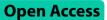
## RESEARCH



# Maximizing phonation: impact of inspiratory muscle strengthening on vocal durations and pitch range

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### Abstract

**Background** This study investigated the acute effects of inspiratory muscle warm-up (IWU) on vocal performance in singers. Proper vocal and respiratory warm-up can enhance vocal range, quality, and endurance. The aim was to determine whether IWU improves maximum phonation time and pitch range, contributing to better voice production efficiency (vocal efficiency) and reduced fatigue.

**Materials and methods** Singers were selected from the Samsun State Opera and the Ballet Directorate (n = 16). This cross-sectional study aimed to investigate the acute effects. The singers in the control group (SC=8) performed only one session of routine voice warm-up, and the experimental group (SE=8) conducted an inspiratory muscle warm-up (IWU) of 2 sets, 30 times/set at 40% maximal inspiratory pressure (MIP) in addition to routine voice warm-up. Subsequently, All participants were then required to perform pre- and post- pulmonary function tests, maximal inspiratory pressure (MIP) and maximal expiratory pressure (MEP), and voice recordings (note high pitch, note low pitch, high pitch durations and low pitch durations sustained with one breath, and maximum phonation duration).

**Results** All pulmonary function and muscle strength parameters improved in the SE group, with the highest increases in MIP (22.9%) and MEP (14.7%). No significant improvements were noted in the SC group (p > 0.05). The Borg Rating of Perceived Exertion showed that the SE group experienced less difficulty with their vocal performance after IWU (-11.6%, p = 0.006), while no significant change was observed in the SC group (p = 0.316). Both warm-up methods used in the study significantly affected the frequencies of high-pitch sounds (SE = 17.8%, SC = 10.9%, p = 0.003); however, the frequency of low-pitch sounds was not significantly affected (p = 0.437). IWU significantly affected the high-pitched note duration (p < 0.001; 32.17%), low-pitched note duration (p < 0.001; 27.11%), and maximum phonation time (p < 0.001; 21%), while routine voice warm-up did not significantly affect any parameter (p > 0.05).

**Conclusions** The combination of IWU with the general body and voice warm-up protocol can acutely improve vocal performance in terms of maximum phonation time, phonation times of the highest and lowest pitched sounds in a single breath, and vocal range levels.

Keywords Inspiratory muscle warm-up, Music, Respiratory muscle strength, Pulmonary function, Vocal performance

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#### Introduction

The production of the human voice is a complex process involving numerous interacting organ systems. While significant attention has been directed towards the impact of vocal fold physiology, the respiratory system also exerts a substantial influence on voice production. Specifically, it is paramount in generating the airflow that circulates between the vocal folds. Consequently, it is instrumental in generating the force that propels the voice. Despite its significance, the intricate mechanisms underlying this process remain to be fully elucidated [1].

The relationship between respiration and the laryngeal system is well established. These two systems work in coordination to regulate the airflow and pressure required by the larynx to produce sounds. This coordination is essential to control the pitch, volume, and quality of sound emitted by the larynx [1].

For singers, the coordination between respiration and the laryngeal system is crucial [2]. Singers should use their voices with the best performance in their reference range to achieve maximum sound production efficiency and productivity with minimum effort [3]. This means maintaining proper breath control and ensuring that laryngeal muscles are not overstrained. Appropriate training and techniques help singers manage airflow and pressure, allowing sustained and controlled vocal performance. Understanding this relationship can lead to better vocal health and longevity, enabling singers to perform better.

Previous studies show that vocal warm-ups should help prepare the vocal tract for exercise and prevent tension [3, 4]. These activities were designed to optimize vocal and acoustic characteristics to reduce both vocal noise and anxiety. A deeper understanding of the physiological and neuromuscular aspects of vocal function will help develop more effective training and rehabilitation programs for voice professionals [4, 5]. The primary aim of vocal exercises and warm-up is to improve vocal function, including increasing vocal range and quality, and the secondary aim is to improve breathing, vocal muscle strength and endurance [5].

It must be highlighted that a critical factor in voice production is the improvement of respiratory muscle endurance to reduce fatigue and enhance vocal performance during prolonged vocalization, such as singing or continuous speech [6, 7]. Singer active use of inspiratory and ekspiratory respiratory muscles is believed to improve sound production [6, 7]. Previous studies have demonstrated the effects of increased respiratory muscle strength on sound production [7, 8]. The functioning of the respiratory system depends largely on the capacity of the respiratory muscles [9]. Inspiratory muscle warm-up (IWU) can enhance the functional capacity of respiratory muscles, improve respiratory muscle oxygenation [10] and enhance inspiratory muscle fatigue resistance [11]. IWU is known to increase the functional capacity of inspiratory muscles (IM), reduce the feeling of shortness of breath, and improve performance [12, 13]. Enhancing respiratory muscle endurance can lead to better control of airflow and pressure, which is essential for maintaining consistent vocal quality over extended periods. This aspect is particularly important for singers and individuals who rely heavily on their voices for professional purposes as it contributes to overall vocal health and longevity. IWU can improve breathing control, expand vocal range, and enhance overall vocal flexibility and performance. Warm-up activities are a widely accepted practice before almost any athletic event to prepare the body for optimal competitive performance [14]. Therefore, this study aimed to assess the acute effects of inspiratory muscle warm-up (IWU) on vocal performance in singers. We hypothesized that the pressure-generating capacity of inspiratory muscles would increase after IWU in singers. Although there are limited studies on the effects of voice and physical exercise on voice therapy and performance [15–17], there is still no consensus on the use of IWU. By evaluating the impact of inspiratory muscle training on vocal performance, we gained insight into the short-term effects of exercise on vocal performance, which is essential for maintaining vocal health over time. This practice not only enhances the physical aspects of voice production, but also improves respiratory muscle endurance, enabling vocal professionals to meet career demands with reduced fatigue and increased voice production efficiency.

#### **Materials and methods** Study design and participants

This study was approved by the Ethics Committee of Gümüşhane University (2023/2; E-95674917-108.99-164965). Prior to the commencement of the study, participants were thoroughly informed about the study's content, purpose, and experimental design. Those who agreed to participate signed the informed consent form. The inclusion criteria were as follows: (a) at least five years of active professional singing experience, (b) age between 18 and 40 years, (c) good general health with no known respiratory or cardiovascular conditions, (d) provision of written informed consent, and (e) affiliation with the Samsun State Opera and Ballet Directorate. Exclusion criteria were as follows: (a) history of lung disease or current upper respiratory tract infections; (b) current voice impairment or vocal cord issues; (c) use of medications that could affect respiratory or vocal performance; (d) current smokers or individuals with a history of smoking within the past year; and (e) participation in similar studies within the last six months. A crosssectional study was conducted, with the sample size

determined using G\*Power software [18, 19]. A power analysis (alpha = 0.05, power = 0.50,  $\eta 2p = 0.8$ ) indicated that a minimum of 13 participants were required [18]. In this study, a quota sampling method was employed, whereby a total of sixteen volunteer singers with a minimum of five years of active working history from the Samsun State Opera and Ballet Directorate were selected.

#### **Experimental design**

Participants visited the laboratory and recording room three times. On the first visit, participants underwent lung function tests (PFT), maximum inspiratory pressure (MIP), expiratory pressure (MEP), voice recordings, and inspiratory muscle warm-up and voice warm-up as a practice procedure 3 times [20]. During the second visit, the PFT and MIP-MEP tests were performed without IWU or vocal warm-up, and voice recordings were recorded. On the third visit, the participants were divided into two groups. The experimental group (SE), which performed both inspiratory muscle and voice warm-ups, and the control group (SC), which performed only voice warmth, were sex-matched with a total of four males and four females in each group (Fig. 1).

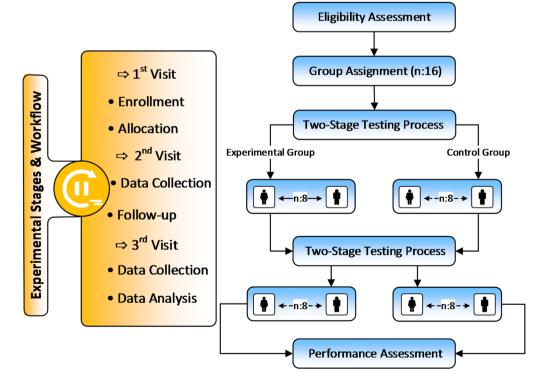
PFT and MIP-MEP tests were reassessed, and audio recordings were taken immediately after the IWU. The visit were conducted simultaneously daily (between 09:00 and 12:00). Participants were provided with a 72-hour notice prior to each visit, during which time they were

instructed to refrain from vocal performance and highintensity physical activity. A 72-hour recovery period was allowed between each visit.

#### **Audio recordings**

Audio recordings were performed in a professional  $12 \text{ m}^2$  audio recording room at room temperature. It was hypothesised that the subjects would not experience vocal fatigue due to the restriction of vocal performances between 72 h of rest. The recordings were conducted in the morning when the singers were free from vocal fatigue. Participants were instructed to abstain from consuming foods that could potentially adversely affect their voice quality at breakfast and were recorded in the studio two hours after the meal [21].

Prior to vocal recordings at all visits, all performers performed a vocal warm-up (a warm-up procedure specific to the individual and vocal range) [22, 23] for at least 15 min to improve their vocal quality, control and flexibility [24, 25] after which two measurements (respiratory and vocal performance) were taken. The singers were asked to fulfil a total of 3 tasks. First task; the artists were asked to take a deep breath and use the vowels /a/ for chest recording and second task; /e-i/ for falsetto [26]. The third task; performers were asked to sustain notes (a piano was used as a reference) to determine the maximum phonation duration and personal pitches with the highest and lowest frequency [27]. The singers were



asked to repeat Staccato phonation consisting of four rising and four descending notes at a 60-metronome rate as long as the maximum phonation time could continue with a single breath [7]. Two measurements were taken from the performers before and after training to familiarise them with the responses to room acoustics.

#### Sound measurements

All sound measurements were performed in a standing position to obtain better respiratory and voice parameters [28]. The microphone stand was customized to suit each participant's needs and was positioned at a distance of 15 cm [17]. To ensure optimal sound quality, we used a professional recording microphone (AKG-C214) known for its high fidelity that explicitly captures the fundamental frequency ( $F_0$ ). The microphone cable was connected to the TL Audio IVORY 2 model preamplifier. The sounds were transferred to the PreSonus sound card and then to digital medium at a sampling rate of 20 kHz and the lowest quantization rate of 16 bits in the model studiolive 32.4.2 AI Cubose pro 8.0 daw software in the computer. Every data related to sound was recorded using the software [7].

#### Pulmonary function tests (PFT)

Peak expiratory flow (PEFmax) and forced expiratory volume (FEV1), which is the volume that has been exhaled at the end of the first second of forced expiration), FEV1 / FVC (Tiffenau index), and After profound inspiration, the total exhaled volume forced vital capacity (FVC) were measured using an MGF Diagnostics CPFS/D USB spirometer. Each participant was fitted with a nose clip and instructed to take as deep a breath as possible through the mouth tube connected to the spirometer and blow as hard and fast as possible. Three trials were obtained and the largest of the three was selected for analysis. Artists with an FEV<sub>1</sub> / FVC <75% and those with a history of lung disease, upper respiratory tract infection, or current voice impairment were excluded. PFT was measured according to the 2002 guidelines of the American Thoracic Society and the European Respiratory Society (ATS/ERS) [29] Subjects were verbally motivated to exert maximal effort in all respiratory tests.

# Maximal inspiratory (MIP) and Expiratory (MEP) pressure measurement

MIP and MEP were measured using a portable hand-held mouth breathing pressure monitor (MicroRPM; CareFusion Micro Medical, Kent, UK) according to the 2002 guidelines of the American Thoracic Society and European Respiratory Society (ATS/ERS) [29]. After appropriate filters and holders were fixed, the nasal airway was closed using a clip. MIP measurements were started with residual volume and MEP measurements were started with total lung capacity. Measurements were repeated until a 5% difference between the two best findings was recorded as the mean cm  $H_2O$  [30].

#### The rate of perceived exertion (RPE)

The rate of perceived exertion (RPE) is a subjective method of measuring an individual's perception of the physical demands of a given activity. In this study, it was used to determine how hard singers labour during vocal performance. The most commonly utilised RPE instrument is the Borg scale, a psychophysical, categorical scale with a rating ranging from 6 (no exertion) to 20 (maximum exertion) (ACSM, 2010). Following the sound performances, the subjects were invited to provide an accurate description of their feelings regarding the intensity of the activity by assigning a number from 6 to 20. This numerical rating was intended to reflect the perceived rate of exertion and the subjective level of fatigue experienced by each participant.

#### Inspiratory muscle warm-up (IWU)

POWERbreathe<sup>®</sup> device (IMT Technologies Ltd., Birmingham, UK) was used for IWU. For IWU, with a 60-s rest between sets, the resistance setting of the POWERbreathe<sup>®</sup> device was determined to be 40% of the MIP, and the subject was asked to breathe 30 (expiratory and inspiratory) breaths in two sets in a standing position [10].

#### Statistical analysis

Statistical analyses were performed using SPSS (version 21.0, IBM Corp., Armonk, NY, USA), with statistical significance set at 0.05. The Shapiro–Wilk normality test was used to assess the normality of the data. The preand post-test differences of each group were determined by a paired comparison test using a paired t-test, and the post-test and pre-test difference values between the groups were assessed using one-way analysis of variance. In addition, in the comparison of paired groups, effect size was calculated according to Cohen's d and was interpreted as: <0.5 (small); 0.05–0.79 (moderate); and >0.8 (large) [31].

#### Results

A total of 16 singers participated in this study, with 8 individuals in the experimental group (SE) and 8 in the control group (SC). Table 1 summarizes the key demographic and physical characteristics of the participants. The participants' ages range with a mean of approximately 30 years across both groups. The Body Mass Index (BMI) across the participants averages to 25.65 kg/m<sup>2</sup>. Experience in years is relatively similar, with the SE group averaging 9.43 years and the SC group averaging 8.71 years (Tables 1, 2), (Fig. 2).

Table 1 Characteristic of the participants

Variable	SE (n=8)	SC (n=8)	Total		
	$mean \pm SD$	$mean \pm SD$	$mean \pm SD$		
Age (year)	31.14±4.91	$29.43 \pm 5.47$	$30.29 \pm 6.40$		
Height (m)	$1.75 \pm 0.11$	$1.73 \pm 0.15$	$1.74 \pm 0.12$		
Body weight (kg)	$81.71 \pm 19.76$	$76.14 \pm 16.69$	$78.93 \pm 19.34$		
BMI (kg/m²)	$26.50 \pm 5.78$	24.81±2.23	$25.65 \pm 4.86$		
Experience (year)	9.43±2.51	8.71±1.70	$9.07 \pm 2.59$		

Note. SE, Singers Experimental; SC, Singers Control

Inspiratory muscle warm-up (IWU) led to substantial improvements in several key respiratory parameters in the SE group, while the SC group showed negligible changes. The most notable increase was in Maximal Inspiratory Pressure (MIP), which increased by 22.9% in the SE group compared to a non-significant change in the SC group (p = 0.005). Additionally, the Maximal Expiratory Pressure (MEP) increased by 14.7% (p = 0.006) in the SE group.

Table 3 presents the various vocal parameters for two groups of singers. There was find significant increases in both high-pitch and low-pitch durations were observed in the SE group, with high-pitch note durations improving by 35.42% (p < 0.001) and low-pitch durations by 28.70% (p < 0.001). The SC group did not exhibit statistically significant changes in these parameters (p > 0.05; Table 3). Improvements in perceived exertion, measured by the Borg Rating, also demonstrated that IWU effectively reduced vocal strain by 11.6% in the SE group (p < 0.006), which was not observed in the SC group. This reduction in perceived exertion underscores the value of IWU as a preparatory tool to ease vocal demands and optimize performance (Fig. 3).

#### Discussion

The findings of this study demonstrated that IWU significantly improved inspiratory and expiratory muscle strength in the SE. Participants reported an 11.6% reduction in difficulty in the Borg Rating of Perceived Exertion after IWU. Additionally, the Singers in IWU showed significant improvements in several vocal parameters, including high pitch frequency, high pitch duration, low pitch duration, and maximum phonation time. In contrast, the control group exhibited minor improvements that were generally smaller and not statistically significant. These findings indicate that the intervention applied to the SE group effectively improved the vocal performance applied in the study.

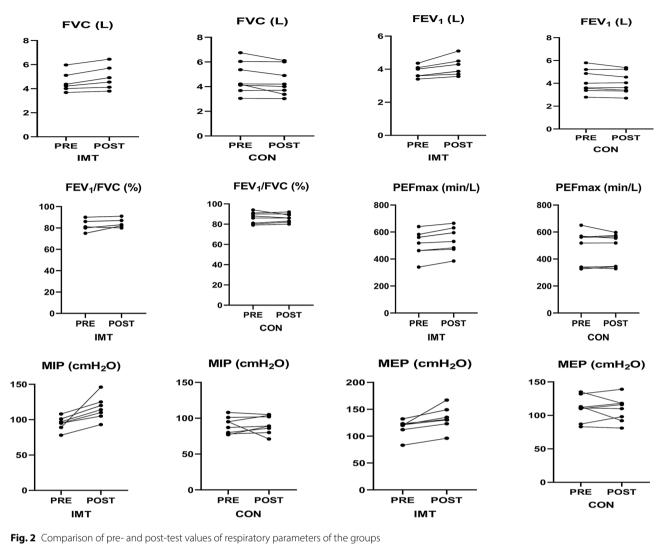
The primary goals of vocal exercises and training are generally to improve vocal function, such as increasing vocal range and enhancing vocal quality. The findings of this study highlight the acute effects of Inspiratory Muscle Warm-Up (IWU) on respiratory muscles and vocal parameters, suggesting a positive impact of exercise on long-term vocal performance, which warrants further investigation. Achieving improved vocal function likely involves altering the muscle activation across multiple subsystems and refining their coordination. In voice therapy or singing training, the main objective is to synchronize the respiratory muscles with the vocal system rather than to strengthen the respiratory muscles. Nonetheless, respiratory muscle endurance is crucial for voice production because it helps reduce fatigue and improves vocal performance during singing or prolonged vocalization [5].

Improved motor unit uptake and increased synergy between active inspiratory muscles may be the dominant mechanisms responsible for the increase in inspiratory muscle strength following IWU [10, 32]. From a physiological perspective, it has been reported that IWU can improve intramuscular coordination and cause more

		SE (n=8)		ES	<b>p</b> <sub>1</sub>	SC (n=8)		ES	p <sub>2</sub>	p <sub>3</sub>
		mean ± SD	(%)	_		mean±SD	mean±SD (%)			
FVC (L)	Pre.	4.53±0.77	8.61	0.463	0.001	4.45±1.35	6.52	0.223	0.063	0.000
	Post	↑4.92±0.91*				↑4.74±1.24				
FEV <sub>1</sub> (L)	Pre	$3.81 \pm 0.35$	8.4	0.703	0.003	$4.03 \pm 1.11$	3.47	0.131	0.087	0.000
	Post	↑4.13±0.54*				↑4.17±1.03				
FEV <sub>1</sub> /FVC (%)	Pre	$81 \pm 3.34$	3.7	1.005	0.032	86±6.04	0.16	0.024	0.885	0.048
	Post	↑84±2.58*				↑86.14±4.51				
PEFmax (min/L)	Pre	$509.29 \pm 98.53$	5.53	0.285	0.001	465.86±134.32	1.26	0.046	0.571	0.003
	Post	↑537.43±98.95*				↑471.71±121.39				
MIP (cmH <sub>2</sub> O)	Pre.	94.71±9.43	22.9	1.594	0.005	88.57±11.41	0.64	0.048	0.877	0.007
	Post	↑116.4±16.77*				↑89.14±12.21				
MEP (cmH <sub>2</sub> O)	Pre	116±15.68	14.7	0.890	0.006	104.71±17.54	2.46	0.160	0.750	0.009
	Post	↑133±21.98*				↑107.29±14.59				

Table 2 Comparison of pre- and post-test values of respiratory parameters and borg scale scores

Note. \* n < 0.05; †increased; | decreased; ES, effect size; SE, Singers Experimental; SC, Singers Control, p1, SE significant value p2, SC significant value; p3 between groups significant value %; rate of change in intervention



		SE(n=8)		ES	p <sub>1</sub>	SC (n = 8)		ES	p <sub>2</sub>	p3
		mean ± SD	%	_		mean±SD	%	-		
Note High-Pitch (Hz)	Pre.	535.01±162.76	17.22	0.467	0.035	$566.59 \pm 204.93$	-8,55	0.239	0.016	0.003
	Post	↑627.13±226.62*				↓518.13±200.22*				
Note Low-Pitch (Hz)	Pre	$222.58 \pm 164.50$	0.94	0.013	0.264	216.13±171.41	-3.30	0.042	0.552	0.437
	Post	224.69±165.64				208.99±162.31				
High-Pitch Duration(sec.)	Pre	$11.63 \pm 5.40$	35.42	0.80	< 0.001	$12.75 \pm 5.53$	4.94	0.118	0.388	0.001
	Post	↑15.75±4.86**				↑13.38±5.13				
Low-pitch Duration (sec.)	Pre	17±1.93	28.70	1.92	< 0.001	15±7.27	4.99	0.119	0.483	0.003
	Post	↑21.88±3.04**				↑15.75±5.06				
Max. Phonation time (sec)	Pre.	$16.63 \pm 3.25$	21.76	1.03	< 0.001	$14.50 \pm 4.66$	7.79	0.241	0.003	0.015
	Post	↑20.25±3.73**				15.63±4.69*				
BORG (6–20)	Pre	$14.86 \pm 0.38$	11.6	1.734	0.006	15.14 <b>±</b> 1.21	0.93	0.284	0.316	0.058
	Post	↓13.14±1.35*				↓14.86 <b>±</b> 0.69				

Note. \*<sub>D</sub><0.05; \*\* <sub>D</sub><0.001; 1increased; 1decreased; E5, effect size; SE, Singers Experimental; SC, Singers Control; p1, SE significant value; p2, SC significant value; p3 between groups significant value; % rate of change in intervention

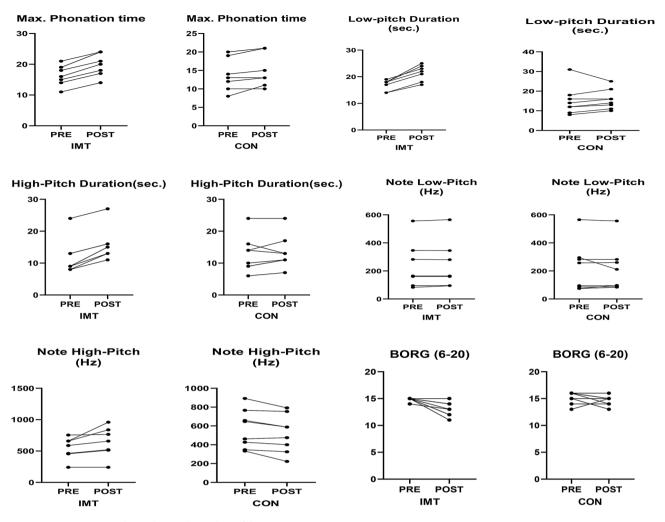


Fig. 3 Note/Frequency value and time relationships of the groups

force generation by eliminating reflex inhibition [33]. In addition, this type of training can help muscles achieve optimal reactive  $O_2$  products [34], which physiologically may help improve conditions for  $O_2$  delivery [35, 36] and improve contractile performance in the respiratory muscles. Furthermore, the beneficial effect of physical warm-up is known to facilitate muscle contraction and relaxation rates and improve movement effectiveness as a result of oxygen delivery and utilization, nerve conduction, and blood flow [37].

The purpose of vocal warm-up is to provide voice therapy or vocal training for singers and to coordinate the three vocal subsystems (breathing, phonation, and resonance). The purpose of the IWU is to prepare the respiratory muscles for exercise or vocal effort, and to make more use of these respiratory parameters. While it was observed that both exercises of the participants in the study affected high-pitch sound frequency, SE reported a higher percentage of improvement by 7.1; however, this effect was not significant in the frequency values of lowpitched sounds. Furthermore, the acute response of the IWU was dominant in the high- and low-pitched note durations and maximum phonation time. This finding highlights the acute positive effect of IWU on short-term respiration [5, 38]. Vocal Function Exercises, Phonation Resistance Training Exercises, and Lee Silverman Voice Therapy [39–42] have been shown to improve one or more vocal functions (maximum phonation time, maximum frequency, or maximum intensity). A previous study showed that vocal warm-up also affects acoustic parameters [3]. Furthermore, the acute response of vocal warm-up was shown to increase phonation threshold pressure after a loud reading task at the highest pitch [43]. However, in this study, the response to vocal warmup contributed to note-High-Pitch only compared to IWU.

The positive effect of IWU on the respiratory muscle and vocal performance can be attributed to neural facilitation. Previous reports have shown that IWU reduces respiratory muscle energy demands, thereby enhancing breathing patterns [11]. Our findings offer acoustic analysis as a valuable tool for demonstrating, evaluating, and measuring the effects of inspiratory muscle warm-up on sound production. It is widely accepted that vocal warmup improves vocal reproduction and facilitates phonation [17, 37, 43–45]. While vocal warm-up prepares the vocal folds and vocal tract for performance and prevents tension [17, 46, 47], it has also been shown to improve pulmonary function [23, 48]. Singers should aim to use their voices with maximum voice production efficiency and effectiveness, while minimizing strain. Therefore, combining vocal warm-up with IWU can better vocal performance, voice production efficiency, and effectiveness.

This study has several limitations. The small sample size restricted their ability to generalize the findings. This research focused on acute effects and did not assess longterm outcomes, limiting the understanding of sustained impacts. All the participants were recruited from a single opera and ballet directorate, thereby restricting the applicability of the results to other singer populations. The study did not address gender differences or account for psychological factors, such as anxiety, which could have influenced the results. Additionally, the research only compared inspiratory muscle warm-up with routine vocal warm-up without exploring varying intensities or types of inspiratory exercises. Each artist has performed vocal warm-ups according to their vocal range and has practiced their unique warm-up routines. Future studies should aim to include larger, more diverse samples, examine long-term effects, consider sex and psychological variables, and investigate a broader range of inspiratory exercise protocols to provide a more comprehensive understanding.

#### Conclusion

This study provides compelling evidence that integrating inspiratory muscle warm-up (IWU) into traditional vocal warm-up protocols significantly enhances various aspects of vocal performance. Our findings indicate that IWU acutely improves maximum phonation time, extends the duration of both high-pitched and lowpitched notes, and broadens the overall vocal range.

These results underscore the critical role of respiratory muscle conditioning in the optimization of vocal performance. The observed improvements in pulmonary function and muscle strength coupled with reduced perceived exertion think IWU as a valuable addition to vocal preparation. This approach not only enhances voice production efficiency, but also reduces the physical strain associated with sustained vocalizations. Given the acute nature of these improvements, further research is warranted to explore the long-term benefits and potential adaptations of regular IWU practice. In addition, expanding the participant pool to include more diverse demographics and examining the impact of varying IWU intensities and protocols will provide a more comprehensive understanding of its efficacy.

In conclusion, our study advocates the incorporation of IWU into standard vocal warm-up regimens, particularly in individuals with lower baseline respiratory function. This practice offers a practical, evidence-based method for enhancing vocal performance, ensuring that singers achieve greater vocal control and endurance with reduced effort.

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#### Author contributions

Conceptualization, C.Y. and Ö.B.; methodology, C.Y.; software, C.Y.; validation, C.Y., Ö.B.; formal analysis, C.Y.; investigation, C.Y., Ö.B., Ö.E., and M.I.A; resources, C.Y.; data curation, C.Y.; writing—original draft prepa-ration, C.Y., ö.B., Ö.E., R.A. and M.I.A; writing—review and editing, C.Y., Ö.B, Ö.E., R.A. and M.I.A; supervision, C.Y. and Ö.B.; project administration, C.Y. All authors have read and agreed to the published version of the manuscript.

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#### Data availability

The supporting data for the findings of this study can be obtained by contacting the corresponding author upon request.

#### Declarations

#### Institutional review board statement

The study was conducted in accordance with the Declaration of Helsinki. This study was approved by the Ethics Committee of the Gümüşhane University (2023/2; dated E-95674917-108.99-164965). All participants had given written informed consent before the data collection began.

#### Informed consent

Informed consent was obtained from all subjects involved in the study.

#### **Competing interests**

The authors declare no competing interests.

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