Nasal Breathing Through a Damp Gauze Enhances Surface Hydration of the Vocal Folds and Optimizes Vocal Function

[1]Maria Borragan, [1]Bruno Gomez Mediavilla, [1]Marian Agudo Legina, [1]M.Jose Gonzalez Fernandez, [2]Dario Strangis, [3]Marco Fantini, [4]Andrea Ricci Maccarini, and [1]Alfonso Borragan Torre, [1]*Santander, Spain, and* [2]*Torino,* [3]*Candiolo, and* [4]*Cesena, Italy*

Abstract: Objective. The present study proposes a direct surface hydration system based on nasal breathing through a damp gauze. The goal is to observe whether such direct hydration procedure positively modifies vocal fold functionality by improving voice quality and enhancing the mobility and pliability of the vocal fold mucosa.
Methods. Sixty-one young adults without voice problems were enrolled and were randomly divided into three double-blinded treatment groups. In the first group (the gauze group), participants breathed through the nose wrapped in a damp gauze for 10 minutes while doing vocal warm-up exercises to facilitate water penetration into the mucosal layers of the vocal folds. The second group (the exercise group) performed the same warm-up exercises as the gauze group for 10 minutes without hydration procedures. The third group (the control group) talked using their normal speaking voice for 10 minutes. The participants were evaluated before and after the treatment tasks with three tests: a laryngostroboscopic examination (ie, the glottic closure, the amplitude of the mucosal wave, and the maximum opening of the glottic space); voice acoustic analysis (multidimensional voice program); and a perceptual voice evaluation (GRBAS scale).
Results. Results showed that after the use of a damp gauze, glottic closure, the amplitude of the mucosal wave, the maximum opening of the glottic space, the shimmer, and the B of GRBAS all improved.
Conclusion. Findings showed significantly better vocal results for the participants of the gauze group, suggesting the damp gauze procedure to be an effective, fast, and economical procedure to improve and optimize vocal fold functionality. It can be hypothesized that the obtained results are related to an improvement in vocal folds surface hydration and viscoelasticity.
Keywords: Vocal folds—Voice disorder—Voice prevention—Hydration—Humidity—Moisturization.

INTRODUCTION

Hydration is the process of providing an adequate amount of water to body tissues. Good body hydration has several positive benefits. As regards the voice, better hydration may help prevent vocal fold damage. It has been observed that excessively dry vocal folds are more susceptible to the negative impact of collision occurring during vibration than hydrated vocal folds.[1,2] This is because hydration performs like a shock absorber to protect vocal folds from injuries. Hydration also protects the airways because it acts as a physical and biochemical barrier against inhaled particulates and pathogens. In addition, vocal fold hydration improves the functionality of the vocal folds.[3] Namely, adequate hydration ensures that the mucosa reaches optimal biomechanical conditions, thus increasing the efficiency of oscillation and mucosal wave.[3]

As reported by several authors, in order to promote a better mucosal wave[4,5] and to improve voice quality,[2,6] vocal folds need good mobility and pliability.[7,8] A good hydration support reduces the viscosity of the mucosal layers of the vocal folds[1,2] meaning that the lower the viscosity, the lower the resistance of the tissues to deformation.[4,9] Therefore, the quality of the mucosal wave may be a good indirect indicator of the hydration of the vocal folds.[1,10] Even if some devices come close to reading the amplitude of the mucosal wave (eg, videokymography),[11] there is still no meter that guarantees a precise measure of the mucosal wave's amplitude. For this reason, several research tools are used to indirectly measure the amplitude of the mucosal wave, as in the case of photoglottography, which measures the glottic space variations, or laryngostroboscopy, that subjectively evaluates vocal fold movements.[12,13]

Recent research has focused on hydration induced improvement of vocal fold functionality. Two types of hydration techniques have been proposed: systemic hydration, referring to an oral intake of water that hydrates the whole body; and direct surface hydration of the vocal folds, obtained by inhaling through humidifiers and inhalers.[14,15] Overall, several studies outlined that hydrated mucosa requires less subglottic pressure than dry mucosa to begin oscillatory movements, determining a decrease of the phonatory threshold pressure (PTP) and favorable changes in some acoustic microperturbation parameters, such as shimmer.[1,2,4,14,16] The results of these studies suggest a direct relationship between improved vocal fold hydration and the ease and smoothness of glottic vibration.

Systemic hydration has been shown to be the easiest and most cost-effective solution to improve voice quality so far.[17] However, though systemic hydration is good for

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From the [1]CFL, Centro de Foniatria y Logopedia, Santander, Spain; [2]Voce InForma – Centro Vocologico Internazionale, Torino, Italy; [3]Head and Neck Oncology Service, Candiolo Cancer Institute, FPO IRCCS, Candiolo, Italy; and the [4]Hospital Maurizio Bufalini, Cesena, Italy.
Address correspondence and reprint requests to Maria Borragan Centro de Foniatria y Logopedia (CFL), Calle Castilla 9 2izq, Santander, Spain 39002. E-mail: mborragan@foniatriaylogopedia.com
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general body hydration, it takes a long time to achieve improved vocal fold hydration. In order to prepare vocal activity in a wide range of conditions factors (eg, talking for hours, high volumes, dry environments, the moment before a vocal performance, feeling dry throat), the vocal fold mucosa needs a large and immediate supply of hydration as it will face intense and prolonged frictions. As a consequence, a quick and efficient hydration system is required to make the vocal tissues more mobile and deformable.[4] Thus, systemic hydration is not sufficient for this purpose since improved vocal hydration is achieved very slowly and unpredictably (the amount of water reaching different areas of the body will vary in accordance with the body's hydric homeostasis). Furthermore, it should be pointed out that ingestion of an excessive amount of water (i.e., recommended daily amount of water intake - around 1.5 liters of water per day for a sedentary adult[18,19]) - could promote reflux (ie, in people with reflux or with hiatal hernia) and, consequently, greater laryngeal dryness.[20] This which in turn can be counterproductive and start a vicious circle of drinking water/increasing reflux and dryness.

For this reason, direct hydration seems to be a great option. Direct surface hydration by means of humidifiers providing water in microparticles in suspension could be ideal. However, some evidences suggest that this may not be as effective as we expect. The reason for this is that the size of water droplets produced by humidifiers (ie, aerosols) entering the nose or the mouth is very small and light, around 5 μm. Once these water microparticles, enter the nose or the mouth, they penetrate easily and quickly into the lower respiratory system, drying rapidly before settling,[21,22] and they may not penetrate into the vocal folds mucosa. Note that this droplet size produced by the humidifiers is necessary for medical purposes (ie, drugs delivery in the lower respiratory tract and alveoli).[23,24] On the contrary, larger water droplets (>10 μm) tend to remain trapped in the nose and throat without drying out completely, permitting longer permanence of the humidity.[25] Then, we hypothesized that water droplets bigger that 10 μm could remain longer without drying completely in the nose and throat, yielding hydration in the vocal folds. Therefore, they may penetrate the vocal fold tissues thanks to the mucosal permeability, promoting favorable changes in the hydration of the mucosal layers and their viscoelastic properties.[7]

Thereby, it aims for direct hydration of the vocal folds by means of water droplets larger than 10 μm. In the present study, we hypothesize that hydration must follow the respiratory path through the nose and we propose a direct surface hydration method by breathing through the nose wrapped in a damp gauze.[26-29] We hypothesize that the damp gauze could produce water droplets bigger than 10 μm, as the water is in its natural state, and nor broken down by machines into less effective smaller droplets. The gauze is chosen as a vehicle for hydration because of its cross-linked structure, which can retain water very well (i.e., water remains in the gauze, despite some water being inhaled with each breath).[26-29] Hydration through the damp gauze should be done accompanied by vocal warm-up exercises performed for 10 minutes in order to promote water penetration in the vocal folds tissues just like an ointment needs massage to help its absorption by the skin.[30,31] Thereby, the present study aims to observe whether direct surface hydration obtained by breathing through the nose wrapped in a damp gauze for 10 minutes combined with vocal warm-up exercises modifies the mucosal wave and the vocal quality in a vocally healthy population. To do so, three groups (gauze, exercise, and control) were tested and compared through laryngostroboscopic examinations; acoustic analysis and perceptual evaluations. The tests were carried out before (pre) and after (post) the treatment.

METHODS

Participants

A total of sixty-one young adults (mean $_{age}$ = 23.49 years, SD $_{age}$ = 2.9) took part in this study. Participants were students recruited from the University Gimbernat-Cantabria (Spain) with no prior or ongoing vocal pathology. None of them referred to having a history of elite voice use (eg, singing, acting, teaching). The study was carried out in the CFL clinic (Centro de Foniatría y Logopedia in Santander) with an evaluator, a phoniatrician with 40 years of experience (phoniatrics is a medical specialty focusing on voice, speech, language, hearing, and swallowing disorders), who blindly evaluated the participants. Five speech therapist's experts in voice disorders had the task of accompanying, supervising and guiding the participants throughout the whole experimental setting.

The experiment was carried out prospectively and double-blinded, because neither the participants nor the evaluator knew the treatment group assigned to each participant. There were three different treatment groups: exercise group (20 participants), gauze group (21 participants), and control group (20 participants). The groups were set up to have the same number of females so as to control gender differences in voice parameters (16 females in each group).

Participants were appropriately informed, and signed consent forms before the experiment. The protocol was carried out according to the guidelines approved by the CFL (Centro de Foniatria y Logopedia) Ethics Committee in line with the Professional College of doctors and of Speech Therapists of Cantabria. Students were rewarded with a present after the experiment.

Procedures

The entire study lasted for about 30 minutes, during which participants followed the same procedure: initial self-assessment, pretest, treatment task, and post-test. Participants were individually tested before (pretest) and after (post-test) a double-blinded assigned treatment task (gauze, exercise, and control) with three tests (laryngostroboscopic examinations, acoustic analysis, and perceptual evaluations). Each participant was accompanied all the time by one speech

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therapist for guiding and supervision. A program, presented on a 21-inch PC monitor running Experiment Builder software, assigned the participant to a treatment group, blindly and randomly by gender. The participants did not know which treatment group they had been placed in.

Firstly, participants carried out an initial self-assessment before they started the study. They filled out a personal survey, in order to evaluate the inflammatory factors that could affect the degree of vocal well-being at the level of the glottis and of the upper respiratory tract, as previous research showed that they could affect the voice quality.[32,33] Those factors should be taken into account in order to control that all participants have the same homogeneity in terms of their voice quality. They had to subjectively rate different parameters according to their own perception. They were asked to rate on an ordinal scale going from 0 to 10 their perception of the degree of dysphonia (ie, the question in Spanish was "tu voz es ronca," which is the informal term, and everybody has a subjective perception, to refer that the voice sounds breathy raspy, strained or will be soften in volume or lower in pitch). Also, they had to rate on ordinal scales from 0 to 4 (where 0 is none and 4 is a lot) the degree of vocal effort while speaking, how much they liked their voice, the amount of tobacco consumed per week, the alcohol consumed per week, the degree of nuisance perceived while speaking, and the propensity of colds suffered per year. Lastly, participants had to fill in the Spanish version of the Voice Handicap Index (VHI-30)[34] with its three subscales: emotional, physical, and functional. A total score (possible range, 0-120 points) and subscale scores (possible range, 0-40) were calculated; the higher the score, the greater the voice handicap.

Before and after the treatment tasks, participants were evaluated through laryngostroboscopies, acoustic analysis and perceptual evaluations. Laryngostroboscopic examinations were carried out by the experienced phoniatrician with a 70° Karl Storz telelaryngoscope and a Karl Storz Pulsar stroboscopic light source[35] and were recorded on a Windows PC Inter Core i5-6400 2.70 GHz using Adobe Premiere Pro 2.0.[36] Laryngostroboscopy creates a low-motion illusion of vocal fold vibration by compiling images at different points (30 frames per second) of each vibratory cycle, helping clinicians to interpret the physiology of the vocal folds (eg, the symmetry and periodicity of glottic vibration, glottic closure, profile of vocal fold, edge, amplitude of vocal folds vibration and mucosal wave).[37–39] Although other techniques can be employed to measure the mucosal wave (eg, high speed digital imaging, Videokymography), we chose this tool because it is the most commonly used by clinicians and has the advantages of a low-cost, quick and easy technique.[40] Furthermore, there are no significant differences found when comparing mucosal wave parameters extracted from laryngostroboscopic images and other techniques in patients with no voice problems.[13]

To evaluate the glottic function, three measures of interest were deducted from the laryngostroboscopic examination: two subjective parameters (the amplitude of the mucosal wave and the glottic closure) and one objective parameter (the maximum opening of the glottic space). The glottic closure parameter measures the degree of glottic competence and can be complete, inconstant, or incomplete. The experienced phoniatrician, blind to the treatment group of the participants, evaluated the degree of the glottic closure rating from 0 (complete closure) to 4 (incomplete closure). The amplitude of the mucosal wave parameter evaluates the progression of the wave generated by the flowing of the vocal fold epithelium on the vocal ligament due to the lax tissue contained in the superficial layer of the lamina propria, driven by subglottic pressure, myoelastic, and aerodynamic forces. Also, the experienced phoniatrician rated this parameter on a scale from 0 (absence of mucosal wave) to 4 (normal mucosal wave). In order to have reliability in those subjective measures, an inter-rater reliability analysis was conducted. Three experienced judges outside the experimental process were asked to evaluate the amplitude of the mucosal wave and the glottic closure. The external judges were three external speech therapists who have an experience of more than 35 years evaluating and treating voice disorders. They were instructed to follow the same evaluation procedure as the phoniatrician, rating from 0 to 4 the laryngostroboscopic examinations recordings.

The objective laryngostroboscopic measure, the maximum opening of the glottic space, was taken into account basing measurements on the width of the vocal folds defined by Hirano,[38] which subjectively measures the distance of the vocal fold's edge from the middle area of the glottis to the side. The width is an indicator of how much the mucosal wave moves during phonation. Trying to avoid this subjective evaluation, the maximum opening of the glottic space in relation to the width of the vocal folds was measured objectively. To do so, each participant's recorded laryngostroboscopic video was frozen at the frame of maximum glottic opening during the vibratory cycle using Adobe Premiere Pro CS5.5. A trained speech therapist identified the line of the maximum glottic space opening and drew a horizontal line going from the Morgagni's ventricle of the right vocal fold to the contralateral one, allowing measurement of the total pixel width of the vocal folds and glottic space. The ratio between the glottic space and the total width of the vocal folds was then calculated and expressed as a percent measurement (glottic space width X 100/VFs total width). This measurement is constant regardless of the distance of the glottis from the endoscopy lens and the variation in the size of the larynx between the participants.

Concerning perceptual evaluation, voices were assessed through the GRBAS scale[41,42] by the phoniatrician and the external experienced judges. Mean values of the perceptual ratings were considered. The GRBAS scale is a widely used perceptual evaluation tool specifically addressing the following parameters of voice quality: grade (G), roughness (R), breathiness (B), asthenicity (A), and strain (S). Each parameter scores from 0 (normal voice) to 3 (severe dysphonia). Lastly, for the acoustic analysis, the multidimensional voice program (MDVP)[43] was used to measure a series of acoustic

parameters of the voice quality, such as microperturbations (Jitter, RAP, PPQ, shimmer, APQ), macroperturbations (SPPQ, sAPQ) and variations of the fundamental frequency (F0), the noise parameters (NHR, VTI, SPI), tremble parameters (FTRI, ATRI) and the subharmonic and broken voice parameters (DVB, DSH, DUV). However, only three of these parameters, the mean fundamental frequency (F0), the frequency variation from cycle to cycle (jitter) and amplitude variation of the sound wave (shimmer), were considered as measurements of interest due to their connection with the sound wave quality. The test was carried out by the speech therapist in a soundproofed room (<30 dB). Each participant's voice was recorded with a Rode NT1-A microphone in standard conditions, with a mouth-to-microphone distance of 30cm and constant gain. The microphone was connected via USB to a computer with a Windows operating system running Sound Forge version 9.0 software. Participants were asked to produce a sustained vowel /a/. A mid-vowel selection of 3 seconds was performed for the acoustic analysis (they were asked to repeat the vowel three times but only one vowel was analyzed chosen by the evaluator by his perception)

In the treatment group, each participant was given proper instructions through a tutorial, presented on a 21-inch PC monitor running Experiment Builder software, and the speech therapists supervised and guided the proper execution of the treatment task that lasted for ten minutes. Previous research showed that vocal warm-up duration is considered to last 10 minutes in order to have some voice perceptual changes.[44,45] Note that after 10 minutes performing a task, attention declines.[46,47] In the gauze group, participants had to use sterile gauze (non-woven swabs). They had to soak the sterile gauze in still water at a temperature of around 25°C inside a glass. In order to remove excess water, participants had to squeeze the gauze, and after that, they had to place the damp gauze around the nose, completely covering the nostrils (see Appendix 1). They were then asked to breathe normally through the nose wrapped in the damp gauze and to produce some vocal warm-up exercises (ie, sustained /i/ and /u/ vowels at different volumes and tones). The following sequence tasks were proposed: breathe normally through the nose (three times); perform an /u/ vowel at a low pitch and high volume as comfortable as possible, sustaining this for three seconds (this helps to create a large and wide mucosal wave); breathe normally through the nose (three times) and perform an /i/ vowel in an ascending-descending glissando one time (this helps to lengthen and narrow the vocal folds). The sequence task lasted around one minute, so participants could repeat the sequences 10 times approximately. Participants were instructed to dampen the gauze after each sequence (lasting for about one minute) in order to always have a wet gauze. They had to repeat the described sequence for ten minutes.

In the gauze group, vocal warm-up exercises were included to improve the effect of water penetration into the mucosal layers. This aspect was taken into account in order to observe whether some effects were influenced by the vocal warm-up exercises and not simply by the surface hydration itself. Therefore, two more experimental groups were included and compared: the exercise group and the control group.

In the exercise group, participants had to produce the same vocal warm-up exercises as described above for 10 minutes, without the damp gauze. Participants were asked to breathe normally through the nose (three times), to perform a low pitch and high volume /u/ vowel (sustained for three seconds); to breathe normally through the nose (three times) and to perform a sustained /i/ vowel in ascending-descending glissando (one time).

In the Control group, participants had to breathe normally through the nose (three times) and respond to some questions in a normal speaking voice (eg, "What did you do yesterday?" or "Count from 1 to 20") for 10 minutes.

Statistical analysis

Statistical analysis was carried out with Jamovi software (version 0.9.6.7 for Windows). Mean and standard deviations (SDs) for initial self-assessment and the three tests (laryngostroboscopic, acoustic and perceptual analysis) were calculated. The normality of the distributions was assessed with the Shapiro-Wilk test. An alpha of 0.05 was considered for statistical procedures. Although data did not have normal distributions, analyses were carried out with parametric tests. Non-parametrical analysis is recommended when data are not normal distributed. However, recent literature has shown that a parametrical test, such as ANOVA, was robust in terms of good control of type 1 errors in non-normal distribution.

Initial self-assessment was used to control the homogeneity of the sample. The different assessments were analyzed separately in order to observe whether participants from the three treatment groups had similar parameters of vocal well-being. To do so, one-way ANOVAs were carried out by the three treatment groups.

The inter-rater reliability of the subjective measurements were analyzed through Kappa statistics[48] to observe the reliability between the three external judges' ratings and the phoniatrician's rating. In case of excellent agreement between the three external judges' and the phoniatrician's ratings, the analysis of the dependent variable was carried out averaging the four ratings, i.e., those of the phoniatrician and the three external judges. Note that the Kappa value above 0.75 reflects excellent agreement (+1 indicates perfect agreement and 0 chance).

The eleven dependent variables (glottal closure, amplitude of mucosal wave and maximum opening of the glottic space, Fo, jitter and shimmer, G - global grade of dysphonia, R – roughness, B – breathiness , A – asthenicity, S – strain) were examined separately to predict how each dependent variable could be influenced by direct hydration. Each dependent variable was analyzed separately through two-way ANOVAs with the within-subjects predictor defined as Test Moment and the between-subjects predictor defined as

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treatment groups. To follow up possible interactions for each dependent variable, consecutive analyses were performed. In order to observe whether the three groups performed equally in the Pre-Test, a one-way ANOVA was carried out. Also, a one-way ANOVA of the post-test condition was conducted to assess if significant differences between the three groups could be observed after the treatment task. Then, unpaired-sample t-tests (ie, if the samples were dependent between them) were carried out to compare the three treatment groups in the post-test condition and observe which group might modify the vocal quality and the mucosal wave.

RESULTS

The initial self-assessment showed none of the parameters of vocal well-being had significant differences between the three treatment groups (see Table 1). This indicated that the three treatment groups had homogenous samples.

Mean and standard deviations of the laryngostroboscopic, perceptual and acoustic parameters before and after the proposed treatment tasks in the three groups (control, exercise, and gauze group) are shown in table 2.

The Kappa test analysis showed that there was excellent agreement between the three external judges and the phoniatrician's ratings (glottic closure: pretest 0.80, CI = 0.68-0.83 and posttest 0.86, CI = 0.75-0.90; amplitude of the mucosa wave: pretest 0.81, CI = 0.69-0.84, and posttest 0.80, CI = 0.68–0.83; G: pretest 0.89, CI = 0.75-0.91 and posttest 0.92, CI = 0.81-0.95; R: pretest 0.83, CI = 0.65-0.83, and posttest 0.85, CI = 0.68-0.85; B: pretest 0.84, CI = 0.67-0.84, and posttest 0.85, CI = 0.69-0.87; A: pretest 0.88, CI = 0.73-0.90, and posttest 0.92, CI = 0.81-0.95; S: pretest 0.88, CI = 0.73-0.90, and posttest 0.88, CI = 0.75-

TABLE 1.
Descriptive Statistics of Initial Self-Assessments

	Control Group	Exercise Group	Gauze Group	ANOVAs	
				F (df)	*P*
Age	23.6 (3.27)	23.3 (3.01)	23.57 (2.84)	*F*[2, 38] = 0.06	0.943
Dysphonia	2.55 (0.83)	2.65 (0.81)	2.19 (0.68)	*F*[2, 38] = 2.21	0.124
Vocal effort	0.6 (0.6)	0.55 (0.6)	0.81 (0.75)	*F*[2, 38] = 0.79	0.462
Like the voice	6.5 (2.44)	6.45 (1.93)	6.33 (1.59)	*F*[2, 38] = 0.04	0.960
Tobacco	0.65 (1.14)	0.6 (1.19)	0.57 (1.5)	F[2, 38] = 0.02	0.981
Alcohol	0.5 (0.61)	0.9 (1.25)	0.9 (1.37)	F[2, 38] = 1.31	0.282
Nuisance	0.7 (0.47)	0.5 (0.51)	0.52 (0.51)	F[2, 38] = 1.01	0.376
Colds	1.5 (1.64)	1.45 (3.27)	1.81 (1.29)	F[2, 38] = 0.27	0.764
VHI	3.7 (3.67)	4.3 (3.06)	4.43 (3.37)	F[2, 38] = 0.24	0.788

Note: Values reported are means and standard deviations in parenthesis of age (in years), subjective degree of dysphonia (0-10 scale), degree of vocal effort (0-4 scale), tobacco per week (0-4 scale), alcohol per week (0-4 scale), acid reflux (0-4 scale), propensity of colds per year (0-4 scale), and VHI (in a scale from 0 to 40). The last column shows the results from one-way ANOVAs comparing the three treatment groups on the different assessments.

TABLE 2.
Descriptive Statistics Before (Pre) and After (Post) the Treatment Tasks in the Three Groups (Control Group, Exercise Group, and Gauze Group)

	Control Group		Exercise Group		Gauze Group	
	Pre	Post	Pre	Post	Pre	Post
Glottic space	19.1 (3.08)	19.3 (2.55)	20.6 (3.7)	21.0 (3.15)	18.81 (4.5)	24.0 (4.99)
Glottic closure	1.72 (1.04)	1.58 (1.07)	1.36 (0.99)	1.27 (0.97)	1.33 (1.11)	0.51 (0.78)
Amplitude	2.51 (1.01)	2.48 (1.01)	2.11 (0.97)	2.4 (0.93)	1.93 (0.75)	2.99 (0.57)
G	0.2 (0.46)	0.1 (0.18)	0.1 (0.23)	0.03 (0.08)	0.07 (0.11)	0.05 (0.17)
R	0.13 (0.24)	0.16 (0.23)	0.16 (0.23)	0.13 (0.23)	0.08 (0.19)	0.11 (0.27)
B	0.56 (0.49)	0.42 (0.59)	0.73 (0.48)	0.57 (0.52)	0.69 (0.55)	0.17 (0.38)
A	0.03 (0.01)	0.05 (0.01)	0.06 (0.11)	0.05 (0.11)	0.08 (0.12)	0.05 (0.1)
S	0.21 (0.32)	0.1 (0.23)	0.16 (0.38)	0.11 (0.24)	0.13 (0.27)	0.08 (0.18)
Fo	201 (31)	204 (39.7)	213 (32.3)	214 (32.2)	207 (33.8)	206 (50.5)
Jitter	0.66 (0.27)	0.68 (0.21)	0.73 (0.24)	0.68 (0.15)	0.75 (0.28)	0.71 (0.27)
Shimmer	3.45 (1.03)	3.21 (0.98)	3.72 (1.01)	3.49 (0.68)	3.55 (0.89)	2.43 (0.57)

Note: Values reported correspond to the means and standard deviations (in parenthesis) of glottis (% of glottic space), glottal closure (0-4 scale), amplitude (0-4 scale), GIRBAS (0-4 scale), Fo (Hz), jitter (%), and shimmer (%).

0.91). Both the phoniatrician and the external judges had similar work experience so this may also influence when evaluating similarity. Then, those results showed that the phoniatrician ratings were as accurate as the three external judges. Therefore, the analysis was carried out with the average ratings of the phoniatrician and the three external judges.

Results regarding Maximum Opening of the Glottic Space showed a significant effect of Test Moment (F[1, 58] = 34.1, $P < 0.001$, $\eta_p^2 = 0.05$) showing more glottic space after all treatments. Although there was no significant effect of treatment groups (F[2, 58] = 2.03, $P = 0.141$, $\eta_p^2 = 0.052$), the interaction between the two variables was significant (F[2, 58] = 26.1, $P < 0.001$, $\eta_p^2 = 0.075$). To follow up this interaction, pretest and posttest conditions were analyzed separately using one-way ANOVAs. The pretest condition showed no significant effect (F[2, 38] = 1.27, $P = 0.293$, $\eta_p^2 = 0.001$), suggesting that the three groups had no significantly different maximum opening of the glottic space before each treatment. However, the post-test showed a significant effect (F[2, 38] = 37.8, $P = 0.001$, $\eta_p^2 = 0.042$) suggesting that groups varied significantly in the posttest condition. An unpaired t-test comparing each group in the posttest was carried out to observe which group differed significantly from the others. Results showed that the gauze group had significant differences compared with the control group and the exercise groups (t[19] = 3.66, $P = 0.002$, Cohen's d = 0.819 and t[19] = 2.26, $P = 0.036$, Cohen's d = 0.505, respectively). The exercises and control groups showed no differences between them (t[19] = 1.70, $P = 0.106$, Cohen's d = 0.379). The described results are shown in Figure 1.

Glottic Closure showed a significant effect of test moment (F[1, 58] = 14.89, $P < 0.001$, $\eta_p^2 = 0.033$), showing that all groups had better glottic closure in the posttest condition, and of treatment groups (F[2, 58] = 3.24, $P = 0.046$, $\eta_p^2 = 0.082$). Also, there was a significant interaction between the two variables (F[2, 58] = 5.02, $P = 0.010$, $\eta_p^2 = 0.022$). To follow up this interaction, one-way ANOVA for pretest and posttest conditions were carried out. The pretest condition did not show a significant effect (F[2, 38] = 0.86, $P = 0.429$, $\eta_p^2 = 0.004$), suggesting that the three groups had similar glottic closure before the treatment. However, in the post-test condition a significant effect was found (effect F[2, 38] = 7.20, $P = 0.002$, $\eta_p^2 = 0.026$) suggesting that in the gauze group glottic closure changed after the treatment. Then, an unpaired t-test of posttest moments comparing the three treatments was carried out showing that gauze group was significantly different from the control group (t [19] = 4.54, $P = 0.002$, Cohen's d = 0.792) and exercise group (t[19] = 2.36, $P = 0.029$, Cohen's d = 0.582), but the other two groups did not show significant differences (t[19] = 1.34, $P = 0.197$, Cohen's d = 0.299) suggesting that after the gauze group there was more glottic closure (Figure 2).

The amplitude of the mucosal wave showed a significant effect of Test Moment (F[1, 58] = 30.5, $P < 0.001$, $\eta_p^2 = 0.067$) suggesting that the three groups had a greater amplitude in the posttest, but with no effect on treatment groups (F[2, 58] = 0.410, $P = 0.665$, $\eta_p^2 = 0.011$). The interaction between the two variables was significant (F[2, 58] = 11.5, $P < 0.001$, $\eta_p^2 = 0.050$). To follow up the interaction, as for the other variables, a one-way ANOVA for pre and posttest conditions was carried out. Results showed that in the pre-test, mean amplitudes were not significantly different (F[2,38] = 1.40, $P = 0.259$, $\eta_p^2 = 0.031$), but in the posttest condition the difference was significant (F[2, 38] = 3.67, $P = 0.036$, $\eta_p^2 = 0.098$). The unpaired t-test showed that the gauze group significantly differed from the other groups (control group: t[19] = 2.12, $P = 0.047$, Cohen's d = 0.476 and exercise group: t[19] = 2.35, P = 0.020, Cohen's d = 0.567), and no significant differences were found between these two groups (t[19] = 0.29, $P = 0.772$, Cohen's d = 0.066), suggesting that the gauze group showed significantly greater amplitude of mucosal wave after the warm-up (Figure 2).

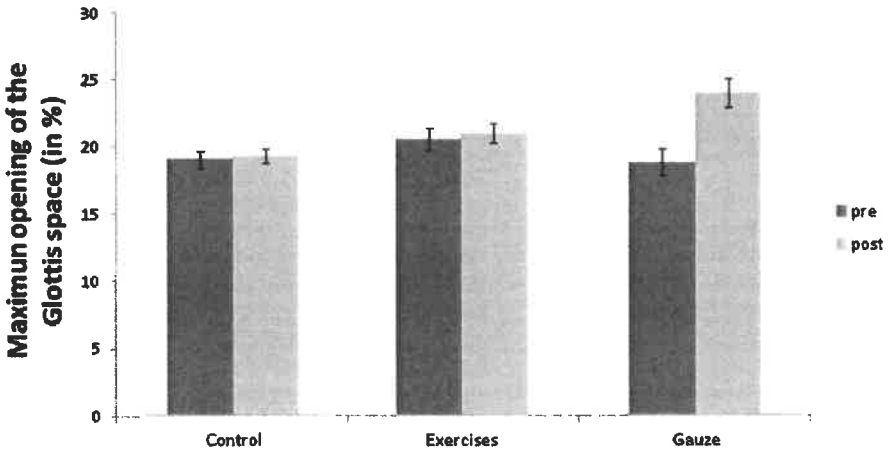


FIGURE 1. Bar plots of the maximum opening of the glottic space (in %) for the pre- and post-test condition of the three groups (control group, exercise group, and gauze group). error bars refer to the standard error (SE) of each mean.

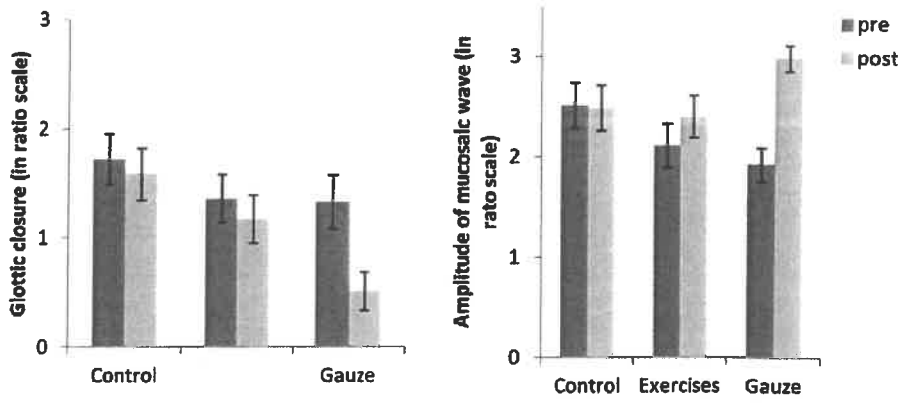


FIGURE 2. Bar plots of the glottic closure and the amplitude of mucosal wave for the pre- and post-test condition of the three groups (control group, exercise group, and gauze group). Error bars refer to the standard error (SE) of each mean value.

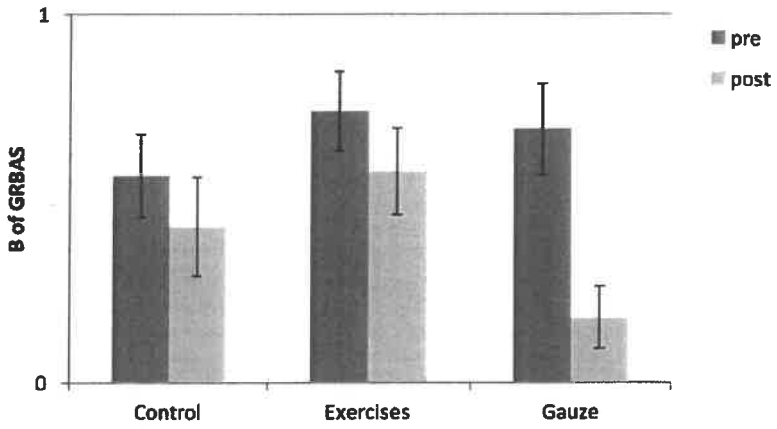


FIGURE 3. Bar plots of the B of GRBAS for the pre- and post-test condition of the three treatments (control group, exercise group, and gauze group). Error bars refer to the standard error (SE) of each mean.

The G of GRBAS did not show any significant effect (all $P > 0.12$), neither the R (all $P > 0.31$), the A (all $P > 0.11$) and the S (all $P > 0.13$). However, the B showed a significant effect of Test Moment ($F[1, 58] = 15.79$, $P < 0.001$, $\eta_p^2 = 0.0565$) and interaction between the two variables ($F[2, 58] = 3.20$, $P = 0.048$, $\eta_p^2 = 0.026$). The pretest one-way ANOVA showed that the three groups were not significantly different ($F[2, 38] = 0.658$, $P = 0.524$, $\eta_p^2 = 0.022$), but the Post-Test one-way ANOVA was significant ($F[2, 38] = 4.01$, $P = 0.027$, $\eta_p^2 = 0.062$). The unpaired sample t-test showed that the gauze group was significantly different from the other two (Control group: $t[19] = 2.17$, $P = 0.043$, Cohen's $d = 0.486$ and exercise group: $t[19] = 3.48$, $P = 0.002$, Cohen's $d = 0.780$) and that the control and the exercise groups did not differ significantly ($t[19] = 0.76$, $P = 0.453$, Cohen's $d = 0.171$), suggesting that only the gauze group improved breathiness after warm-up (Figure 3).

Acoustic analysis showed that the F0 variations were not significant (all $P > 0.63$) nor the Jitter variations (all $P > 0.53$). However, the shimmer showed a significant effect of test moment ($F[1, 58] = 20.55$, $P < 0.001$, $\eta_p^2 = 0.079$) and treatment groups ($F[2, 58] = 3.69$, $P = 0.031$, $\eta_p^2 = 0.073$) with a significant interaction between the two variables ($F[2, 58] = 6.48$, $P = 0.003$, $\eta_p^2 = 0.050$), suggesting that the gauze group was different from the other two. The pretest one-way ANOVA analysis showed that the three groups were not significantly different before the treatment ($F[2, 38] = 0.35$, $P = 0.703$, $\eta_p^2 = 0.026$), but after the treatment they differed significantly ($F[2, 38] = 15.41$, $P < 0.001$, $\eta_p^2 = 0.018$). The unpaired t-test showed that the gauze group differed from the other two groups after the treatment (control group: $t[19] = 3.31$, $P = 0.004$, Cohen's $d = 0.741$ and exercise group: $t[19] = 5.52$, $P < 0.001$, Cohen's $d = 1.235$), while no significant differences were differences were found between exercise and control group ($t[19] = 0.97$, $P = 0.341$,

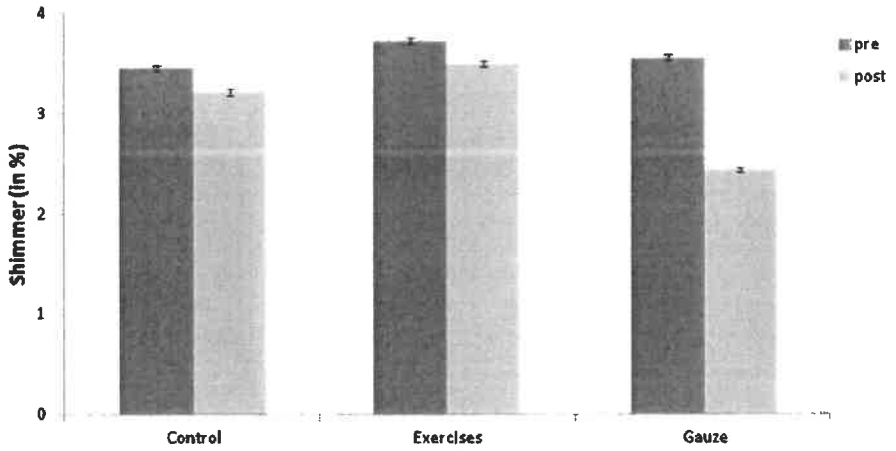


FIGURE 4. Bar plots of the shimmer (%) for the pre- and post-test condition of the three groups (control group, exercise group, and gauze group). Error bars refer to the standard error (SE) of each mean.

Cohen's d = 0.218), suggesting that after the gauze treatment task participants had significantly better shimmer values (Figure 4).

DISCUSSION

The aim of the present study was to investigate whether breathing through the nose with a damp gauze for ten minutes improves vocal folds functionality and, as a consequence, mucosal wave and voice quality. For this purpose, participants were recruited and divided into three treatment groups (gauze group, control group, and exercise group) and evaluated before (pretest) and after (posttest) a group-specific task through laryngostroboscopic examinations, acoustic analysis, and perceptual evaluations. Results showed that, after the damp gauze treatment, significant changes occurred concerning vocal fold functionality, mucosal wave quality (the glottic closure, the amplitude of mucosal wave, and the maximum opening of the glottic space), and perceptual and acoustic voice quality (B – breathiness- of GRBAS scale and shimmer form the MDVP).

Referring to the present results, only after the damp gauze treatment vocal fold functionality and voice quality significantly improved. We attempted to control all variability possible between participants. Thus, we evaluated the inflammatory factors background with an initial self-assessment of vocal well-being, which showed that all treatment groups were homogenous. This suggested that results driven by the treatments could not be influenced by differences between participants because all had similar voice backgrounds (eg, no elite use of voice, inflammatory factors), but rather for the treatment itself.[49]

Also, in order to improve the effect of water penetration into the mucosa, participants of the gauze group were asked to emit sounds while breathing through the damp gauze. The movement of the mucosa might increase water penetration into the tissue, just like an ointment needs massage to help its absorption by the skin.[30,31] For this reason, the proposed vocal warm-up exercises consisted of ascending-descending glissandos with the /i/ vowel (favouring a longitudinal stretching of the vocal folds) and very low and high volume sustained /u/ vowels (promoting a transversal vocal folds stretching). However, such modifications are not related to the vocal warm-up exercises (the Exercise groups did not show significant changes after ten minutes performing the same warm-up tasks), but with the combination of both, vocal warm-up exercise and surface hydration.

Thereby, these results suggest that direct surface hydration through damp gauze improve the vocal fold functionality and perceptual and acoustic voice quality. This change was made after ten minutes of using the damp gauze. Previous research explored the benefits of hydrate directly the vocal folds through breathing through the nose with humidifiers.[14,15] The task was to breathe normally in a room with a humidifier for a period of four hours. Humidifiers are a good solution to hydrate the vocal folds; however, the difference between the damp gauze and the humidifiers resides in the time of administration. Water droplets expelled by the humidifiers are smaller than 10 μm, and it is known that droplets of this size fall into the lower respiratory tract rapidly and do not stay in the upper tract long.[23,24,50] However, droplets bigger than 10μm remain longer in the upper respiratory tract.[23,24,50] Then, it seems, that droplets of the damp gauze may be bigger than 10 μm as these may stay longer in the throat and after breathing those droplets for ten minutes. This may suggest the fact that administering bigger droplets may benefit the amount of time required for physiological and perceptual changes.

Other researchers exploring direct hydration used different substances in order to hydrate vocal folds.[14] But the majority previously dehydrated the upper respiratory tract in order to observe changes. In this study we do not consider previous dehydration as we seek the benefit of treatment on daily life. But highlighting our rationale of the use of the damp gauze it should be noted that previous research evidenced that nasal

breathing increased the humidity of inspired air compared with oral breathing.[51] Then, breathing water through the nose with damp gauze might represent an advantageous procedure to provide hydration for vocal folds rapidly and efficiently. Such positive effects could be very helpful for those who use their voice professionally in dry environments; for those who want to maintain an optimal hydration during long travels by plane (where dry and conditioned air normally favors mucosal dryness); and for those who need to put their voice in optimal hydration conditions quickly and effectively before performances (eg, actors and singers).

Specifically, our results show changes in vocal fold functionality (mucosal wave), perceptual quality (breathiness) and acoustic quality of voice (shimmer and jitter) after using the damp gauze. It could be hypothesized that breathing through a damp gauze promotes water penetration into the vocal fold mucosa, thus changing its viscoelastic properties and promoting improved tissue pliability and mobility.[7,9] This effect might be due to the interaction between water molecules and mucosal glycoaminoglycans (GAGs)[52,53] located in the lamina propria, producing a swelling effect on the superficial layers of the vocal folds. As a consequence, vocal fold mucosa might become more turgid, flexible and elastic.[9,11] The effects of these events can be observed through laryngostroboscopy, as the maximum glottic opening is greater, the glottic closure is better, and the amplitude of the mucosal wave is higher, as reported in the present study.

Previous research already showed that proper hydration reduces viscosity of the vocal fold mucosa, producing favorable changes in voice quality (ie, jitter and shimmer) and reducing subglottic pressure[1,2,15,16,54] and our findings seems to be in line with the literature. It is known that higher subglottic pressure is needed to sustain effective phonation in conditions of mucosal dryness. To this respect, thanks to hydration, it seems possible that hydrated vocal folds favor less transglottic airflow leakage and – as a consequence - reduced breathiness. Since laryngostroboscopic parameters used for the present study (glottic closure, amplitude of the mucosal wave, maximum opening of the glottic space) showed significant improvements after breathing through a damp gauze, they may be considered promising indirect indicators for vocal folds surface hydration status.

Some considerations and limitations of the present study should be mentioned. One limitation is represented by the small number of recruited subjects and the lack of medium- and long-term analysis after hydration procedures. Another not negligible limitation is represented by the lack of direct measures of surface hydration status of the vocal folds. Future research should look for strategies for quantifying surface hydration and observing whether damp gauze hydration benefits by itself or by the combination of both damp gauze breathing and vocal exercises. Also, future research should better understand the role hydration plays for the phonatory system and its short- and long-term effects on larger populations (including subjects suffering from voice pathologies), as well as direct lubrication techniques and specific effects of vocal folds lubrication.

CONCLUSIONS

The present study shows that breathing through the nose wrapped with a damp gauze for ten minutes promotes favorable effects on laryngostroboscopic, perceptual and acoustic voice parameters. Proper hydration of the vocal folds not only favors the production of a better voice, but may also increase the turgidity of the mucosa. Even if surface hydration level was not directly measured, it can be hypothesized that vocal folds surface hydration obtained through the described technique may help reach maximum vocal performance with minimum effort, producing a feeling of vocal well-being and a reduction in vocal fatigue.

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SUPPLEMENTARY DATA

Supplementary data related to this article can be found online at doi:10.1016/j.jvoice.2021.06.023.

Supplementary data this video link: https://www.youtube.com/watch?v=JZw6TFsTVGQ.

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