diseases, a positive history of laryngeal pathologies was reported by 27.81% of ACS and 25.44% of control subjects, with no significant difference between the two study groups regarding the screened pathologies (corditis, nodules, polyps, cysts, Reinke's edema, paralysis). Nevertheless, a significant difference was highlighted regarding the relative treatment options. In fact, while subjects in the control group most frequently resorted to medications (68.10% versus 26.61%, p=0.001 at Chi-Squared test), ACS most frequently underwent voice therapy (SLT), surgery, or SLT and surgery combined. Voice rest appeared to be a more frequent therapeutic option among ACS, compared to control subjects (15.60% versus 0.86%, p=0.001 at Chi-Squared test).

3.2 Training in singing and singing experience

Data regarding singing experience and training for ACS are depicted in Table 5. Most respondents reported an overall singing experience of 5 years or more (N=315, 80.36%), with weekly singing sessions (N=284, 72.45%) and performances lasting 60 minutes or more (N=296, 75.51%). In most cases, ACS declared not to use instruments for amplification (N=316, 80.61%). More than half of ACS underwent training in singing (N=217, 55.08%), with a total duration between 1 and 5 years in most cases (N=113, 52.07%). Specifically, most of them received their training from private teachers (N=112, 51.61%), followed by music schools or academies (N=50, 23.04%), conservatories (N=39, 17.97%) and individual training (N=16, 7.37%). With regards to singing as a potential secondary source of income, only the minority of ACS declared to be sporadically involved in occasional professional singing (OPS) activities (N=74, 18.88%) for which they were paid.

3.3 History of GERD and LPR

Data regarding GERD and LPR for both study groups are reported in Table 6. No differences were found between the 2 groups in terms of previous diagnoses of GERD and LPR. Interestingly, GERD major symptoms were less frequent among ACS in comparison with controls (p=0.028 at Chi-Squared test). A relevant difference was also highlighted for esophagogastroduodenoscopy, which appeared to be more frequently prescribed among control subjects (p=0.029 at Chi-Squared test). Finally, medical treatments were globally reported as more frequent among the control group (p=0.012 at Chi-Squared test).

3.4 Patient-reported outcome (PRO) instruments (questionnaires)

Results of the administered PRO instruments (RSI, VTDS, VoiSS) are reported in Figures 1 to 3. Regarding the RSI (Figure 1), no significant differences were found between the two study groups. The RSI mean total score was 5.83 ± 6.03 for ACS and 5.47 ± 6.08 for controls subjects (p = 0.403 at Student's t test). An RSI score higher than 13 (cut-off score) was reported by 45 ACS (11.48%)

and 46 control subjects (10.09%), with no significant difference between the two groups (p=0.481 at Chi-square test).

Conversely, significant differences were highlighted for both the VTDS and the VoiSS. As far as the VTDS is concerned (Figure 2), ACS reported higher scores for both the Frequency (7.53 ± 7.99 versus 6.17 ± 7.05) and the Severity (7.26 ± 8.18 versus 5.73 ± 6.85) subscales (p<0.01 for both comparisons at Student's t test). A similar trend was also demonstrated for the VoiSS (Figure 3), as both the total score and the three subscales' scores were significantly higher among ACS in comparison with controls (p<0.05 for all comparisons at Student's t-test). However, the percentage of subjects reporting a VoiSS total score higher than 15 (cut-off score) did not significantly differ between ACS (N=121, 30.87%) and controls (N=125, 27.41%) (p = 0.059 at Chi-Squared test).

3.5 Correlations between Patient-reported Outcome (PRO) Measures

The correlations between the three PRO instruments, analyzed using Pearson's test, are reported in Tables 7 (for group A) and 8 (for group B). All the examined correlations resulted statistically significant at Pearson's test. Specifically, the most relevant correlations in both groups were the following ones: RSI score and VTDS frequency score (r=0.722 in Group A, r=0.696 in Group B); RSI score and VoiSS physical subscale score (r=0.674 in Group A; r=0.684 in Group B).

3.6 Influence of singing-related variables on Patient-reported Outcome (PRO) Measures

A simple linear regression analysis (Cox model) was conducted to assess the influence of singing-related variables on the PRO measures which resulted significantly different between the two study groups (VTDS, VoiSS). The results are depicted in Table 9. The first more relevant variable which significantly influenced both the VoiSS and VTDS scores was OPS. The second most relevant variable was the one related to the type of training in singing, which significantly influenced the scores of the Emotional, Physical subscales of the VoiSS, as well as the Frequency and Severity subscales of the VTDS. Other variables appeared to influence the scores of the PRO instruments,

such as actual training in singing (influence on the Emotional subscale of the VoiSS), frequency of singing (influence on the Emotional subscale of the VoiSS), average duration of singing session (influence on the Frequency and Severity subscales of the VTDS) and amplification (influence on the Physical subscale of the VoiSS). Considering the significant influence of OPS on the PRO measures scores, the differences between PRO measures scores in OPS and non-OPS ACS were investigated (Table 10). OPS ACS reported significantly higher scores for the VoiSS Emotional subscale (p=0.003 at Student's t-test) and the VoiSS total score (p=0.043 at Student's t-test), as well as for the Frequency subscale of the VTDS (p=0.047 at Student's t-test). No significant differences were found between the two subgroups for the RSI (p=0.682 at Student's t-test).

4. Discussion

To the best of our knowledge, the present research is the first cohort study specifically aimed at investigating the prevalence of VTD, dysphonia and LPR symptoms simultaneously in ACS, in comparison with a general population sample. The large number of enrolled subjects is a strength of this study, while the high response rate suggests that the evaluation of these symptoms is perceived as important.

4.1 Clinical and demographic characteristics

No significant gender nor age differences were found between ACS and controls. In addition, similarly with the study by Dietrich et al. [40], no differences were highlighted also for the BMI. In accordance with the study of Chai et a. [41], a global tendency among ACS to adopt a healthier lifestyle was demonstrated regarding the frequency of smoking and alcohol consumption which appeared to be less frequent in comparison with controls. In addition, the habit of late-night dinner was less frequent in ACS than in controls. Previous reports demonstrated that dinner late at night is common among opera choristers and soloists [2, 25]. This difference may be related to the stressful life of professional singers, who often refrain from eating before performances and compensate at post-performance gatherings [42]. In addition, ACS may not require postponing evening meals to avoid the repercussions of food intake on vocal tasks, and amateur performances and rehearsals are typically planned either late in the evening or on weekends, to allow the participation of all choir members.

Literature about professional singers reveals a higher prevalence of pathologies of the vocal folds (e.g., edema, polyps) within this population [27,43,44]. The substantial overlap in terms of prevalence of laryngeal pathologies between ACS and controls found in the present study might be related to several factors: (a) higher vocal demands in comparison with non-singing subjects, but still lower than professionals; (b) superior awareness of vocal health and stronger predisposition towards healthy habits, in comparison with controls. However, no final inferences can be drawn, as no endoscopic

evaluation of the vocal folds was performed. Significant differences were demonstrated regarding treatment options, with control subjects more frequently resorting to medications and ACS more frequently undergoing voice-rest, SLT, surgery, or SLT and surgery combined. This difference might be explained considering that ACS might be more willing to endorse treatments directly aimed at reducing their vocal impairment, as they develop a higher self-perception of their vocal function [45].

4.2 Training in singing and singing experience

As far as training in singing and singing experience are concerned, most of the ACS reported to have been singing for more than 5 years. However, only 25.26% of them declared to sing every day, while daily singing is almost routinary for their professional counterparts. Singing training was reported by 55.08% of ACS. The majority of them relied on private teachers, while usually most professional singers face intense and full-time training in conservatories. OPS was reported in only 18.88% of cases. Given the well-acknowledged repercussions of professionalism on voice-related QOL [46], we believe that OPS should always be screened in ACS, since higher demands of professional settings might lead to higher vocal impairments in scarcely trained performers [45].

4.3 History of GERD and LPR

Similar to the study of Dietrich *et al.* [40], ACS less frequently reported GERD symptoms, underwent EGDS, and used medical therapy for reflux symptoms. Contrariwise, literature regarding GERD symptoms in professional singers reveals a higher frequency of heartburn and regurgitation, as well as an increased risk of GERD [27]. Cammarota et al. [24] demonstrated an association between GERD symptoms and lifetime duration (years) of professional singing, configuring GERD as an actual work-related disease in professional singers, possibly related to abdominal muscle and diaphragmatic tension to support voice projection. Lenti et al. [25], reported that among opera soloists the prevalence of major GERD symptoms was even higher than the one highlighted in choristers. To the best of our knowledge, the only study reporting a higher prevalence of heartburn in amateur

singers was the one conducted by Loor et al. [47] who compared 30 vocal opera students (mean age 27 years) and 20 healthy controls (mean age 23 years). However, since GERD tends to be more frequent among adolescents [48,49], the prevalence of heartburn and regurgitation in this population might have been overestimated.

It is possible that the healthier and more regular habits found in ACS may have played a role in reducing the prevalence of major GERD symptoms in this population. However, as suggested by Dietrich *et al*. [40], also moderate singing and adequate breathing exercises (like those performed within an amateur choir) may be helpful in reducing GERD symptoms by reinforcing the crura of the diaphragm, thus lowering the frequency of non-paraphysiological reflux episodes. As a matter of fact, a recent systematic review highlighted the potentially beneficial effects of breathing training on the reinforcement of the anti-reflux barrier at the level of the gastro-esophageal junction [50].

4.4 Patient-reported outcome (PRO) instruments (questionnaires)

Neither the RSI score nor the portion of subjects with a score suggestive for LPR (RSI>13) appeared to be significantly different between ACS and control. Additionally, even when discriminating between OPS and non-OPS singers, no differences were demonstrated for the RSI. These results diverge from those reported by Nacci et al. [51] who found higher RSI scores in 56 singing students, in comparison with 60 controls. Similarly, Hočevar-Boltežar *et al*. [52] highlighted higher RSI scores in 119 professional opera choristers in comparison with 70 teachers and 111 controls. Lloyd et al. [53] found an RSI score suggestive for LPR in 13 out of 20 (65%) professional singers and mild reflux findings at the pH probe (ResTech®) in 19 of them (95%). For this reason, the authors concluded that the RSI might not be sensitive enough to evaluate reflux-related impairments in singers. As a matter of fact, singers may actually refer symptoms which may be suggestive for LPR (e.g., dysphonia, throat clearing, difficulty in changing register), but scoring lower than 13 at the RSI [54]. Moreover, since significant flaws in the development of the RSI can be acknowledged [55], there might be LPR cases which remain undetected basing solely on the RSI score. Considering these

assumptions, it is possible to speculate that the prevalence of LPR among ACS emerged from the present investigation (11.48%), based solely on the RSI score, might be underestimated. Further studies including pH-probe testing in the analyses could better quantify the actual burden of LPR in settings of amateur singing.

Relevant differences between ACS and controls were demonstrated regarding the VTDS and the VoiSS, with significantly higher values among singers for both measures. The higher vocal impairment and vocal tract discomfort in ACS could be related to a higher self-perception of their vocal functioning [45], and to a superior inclination towards vocal abuse and/or misuse [56] which might be favored by the lack of proper musical training and vocal technique classes. In fact, even if singing is practiced just for leisure or pleasure, several risks and factors can potentially impair vocal health in ACS [57].

4.5 Correlations between Patient-reported Outcome (PRO) Measures

Significant correlations were highlighted between the employed PRO instruments (RSI, VTDS, VoiSS). In addition, most correlations scored as “moderate”, “strong” or “very strong”, with only three correlations for each study group scoring as “weak”. These results agree with previous reports [20-22,58] and reinforce the hypothesis that LPR symptoms, VTD and dysphonia are related to each other [20].

4.6 Influence of singing-related variables on Patient-reported Outcome (PRO) Measures

Interestingly, OPS was the variable which influenced VoiSS and VTDS scores the most. As a matter of fact, OPS ACS reported higher mean values for both the VoiSS (Emotional subscale, total score) and the VTDS (Total and Frequency subscale). This finding might be related to the higher demands of professional settings, which might lead to higher self-perceived vocal impairments in unexperienced performers [45]. On the other hand, it is also possible that OPS might be more aware of VTD and dysphonia since the presence of these symptoms might negatively impact on their ability

to work. Regardless of the cause, the results of this analysis underline the importance of screening OPS when investigating voice and vocal tract disturbances in ACS.

4.7 Study limitations

The present study has several limitations. First, being a web-based survey study, only ACS able to access the Internet and confident with web browsing were able to participate. Therefore, this might have had an impact on the representativeness of the sample. Moreover, setting all items as mandatory might have increased dropout rates and slightly decreased overall quality of answers [59]. Future survey studies should then be designed integrating multiple recruitment strategies (i.e., in person and web based), also granting a reasonable balance between mandatory and optional items, possibly yielding overall better data quality. Second, no objective data regarding GERD, LPR, vocal folds and voice is available. Further studies should be aimed at objectively evaluate the prevalence and the severity LPR, dysphonia and VTD in this peculiar population of voice professionals.

5. Conclusions

The findings of the present study reveal that ACS do not evidently manifest a higher impairment connected to LPR (RSI score), in comparison with a sample of the general population. Contrariwise, they do report higher levels of voice (VoiSS score) and vocal tract (VTDS score) impairments, in comparison with control subjects. For its significant influence on the dysphonia and vocal tract symptoms, OPS should be screened in amateur singers.

6. Acknowledgments

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7. Figure captions

Figure 1: Reflux Symptom Index (RSI) scores of the two study groups.

Figure 2: Vocal Tract Discomfort Scale (VTDS) scores of the two study groups.

Figure 3: Voice Symptom Scale (VoiSS) scores of the two study groups.

8. Tables

Table 1 Demographic characteristics and Body Mass Index (BMI) of the two study groups.

Gender				
		Singers (*n* = 392)	Controls (*n* = 456)	*p*
Male (n, %) Female (n, %)		129 (32.9%) 263 (67.1%)	179 (39.25%) 277 (60.75%)	0.078
Age				
		Singers (*n* = 392)	Controls (*n* = 456)	*p*
Mean age ± SD (years)		39.53 ± 15.35	41.83 ± 13.64	0.103
Age range (years)		15 – 78	15 – 75	
Age groups (n, %)	15-29 years	135 (34.44%)	97 (21.27%)	0.051
	30-44 years	92 (23.47%)	152 (33.33%)	0.077
	45-59 years	119 (30.36%)	153 (33.55%)	0.084
	60-75 years	46 (11.73%)	53 (11.84%)	0.103
BMI				
		Singers (*n* = 392)	Controls (*n* = 456)	*p*
Mean BMI ± SD (kg/m2)		23.56 ± 4.23	23.88 ± 4.32	0.201
BMI range (kg/m2)		16.53 – 40.35	16.46 – 45.31	
BMI groups (n, %)	Severely underweight (<16.50)	0 (0.00%)	1 (0.22%)	0.304
	Underweight (16.50-18.49)	27 (6.89%)	20 (4.39%)	0.221
	Normal (18.50-24.99)	250 (63.77%)	293 (64.25%)	0.103
	Overweight (25.00-29.99)	84 (21.43%)	102 (22.37%)	0.201
	Obesity stage I (30.00-34.99)	23 (5.87%)	29 (6.36%)	0.114
	Obesity stage II (35.00-39.99)	6 (1.53%)	8 (1.75%)	0.211
	Obesity stage III (≥40.00)	2 (0.51%)	3 (0.66%)	0.186

Table 2. Data regarding smoking and drinking habits, respiratory infections, allergies and physical exercise in the two study groups.

Smoking			
	Singers (*n* = 392)	Controls (*n* = 456)	*p*
Non-smoker (n, %)	**295 (75.25%)**	**285 (62.50%)**	**0.032***
Former smoker (n, %)	58 (14.79%)	84 (18.42%)	0.134
Smoker (n, %)	**39 (9.96%)**	**87 (19.08%)**	**0.001***
Alcohol			
	Singers (*n* = 392)	Controls (*n* = 456)	*P*
Never (n, %)	135 (34.44%)	146 (32.02%)	0.097
Occasionally (n, %)	226 (57.65%)	249 (54.61%)	0.111
Habitually (n, %)	**31 (7.91%)**	**61 (13.38%)**	**0.038***
URTI[1] and LRTI[2] (last 12 months)			
	Singers (*n* = 392)	Controls (*n* = 456)	*p*
No (n, %)	266 (67.86%)	318 (69.74%)	0.158
Yes (n, %)	126 (32.14%)	138 (30.26%)	
Allergies			
	Singers (*n* = 392)	Controls (*n* = 456)	*p*
None (n, %)	236 (60.20%)	284 (62.28%)	0.187
Respiratory (n, %)	76 (19.39%)	73 (16.01%)	0.134
Food (n, %)	26 (6.63%)	37 (8.11%)	0.102
Medications (n, %)	25 (6.38%)	35 (7.68%)	0.178
Asthma (n, %)	29 (7.40%)	27 (5.92%)	0.109
Physical Activity			
	Singers (*n* = 392)	Controls (*n* = 456)	*p*
No (n, %)	179 (45.66%)	182 (39.91%)	0.202
Yes (n, %)	213 (54.34%)	274 (60.09%)	

[1] URTI = Upper Respiratory Tract Infections
[2] LRTI = Lower Respiratory Tract Infections

Table 3. Data regarding diet and eating habits in the two study groups.

Diet				
		Singers (*n* = 392)	Controls (*n* = 456)	*p*
Chocolate	Never (n, %)	25 (6.38%)	26 (5.70%)	0.334
	Rarely (n, %)	226 (57.65%)	243 (53.29%)	0.285
	Often (n, %)	126 (32.14%)	170 (37.28%)	0.111
	Daily (n, %)	15 (3.83%)	17 (3.73%)	0.603
Mint	Never (n, %)	177 (45.15%)	211 (46.27%)	0.553
	Rarely (n, %)	187 (47.70%)	221 (48.46%)	0.302
	Often (n, %)	25 (6.38%)	22 (4.82%)	0.201
	Daily (n, %)	3 (0.77%)	2 (0.44%)	0.504
Tea	Never (n, %)	55 (14.03%)	93 (20.39%)	0.078
	Rarely (n, %)	182 (46.43%)	207 (45.39%)	0.233
	Often (n, %)	115 (29.34%)	130 (28.51%)	0.419
	Daily (n, %)	40 (10.20%)	26 (5.70%)	0.082
Coffee	Never (n, %)	55 (14.03%)	60 (13.16%)	0.302
	Rarely (n, %)	70 (17.86%)	63 (13.82%)	0.285
	Often (n, %)	160 (40.82%)	210 (46.05%)	0.333
	Daily (n, %)	107 (27.30%)	123 (26.97%)	0.413
Citrus fruits	Never (n, %)	25 (6.38%)	42 (9.21%)	0.143
	Rarely (n, %)	178 (45.41%)	234 (51.32%)	0.095
	Often (n, %)	173 (44.13%)	169 (37.06%)	0.105
	Daily (n, %)	16 (4.08%)	11 (2.41%)	0.111
Fried food	Never (n, %)	54 (13.78%)	58 (12.72%)	0.203
	Rarely (n, %)	298 (76.02%)	341 (74.78%)	0.178
	Often (n, %)	39 (9.95%)	55 (12.06%)	0.223
	Daily (n, %)	1 (0.25%)	2 (0.44%)	0.643
Onion	Never (n, %)	59 (15.05%)	63 (13.82%)	0.345
	Rarely (n, %)	145 (36.99%)	197 (43.20%)	0.105
	Often (n, %)	176 (44.90%)	188 (41.23%)	0.289
	Daily (n, %)	12 (3.06%)	8 (1.75%)	0.088
Garlic	Never (n, %)	91 (23.21%)	100 (21.93%)	0.354
	Rarely (n, %)	166 (42.35%)	210 (46.05%)	0.299
	Often (n, %)	131 (33.42%)	138 (30.26%)	0.156
	Daily (n, %)	4 (1.02%)	8 (1.75%)	0.496
Tomatoes	Never (n, %)	21 (5.36%)	24 (5.26%)	0.677
	Rarely (n, %)	77 (19.64%)	103 (22.59%)	0.298
	Often (n, %)	278 (70.92%)	309 (67.76%)	0.139
	Daily (n, %)	16 (4.08%)	20 (4.39%)	0.495
Soft drinks	Never (n, %)	97 (24.74%)	96 (21.05%)	0.233
	Rarely (n, %)	209 (53.32%)	260 (57.02%)	0.287
	Often (n, %)	82 (20.92%)	91 (19.96%)	0.539
	Daily (n, %)	4 (1.02%)	9 (1.97%)	0.559
Late-night dinner				
		Singers (*n* = 392)	Controls (*n* = 456)	*p*
No (n, %) Yes (n, %)		**269 (68.62%)** **123 (31.38%)**	**279 (61.18%)** **177 (38.82%)**	**0.014***

Table 4. Data regarding previous pathologies of larynx and relative treatment options in the two study groups.

History of pathologies of the vocal folds			
	Singers (*n* = 392)	Controls (*n* = 456)	*p*
Never (n, %)	283 (72.19%)	340 (74.56%)	0.493
Vocal corditis (n, %)	2 (0.51%)	2 (0.44%)	0.699
Laryngitis (n, %)	93 (23.72%)	90 (19.74%)	0.486
Vocal nodules (n%)	6 (1.53%)	9 (1.97%)	0.583
Polyp (n, %)	4 (1.02%)	3 (0.66%)	0.385
Cyst (n, %)	1 (0.26%)	4 (0.88%)	0.204
Reinke's edema (n, %)	2 (0.51%)	6 (1.32%)	0.411
Paralysis (n, %)	1 (0.26%)	2 (0.44%)	0.489
Treatment options			
	Singers (*n* = 109)	Controls (*n* = 116)	*p*
No therapy (n, %)	9 (8.26%)	17 (14.66%)	0.059
SLT[1] (n, %)	**25 (22.94%)**	**14 (12.07%)**	**0.009***
Surgery (n, %)	**15 (13.76%)**	**3 (2.59%)**	**0.018***
SLT[1] + surgery (n, %)	**14 (12.84%)**	**2 (1.72%)**	**0.027***
Medications (n, %)	**29 (26.61%)**	**79 (68.10%)**	**0.001***
Vocal rest (n, %)	**17 (15.60%)**	**1 (0.86%)**	**0.001***

[1] SLT = Speech and language therapy

Table 5. Training in singing and singing experience of non-professional singers (Group 1).

Overall singing experience		
		Singers (*n* = 392)
Less than 1 year (n, %)		12 (3.06%)
Between 1 and 5 years (n, %)		65 (16.58%)
More than 5 years (n, %)		315 (80.36%)
Frequency of singing		
		Singers (*n* = 392)
Daily (n, %)		99 (25.26%)
Weekly (n, %)		284 (72.45%)
Monthly (n, %)		9 (2.30%)
Average duration of singing sessions		
		Singers (*n* = 392)
Less than 30 minutes (n, %)		15 (3.83%)
Between 30 and 60 minutes (n, %)		81 (20.66%)
Over 60 minutes (n, %)		296 (75.51%)
Amplification (microphone)		
		Singers (*n* = 392)
Never (n, %)		316 (80.61%)
Sometimes (n, %)		76 (18.88%)
Always (n, %)		2 (0.51%)
Vocal type		
		Singers (*n* = 392)
Female voices (n = 263, 67.09%)	Soprano (n, %)	131 (33.42%)
	Mezzo-soprano (n, %)	68 (17.35%)
	Alto (n, %)	64 (16.33%)
Male voices (n = 129, 32.91%)	Tenor (countertenor) (n, %)	63 (16.07%)
	Baritone (n, %)	44 (11.22%)
	Bass (n, %)	22 (5.61%)
Training in singing		
		Singers (*n* = 392)
No (n, %)		175 (44.42%)
Yes (n, %)		217 (55.08%)
Type of training in singing		
		Singers (*n* = 217)
Self-taught (n, %)		16 (7.37%)
Private teachers (n, %)		112 (51.61%)
Music school or academy (n, %)		50 (23.04%)
Conservatory (n, %)		39 (17.97%)
Duration of training in singing		
		Singers (*n* = 217)
Less than 1 year (n, %)		34 (15.67%)
Between 1 and 5 years (n, %)		113 (52.07%)
More than 5 years (n, %)		70 (32.26%)
Occasional Professional Singing (OPS)		
		Singers (*n* = 392)
No (n, %)		318 (81.12%)
Yes (n, %)		74 (18.88%)

Table 6. History of gastroesophageal reflux disease (GERD) and laryngopharyngeal reflux disease (LPR) in the two study groups.

Former diagnosis of GERD and/or LPR				
		Singers (*n* = 392)	Controls (*n* = 456)	*p*
No (n, %)		281 (71.68%)	301 (66.01%)	0.105
GERD (n, %)		105 (26.79%)	140 (30.70%)	0.325
LPR (n, %)		2 (0.51%)	9 (1.97%)	0.466
GERD and LPR (n, %)		4 (1.02%)	6 (1.32%)	0.397
GERD major symptoms				
		Singers (*n* = 392)	Controls (*n* = 456)	*p*
Heartburn (n, %)		**22 (5.61%)**	**38 (8.33%)**	**0.021***
Acidic regurgitation (n, %)		**54 (13.78%)**	**82 (17.98%)**	**0.048***
Both (n, %)		**35 (8.93%)**	**55 (12.06%)**	**0.041***
None (n, %)		**281 (71.68%)**	**281 (61.62%)**	**0.028***
Instrumental examinations				
		Singers (*n* = 392)	Controls (*n* = 456)	*p*
EGDS[1]	Never (n, %)	**314 (80.10%)**	**336 (73.68%)**	**0.029***
	Negative (n, %)	**29 (7.40%)**	**54 (11.84%)**	**0.021***
	Positive (n, %)	**49 (12.50%)**	**66 (14.47%)**	**0.038***
MII-pH[2]	Never (n, %)	378 (96.43%)	446 (97.81%)	0.203
	Negative (n, %)	6 (1.53%)	5 (1.10%)	0.178
	Positive (n, %)	8 (2.04%)	5 (1.10%)	0.223
Laryngoscopy	Never (n, %)	328 (83.67%)	390 (85.53%)	0.302
	Negative (n, %)	33 (8.42%)	31 (6.80%)	0.201
	Positive (n, %)	31 (7.91%)	35 (7.68%)	0.504
GERD and/or LPR medical therapy				
		Singers (*n* = 392)	Controls (*n* = 456)	*p*
Never (n, %)		**235 (59.95%)**	**207 (45.39%)**	**0.012***
Yes, in the past (n, %)		**99 (25.26%)**	**185 (40.57%)**	**0.008***
Yes, currently (n, %)		58 (14.80%)	64 (14.04%)	0.063
GERD and/or LPR medications				
		Singers (*n* = 157)	Controls (*n* = 249)	*p*
Proton pump inhibitors (n, %)		116 (73.89%)	171 (68.67%)	0.098
H2 antihistamines (n, %)		13 (8.28%)	19 (7.63%)	0.284
Alginates (n, %)		83 (52.87%)	161 (64.66%)	0.065
Antacids (n, %)		67 (42.68%)	104 (41.77%)	0.204
Prokinetics (n, %)		25 (15.92%)	51 (20.48%)	0.088

[1] EGDS = Esophagogastroduodenoscopy

[2] MII-pH = Esophageal 24-hour multichannel intraluminal impedance and pH test

Table 7. Correlations between the adopted outcome measures calculated using Pearson correlation coefficient (PCC) in the group of amateur choir singers (ACS, group A).

		RSI	VTDS		VoiSS			
			Frequency	Severity	Impairment	Emotional	Physical	Total
RSI		1						
VTDS	Frequency	0.722*	1					
	Severity	0.546*	0.795*	1				
VoiSS	Impairment	0.492*	0.479*	0.396*	1			
	Emotional	0.397*	0.449*	0.377*	0.643*	1		
	Physical	0.674*	0.661*	0.549*	0.589*	0.465*	1	
	Total	0.601*	0.604*	0.501*	0.939*	0.790*	0.775*	1

* $p = 0.01$

Table 8. Correlations between the adopted outcome measures calculated using Pearson correlation coefficient (PCC) in the group of non-singing control subjects (group B).

		RSI	VTDS		VoiSS			
			Frequency	Severity	Impairment	Emotional	Physical	Total
RSI		1						
VTDS	Frequency	0.696*	1					
	Severity	0.649*	0.857*	1				
VoiSS	Impairment	0.463*	0.451*	0.443*	1			
	Emotional	0.356*	0.264*	0.308*	0.711*	1		
	Physical	0.684*	0.598*	0.612*	0.622*	0.535*	1	
	Total	0.564*	0.510*	0.520*	0.954*	0.818*	0.794*	1

* $p = 0.01$

Table 9. Simple linear regression analysis assessing the influence of singing-related variables on patient-reported outcome (PRO) instruments (VTDS, VoiSS).

	VoiSS-I (R^2, F, p)	**VoiSS-E** (R^2, F, p)	**VoiSS-P** (R^2, F, p)	**VoiSS-Total** (R^2, F, p)	**VTDS-F** (R^2, F, p)	**VTDS-S** (R^2, F, p)
OPS[1]	0.035 0.474 0.491	**0.171** **11.799** **0.001***	**0.119** **5.597** **0.018***	**0.103** **4.204** **0.041***	**0.181** **13.150** **0.001***	**0.164** **10.828** **0.001***
Training in singing (yes/no)	0.020 0.149 0.700	**0.164** **10.774** **0.001***	0.068 1.813 0.179	0.077 2.315 0.129	0.086 2.882 (1-391) 0.090	0.073 2.077 (1-391) 0.150
Type of training in singing	0.008 0.025 0.873	**0.128** **6.528** **0.011***	**0.092** **3.327** **0.049***	0.068 1.795 0.181	**0.129** **6.552** **0.011**	**0.133** **7.047** **0.008***
Overall singing experience	0.082 2.621 0.106	0.020 0.155 0.694	0.026 0.263 0.608	0.061 1.460 0.228	0.009 0.032 0.858	0.048 0.909 (1-391) 0.341
Frequency of singing	0.002 0.002 0.964	**0.115** **5.241** **0.023***	0.004 0.005 0.943	0.034 0.497 0.504	0.049 0.949 0.331	0.061 1.468 (1-391) 0.226
Average duration of singing sessions	0.068 1.404 0.237	0.017 0.113 0.737	0.067 1.756 0.186	0.060 1.410 0.236	**0.104** **4.284** **0.039***	**0.117** **5.456** **0.020***
Amplification (microphone)	0.045 0.775 0.379	0.057 1.288 0.257	**0.106** **4.406** **0.036***	0.022 0.185 0.668	0.099 3.831 0.051	0.050 0.978 0.325

[1] OPS = Occasional professional singing

Table 10. Patient reported outcome (PRO) instruments scores in singers involved in occasional professional singing (OPS) and in singers not involved in OPS.

	OPS singers (*n* = 74, 18.88%)	**Non-OPS singers** (*n* = 318, 81.12%)	***p***
RSI	6.23 ± 5.04	5.80 ± 6.09	0.682
VoiSS-I	8.00 ± 7.07	7.17 ± 7.16	0.450
VoiSS-E	**3.62 ± 4.59**	**1.52 ± 3.19**	**0.003***
VoiSS-P	6.31 ± 4.16	4.81 ± 3.67	0.344
VoiSS-Total	**17.92 ± 13.08**	**13.49 ± 13.13**	**0.043***
VTDS-F	**10.42 ± 8.86**	**7.32 ± 7.89**	**0.047***
VTDS-S	9.73 ± 8.88	7.08 ± 8.11	0.381

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