

The Effects of Menopause on Vocal Folds' Vibratory Characteristics of Female Professional Voice Users

*¹Mauro B. Fiuza, ^{†2}Filipa M.B. Lã, ^{‡3}Pluvio J. Coronado, and ^{§,4}Magda Sofia Roberto, *^{†‡}Madrid, Spain, and [§]Lisbon, Portugal

Summary: Introduction. Menopause has been associated with changes in the vocal folds; however, whether these changes impact on vocal folds' oscillation and collision patterns is still unknown.

Materials and Methods. Phonation and collision threshold pressures (PTP and CTP, respectively) were compared between premenopausal and postmenopausal female professional voice users (FPVUs), allocated into levels 1 and 2 according to professional use of their voice (ie, singers/singing teachers and schoolteachers, respectively). Audio, electroglottographic, and intraoral pressure signals were recorded while participants performed diminuendo sequences on the syllable /pa/ at pitches A3, E4, and A4. Hormonal profiles and self-perceived voice symptoms were also assessed.

Results. Voice-related menopausal symptoms were self-perceived as mild in postmenopausal FPVUs. No statistically significant differences in PTP and CTP were found between professional groups for the interaction between reproductive status (ie, premenopausal and postmenopause) and professional level (ie, singers/singing teachers and schoolteachers) for any of the analyzed pitches, despite significant differences in concentrations of pituitary hormones and estradiol. No significant correlations between hormones and voice metrics could be found.

Conclusions. The increasing numbers of menopausal FPVUs and the great individuality in degree of severity of menopause-related voice symptoms justify further investigations, including professional voice users, especially those complaining of more severe menopause-related voice symptoms.

Key Words: Menopause—Female professional voice users—Vocal folds' oscillation patterns—Phonation threshold pressure—Collision threshold pressure.

INTRODUCTION

Menopause is characterized by a significant reduction in concentrations of sex steroid hormones (ie, estrogens, progesterone, and testosterone) and an increase in concentrations of pituitary hormones, especially follicle-stimulating hormone (FSH).^{1,2} These hormonal variations have been associated with several bodily symptoms, including changes in voice quality.³ For example, the fundamental frequency (f_0) was found to be lowered in post- as

compared with premenopausal women for both speech and sustained vowel /a/.^{4,5} In addition, postmenopausal singers have complained of decreased singing range and changes in timbre, endurance, flexibility, stability, and breath management.^{6–8} Moreover, it is possible that these symptoms have contributed to the reduced self-efficacy with respect to voice function perceived by postmenopausal singers when singing high, middle, and transitional regions of their voices.⁹

After menopause and especially with the onset of biological senescence, the vocal folds tend to become thicker¹⁰ and to show a higher incidence of an anterior glottal gap at closure.¹¹ Also, edema, dryness, stiffness, and increased vascularity are observations commonly associated with postmenopause.^{12–14} Given that both tissue viscosity and hydration levels, both reported as potential menopause-related voice alterations, affect vocal folds' vibratory capacity,¹⁵ one may argue that vocal folds of postmenopausal women will require a higher pressure to initiate phonation and collision.

The depletion in concentrations of estradiol (E2) with menopause has been associated with loss of collagen content and elastin fibers in several bodily tissues, such as, for example, the skin¹⁶ and laryngeal membranes.⁶ In ovariectomized rats (ie, menopause-induced group), the vocal folds display a significant edema, and diminished collagen and elastin content as compared with controls (estrus group).¹⁷ In human vocal folds, such alterations could result in a reduction of vocal folds' vibratory willingness, and, consequently, increased phonation and collision threshold pressures (PTP and CTP, respectively). The

Accepted for publication May 12, 2025.

From the *Escuela Internacional de Doctorado de la Universidad Nacional de Educación a Distancia (EIDUNED), Faculty of Education, National Distance Education University (UNED), Madrid, Spain; †Department of Didactics, School Organization and Special Didactics, Faculty of Education, National Distance Education University (UNED), Madrid, Spain; ‡Women's Health Institute, Hospital Clinico San Carlos, IDISSC, School of Medicine, Complutense University, Madrid, Spain; and the §CICPSI, Faculty of Psychology, Lisbon University, Lisbon, Portugal

¹ National Distance Education University (UNED), Escuela Internacional de Doctorado de la Universidad Nacional de Educación a Distancia (EIDUNED), Faculty of Education, Calle Juan del Rosal 14, 28040 Madrid, Spain.

² National Distance Education University (UNED), Faculty of Education, Department of Didactics, School Organization and Special Didactics, Calle Juan del Rosal 14, 28040 Madrid, Spain.

³ Women's Health Institute, Hospital Clinico San Carlos, IDISSC, School of Medicine, Complutense University of Madrid, Martín Lagos, s/n, 28040, Madrid, Spain.

⁴ CICPSI, Faculdade de Psicologia, Universidade de Lisboa, Alameda da Universidade, 1649-013 Lisboa, Portugal.

Address correspondence and reprint requests to: Filipa M.B. Lã, Faculty of Education, Department of Didactics, School Organization and Special Didactics, UNED Voice Lab, National Distance Education University (UNED), Calle Juan del Rosal 14, 28040 Madrid, Spain. E-mail: filipa.la@edu.uned.es

Journal of Voice, Vol xx, No xx, pp. xxx–xxx
0892-1997

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<https://doi.org/10.1016/j.jvoice.2025.05.014>

present investigation aims at testing this hypothesis, comparing PTP and CTP between premenopausal and postmenopausal female professional voice users (FPVUs).

PTP corresponds to the minimum air pressure in the lungs that is required to initiate vocal folds vibration.¹⁸ PTP increases with increasing vocal folds viscosity, mucosal wave velocity, and prephonatory glottal width.^{18,19} On the contrary, it decreases with increasing vocal fold thickness.¹⁹ CTP is defined as the least amount of air pressure required to initiate vocal folds collision and it is strongly correlated with PTP.²⁰ As CTP is determined by using higher pressures, this metric is easier to measure as compared with PTP, especially for nontrained voices, who generally have difficulties in performing the softest phonation possible without changing pitch.²¹ Thus, CTP seems to be a relevant complementary measure to PTP, especially when these metrics are extracted from diminuendo sequences of a given pitch.²²

To assess whether PTP and CTP change with menopause seems relevant to FPVUs, especially to singers and schoolteachers. These professional groups both depend on the quality of their voices to work. For singers, even mild changes in voice quality may interfere with their ability to mastering their voices as an artistic mean to convey expressivity. For schoolteachers, the quality of their voices is crucial to the comprehension ability and learning possibilities of their students.²³ Schoolteachers are often identified as professionals with a high risk of developing occupational voice pathologies, partially due to the lack of voice-related educational programs and preventive strategies.⁷ It is therefore expected that teachers with voice complaints may exhibit higher PTP and CTP when compared with singers. Singers are generally highly aware of voice care strategies and often regulate them to increase vocal strength, endurance, and power.²⁴ According to vocal exercise physiology, training the coordination between vocal subsystems within a wide range of frequencies, intensities, and velocity, helps maintaining healthy voice function.²⁵ In addition, voice training might help to reduce voice changes related to menopause,²⁶ and to mitigate adverse age-related laryngeal changes,²⁷ partially due to breath management and laryngeal effort optimization.^{28,29} On the other hand, it is also possible that singers may have higher PTP and CTP as compared with schoolteachers. The high levels of vocal demand and vocal loading associated with certain singing genres may result in an increase in both PTP and CTP. To clarify these questions, the present study tests two hypotheses: (1) postmenopausal FPVUs require higher PTP and CTP as compared with premenopausal professionals; and (2) the impacts of menopause on singers' PTP and CTP are milder as compared with schoolteachers.

MATERIALS AND METHODS

Participants

Ethical approval was granted by the Ethical Committee for Research at the National Distance Learning University (UNED) (Ref. Voice@menopause) and by the Ethical

Committee for Scientific Research at the Hospital Clínico San Carlos (CEIC) (ref. 20/674-E) to conduct this cross-sectional study comparing premenopausal and postmenopausal FPVUs. The recruitment procedure started in January 2021 and lasted about 6 months and was made through (i) authors' personal contacts; (ii) emails to schools, professional choirs, and music conservatoires; (iii) social media; (iv) Facebook groups of singers; and (v) on-line music teachers' platforms in Madrid area. Recruitment targeted female teachers and singers who were healthy and had no current untreated endocrinological, respiratory, nor neurological pathologies. All participants were Caucasian and Spanish native speakers. They belonged to either one of the two professional levels of professional voice users' classification system based on voice use and vocal demand:³⁰ level 1, singers and teachers of singing; and level 2, schoolteachers. As with respect to reproductive stage, participants were recruited based on menstrual cycle regularity and history of amenorrhea, following criteria proposed by the stages of reproductive aging workshop (STRAW). Although hormonal concentrations were measured to confirm correct allocation of participants into premenopausal and postmenopausal groups, the remaining criteria recommended by STRAW, namely antral follicle count and concentrations of Inhibin B, were not financially viable and therefore were not included.³¹ According to the STRAW system, reproductive aging is represented in a continuous timeline where menopause is the reference point (stage 0). Menopause is preceded by stages -5, -4 and -3, corresponding to early, peak, and late reproductive stages, respectively, and by stages -2 and -1, which correspond to early and late perimenopause, respectively. Stages +1 and +2, on the other hand, follow menopause and correspond to early and late postmenopause, respectively.³¹

Participants included in the premenopause group presented (i) regular menstrual cycles (in cases of irregular menstrual cycles, amenorrhea did not surpass more than three consecutive months); (ii) concentrations of E2 higher than 25 pg/mL; and (iii) concentrations of FSH lower than 30 IU/L. Participants included in the postmenopausal group should have (i) 12 or more consecutive months of amenorrhea without surpassing five consecutive years; (ii) concentrations of E2 lower than 25 pg/mL; and (iii) concentrations of FSH higher than 30 IU/L. The cutoff values for these hormones followed the results of previous literature on premenopausal and postmenopause hormonal profiles. According to the STRAW aging system, women in late perimenopause should have concentrations of FSH higher than 25 IU/L.³¹ As to what concerns E2 concentrations, these should be lower than 30 pg/mL.³² To guarantee that only clear cases of premenopausal and postmenopausal voices were included in the present investigation, cutting values used to guide participant's classification into postmenopause included FSH concentrations higher than 30 IU/L and of E2 lower than 25 pg/mL. To minimize possible confounding effects of age on menopause sole-related vocal characteristics, only

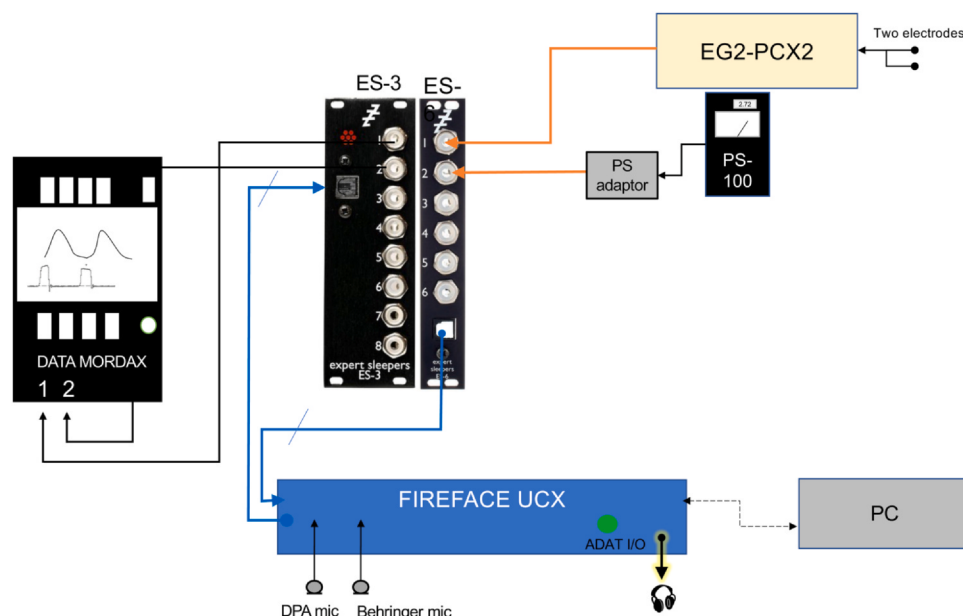


FIGURE 1. Recording setup.

females with more than 40 and less than 65 years old were recruited. Exclusion criteria included the presence of self-reported untreated endocrinological dysfunctions (eg, hypo-/hyperthyroidism), ovariectomy, voice problems, and the use of hormonal-based contraception or medication, such as oral contraceptive pill (OCP) and hormone replacement therapy (HRT). Information on voice education, years of professional experience, hours of occupational daily voice use, menopause-related symptoms (including vocal), and general health status was also collected. A clinical evaluation was carried out by an expert gynecologist in menopause (author PC) for all participants prior to the beginning of the study. Blood samples and multichannel voice recordings were both carried subsequently for all participants on the same day.

Recording procedures

A custom-made sound-treated tent was set up in a room at the Gynecology Department of the Hospital Clínico San Carlos to ensure reverberation time and noise levels suitable for voice recordings. Synchronous recordings of audio, electroglottographic (EGG), and intraoral pressure signals were carried out. For participants in the premenopausal group, recordings were made at middle follicular and luteal phases of the menstrual cycle, according to participants' self-reports of day of last menstruation. The audio signals were captured by means of two microphones attached to an external sound card (Fireface UCX, RME Audio, Germany): an omnidirectional condenser headset microphone (4066 DPA Microphones, Denmark), placed at 5 cm from the center of the participant's mouth, and an omnidirectional condenser measurement microphone (ECM8000, Behringer, Germany), placed on a stand at 30 cm from the front of the participant's mouth. The EGG

signal was picked up by two electrodes held against the neck, one at each side of the thyroid notch, by means of an elastic band. They were connected to an EG2-PCX 2 device (Glottal Enterprises, USA). Contact gel was applied when necessary to improve electric conductivity between the two electrodes, observed by the quality of the live EGG shape displayed in real time by the recording software (version 2.4, by Sten Ternström, Sweden).³³ Intraoral pressure peaks were captured via a small plastic tube introduced in the corner of the mouth and attached to a PS-100 subglottal pressure monitor (Glottal Enterprises, USA). Both EGG and PS-100 devices were connected to an AC/DC converter ES-6 interface (Expert Sleepers Limited, UK), and their signals sent by an ES-3 interface (Expert Sleepers Limited, UK) to an oscilloscope (Data Mordax Systems, USA) for their visualization in real time. The ES-3 interface also allowed the synchronization between the ES-6 interface and the external sound card, from which all signals were sent to a PC via a USB cable. [Figure 1](#) shows the recording setup. The recording software FonaDyn allowed concomitant recordings of both alternative (AC) and direct (DC) current signals, recorded at sampling rates of 44.1 kHz and 100 Hz, respectively.

Except for the EGG, all signals were calibrated prior to the recordings. A sound-level calibrator (Extech Instruments, Germany) was used to calibrate the audio signal. It produced a constant sinusoidal 1-kHz tone with a sound pressure level (SPL) set for 94 dB. This tone was captured by the ECM8000 microphone that was completely sealed inside the calibrator, and its gain adjusted to match the sound level of the calibrator in the RME sound mixer interface. To calibrate the DPA microphone, the participant was asked to sustain the vowel /a/ on a constant pitch and loudness; this microphone gain was then adjusted in

the RME sound mixer interface to match the SPL captured by the ECM8000 microphone. Intraoral pressure signals were calibrated by inserting the end of the plastic tube in a container filled with a known volume of water. The associated volume change and corresponding pressure peak were recorded, and the corresponding pressure value in cmH₂O indicated in the PS-100 monitor announced in the recording.

For measurements of both PTP and CTP, participants were asked to perform a set of diminuendo sequences using the syllable /pa/ while holding a plastic tube inside the corner of the mouth at one hand, and attached to the PS-100 monitor at the other end. To produce flat pressure peaks, participants were trained prior to the recording to perform the diminuendo sequence as legato as possible and voided of aspirated and soft /p/ or /b/ pronunciations, thus following a similar methodological approach as described elsewhere concerning subglottal pressure (P_{sub}) measures as estimates of intraoral pressure.^{34,35} The intraoral pressures peaks resulting from /p/ occlusions were recorded and then measured.³⁶ This vocal task was repeated at least five times for each of the three recorded pitches: A3 (± 220 Hz), E4 (± 333 Hz), and A4 (± 440 Hz). The reasons for these pitches' selection were twofold. First, they fall within and above the speaking fundamental frequency range reported elsewhere for female voices of comparable ages: between 199.7 and 220.8 Hz.³⁷ Second, the recording protocol was tested prior to the beginning of this study with four female nonsingers. In these pilot recordings, the performance of diminuendo sequences in pitches higher than A4 was vocally taxing.

Voice analysis

The custom-made Sopran software (Svante Granqvist, Tolvan Data, Sweden) was used to extract PTP and CTP values. It allows visualization of both pressure peaks and derivatives of the squared EGG signal, the latter used to guide the choice of pressure peaks for PTP and CTP measurements. PTP was computed as the average between the last pressure peak in the diminuendo sequence that produced voicing and the immediately following peak producing absence of voicing. CTP was calculated as the average between the last pressure peak in the diminuendo sequence producing vocal fold contact and the immediately following peak voided of vocal fold contact. The choice of peaks to extract both PTP and CTP, represented in Figure 2, was confirmed by visualization of both audio and derivative of the squared EGG signals, following the recommendations of previous studies extracting these metrics also from diminuendo sequences.^{35,38,39} As each diminuendo sequence was performed at least five times, the values of PTP and CTP were averaged between renderings for each of the three recorded pitches.

Clinical evaluations and hormonal samples

Prior to the recordings, all participants were interviewed by a trained clinical psychologist and observed by an expert

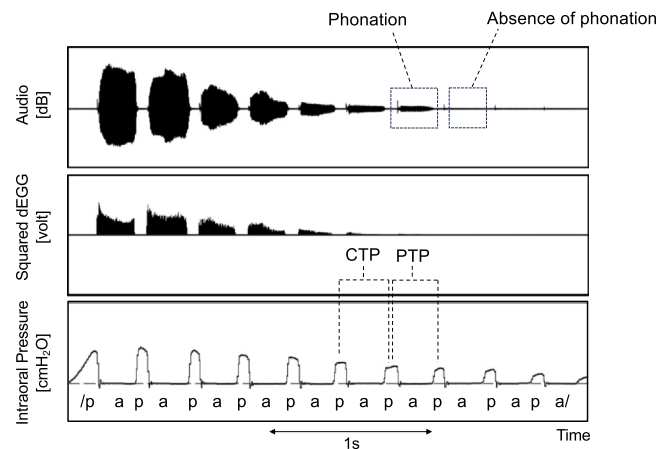


FIGURE 2. Example of the recorded signals in a diminuendo sequence. From top to bottom: audio, squared EGG derivative (dEGG), and intraoral pressure peaks. Pressure peaks selected to extract phonation threshold pressure (PTP) and collision threshold pressure (CTP) are also indicated.

clinician in menopause (author PC). The interview included questions related to reproductive status, such as regularity of menstrual cycles, presence/absence and duration of amenorrhea, questions related to general health status and body mass index (BMI), menopause-related symptoms, including those concerning voice, demographic questions (such as age, profession, marital status, and term pregnancies), and information on occupational voice use, such as years of professional experience and daily voice use. Clinical evaluations included menopausal assessment.⁴⁰ To confirm that participants have been correctly allocated into premenopausal and postmenopausal groups, all participants were also observed by an authorized nurse who took a blood sample for the assessment of hormonal profiles. Blood samples included evaluations of concentrations of gonadotropins, ie, FSH, and luteinizing hormone (LH), and of sex steroid hormones, ie, E2, progesterone (P), and testosterone (T). In addition, other complementary hormones to evaluate androgenic profile were also measured, namely antimüllerian hormone (AMH), androstenedione (A), and dehydroepiandrosterone sulfate (DHEAS). The free androgen index (FAI), representing the bioavailable testosterone in women,⁴¹ was also calculated as the ratio between testosterone and sex-hormone-binding globulin (SHBG).⁴² These specific hormones were chosen because they allow, on the one hand, the correct identification of reproductive status when information on follicle production is absent, particularly with respect to information on concentrations of FSH and E2,³¹ and, on the other hand, inferences on possible changes in circulating androgens.⁴² All hormonal concentrations were taken from blood samples and measured in serum. In vitro quantification of A was made using Immulite 2000XPi analyzer (Siemens, Munich, Germany). Access DXI-800 analyzer (Beckman Coulter, Brea, California, USA) was used for in vitro quantification of all remaining hormones.

Statistical analysis

A descriptive statistical analysis was carried out to characterize FPVUs in both reproductive and professional groups. To compare professional and menopausal groups with respect to categorical variables (such as singing genre, type of teaching institution, voice education, BMI, smoking habits, and history of past health issues), Fisher exact tests were applied when more than 20% of expected cell counts were less than 5. To compare age, days of amenorrhea, professional experience, hours of professional voice use, history of past pregnancies, and current presence of voice and menopause-related symptoms, a robust ANOVA test with median method was used. The same test was applied to compare PTP and CTP between groups and for the three analyzed pitches (A3, E4, and A4). A Spearman correlation test was carried out to investigate relationships between voice metrics and hormonal concentrations. Statistical analyses were carried out using the statistical package for social sciences SPSS (v.30.0.0.0) and the software jamovi (v. 2.2.5). The robust and nonparametric estimation carried out in the latter software was considered to take into account the small sample size used. The statistical level of significance considered for all analysis was $P < 0.05$.

RESULTS

Participants' characteristics

A total of 65 FPVUs were recruited. However, 17 participants were excluded from analysis: (i) one withdrawal prior to the beginning of the study; (ii) two presented sinusoidal EGG signals and/or high-frequency noise components; (iii) seven did not present hormonal profiles concordant with the expected values for clear cases of premenopausal or postmenopause; (iv) four where neither level 1 nor 2 FPVUs; (v) three produced intraoral pressure peaks that, according to what has been described in the literature, could not provide good estimates of P_{sub} .⁴³ Therefore, analyses were carried out only for 48 females (49.4 ± 6.1 years.; age range between 40 and 63 years).

Participants were divided into four groups, according to their reproductive status and voice-related profession: (i) level 1 premenopause ($n = 10$); (ii) level 1 postmenopause ($n = 10$); (iii) level 2 premenopause ($n = 13$); and (iv) level 2 postmenopause ($n = 15$). Table 1 displays participants' characteristics, distributed according to these four groups. Information on significant differences between groups with respect to reproductive status, occupational level, and the interaction between these two factors is also provided. As expected, postmenopausal FPVUs are older, have more years of professional experience, have more days of amenorrhea, and complain more about hot flushes. Also, postmenopausal females have had more pregnancies in both professional groups. With respect to differences between occupational levels, as expected, level 1 professionals show higher voice education as compared with level 2. In addition, level 2 FPVUs complained more of voice

symptoms as compared with level 1, with majority of reports being a "weaker" voice.

Hormonal concentrations

As to what concerns hormonal concentrations, the results suggest differences between reproductive status. As expected, postmenopausal FPVUs presented higher concentrations for both pituitary hormones, ie, LH and FSH, whereas concentrations of E2 were lower (Table 2).

Phonation and Collision threshold pressures

PTP and CTP datasets collected in this study were plotted as a function of f_0 and compared with PTP values calculated using Titze's¹⁸ equation for female nonsingers. As shown in Figure 3, for both premenopausal and postmenopausal datasets, PTP values followed those predicted by Titze's equation: the higher the pitch, the greater the PTP. Moreover, CTP tended to be between 2- and 3-cmH₂O higher than PTP, corroborating previous findings reported elsewhere, discussing the relationship between CTP and PTP.²¹

The violin plots presented in Figure 4 display distributions of PTP for premenopausal and postmenopausal FPVUs, according to their occupational group (level 1 and level 2) and for the three analyzed pitches, using density curves. The curve's width represents the approximate frequency of each data point. Also, the marker within the boxplot represents the median of the data, and the whisker the range.

As can be observed in Figure 4 and by the results of the statistical tests displayed in Table 3, there are no significant differences between groups for all the three analyzed pitches.

Figure 5 displays the violin plots comparing CTP values between participants in both reproductive groups (ie, premenopausal and postmenopause) and according to their occupational level for the three analyzed pitches.

As observed, no statistically significant differences were found between groups for the three analyzed pitches; the results of the corresponding statistical tests are displayed in Table 4.

Relationship between voice metrics and endocrinological profiles

To investigate possible relationships between voice metrics, hormonal profiles of premenopausal and postmenopausal FPVUs (ie, FSH, LH, and E2), and age, Spearman correlations were carried out. As shown in Table 5, no statistically relevant correlations were found for any of these parameters.

DISCUSSION

The present study investigates the impacts of menopause on vocal folds' oscillation and collision patterns. The rationale behind this research is related to the fact that menopause has been associated with changes in the vocal

TABLE 1.
Participants' Characteristics, Distributed According to Reproductive Status (Premenopause and Postmenopause) and Professional Voice Use Occupation (Levels 1 and 2)

	n (%) or Median (IQR)				Q (p _{menopause})	Q (p _{professional})	Q (p _{interaction})
	Level 1		Level 2				
	Pre	Post	Pre	Post			
	(n = 10)	(n = 10)	(n = 13)	(n = 15)			
Age	44.5 (2)	56 (5)	43 (5)	53 (3)	45.61 (<0.001)	2.00 (0.158)	3.85 (0.050)
Prof. experience [yrs.] †	17 (5)	32 (12)	14 (9)	27 (16)	9.47 (0.002)	0.77 (0.379)	1.36 (0.244)
Voice use [h/d]	4 (2)	4 (2)	4 (1)	4 (1)	0.00 (>0.999)	0.00 (>0.999)	0.00 (>0.999)
Singing Style							
Modern singer	6 (30)	1 (5)			0.087		
Classical singer	2 (10)	6 (30)					
Choir singer	2 (10)	2 (10)					
Singing teacher only	0 (0)	1 (5)					
Teaching Institution							
Primary			1 (2.8)	3 (8.4)	0.874		
Secondary			8 (22.4)	8 (22.4)			
Primary and secondary			1 (2.8)	2 (5.6)			
University			0 (0)	1 (2.8)			
Professional and adult			1 (2.8)	1 (2.8)			
Special education			1 (2.8)	0 (0)			
Conservatory			1 (2.8)	0 (0)			
Voice education					<0.001		
No	0 (0)	0 (0)	10 (20.8)	9 (18.8)			
Yes, in the past	0 (0)	1 (2.1)	0 (0)	4 (8.3)			
Yes, currently	10 (20.8)	9 (18.8)	3 (6.3)	2 (4.2)			
Voice Education (4/5, very often)							
Voice training	7 (14.6)	6 (12.5)	1 (2.1)	1 (2.1)	0.091		
Singing lessons	2 (4.2)	1 (2.1)	1 (2.1)	0 (0)			
Sing in a choir	2 (4.2)	5 (10.4)	0 (0)	1 (2.1)			
Training with SLP	0 (0)	0 (0)	0 (0)	0 (0)			
Lifelong education	1 (2.1)	1 (2.1)	1 (2.1)	0 (0)			
Days of amenorrhea	17 (9)	1461 (529)	14 (11)	731 (822)	33.92 (<0.001)	3.90 (0.048)	3.84 (0.050)
BMI					0.462		
Underweight	0 (0)	0 (0)	1 (2.1)	2 (4.2)			
Normal weight	5 (10.4)	5 (10.4)	9 (18.8)	6 (12.5)			
Overweight	4 (8.3)	3 (6.3)	1 (2.1)	7 (14.6)			
Obese	1 (2.1)	2 (4.2)	2 (4.2)	0 (0)			
Smoker							
No, never	7 (14.6)	9 (18.8)	11 (22.9)	9 (18.8)	0.860		
No, but yes in the past (>6 months)	2 (4.2)	1 (2.1)	2 (4.2)	5 (10.4)			
Yes (<10 u/d)	1 (2.1)	0 (0)	0 (0)	1 (2.1)			
Thyroid Problems							
No	9 (18.8)	7 (14.6)	11 (22.9)	13 (27.1)	0.703		

TABLE 1 (Continued)

	n (%) or Median (IQR)				Q (p _{menopause})	Q (p _{professional})	Q (p _{interaction})
	Level 1		Level 2				
	Pre	Post	Pre	Post			
	(n = 10)	(n = 10)	(n = 13)	(n = 15)			
Yes, treated with drugs	1 (2.1)	3 (6.3)	2 (4.2)	2 (4.2)			
<i>Endocrinological Problems</i>							
No	9 (18.8)	10 (20.8)	10 (20.8)	15 (31.3)	0.631		
Polycystic ovary syndrome	1 (2.1)	0 (0)	3 (6.3)	0 (0)			
<i>Other Health Problems</i>							
Autoimmune diseases	0 (0)	0 (0)	1 (2.1)	0 (0)	> 0.999		
Respiratory allergies	0 (0)	1 (2.1)	0 (0)	0 (0)			
Thyroidectomy	0 (0)	0 (0)	1 (2.1)	0 (0)			
<i>Voice Symptoms (4/5—always)</i>							
Hoarseness	0 (0)	0 (0)	1 (2.1)	2 (4.2)	0.185		
Vocal fatigue	1 (2.1)	2 (4.2)	2 (4.2)	1 (2.1)	0.223		
Dryness	1 (2.1)	4 (8.4)	3 (6.3)	8 (16.8)	0.269		
Pain	0 (0)	0 (0)	0 (0)	0 (0)	0.313		
Weak voice	0 (0)	0 (0)	3 (6.3)	3 (6.3)	0.042		
Burning sensation	1 (2.1)	0 (0)	0 (0)	0 (0)	0.729		
Shortness of breath	0 (0)	2 (4.2)	0 (0)	1 (2.1)	0.247		
Vocal discomfort	0 (0)	2 (4.2)	0 (0)	1 (2.1)	0.368		
Difficulty speaking	0 (0)	0 (0)	0 (0)	0 (0)	0.632		
Aphonia	0 (0)	0 (0)	1 (2.1)	3 (6.3)	0.080		
Changes in timbre	0 (0)	0 (0)	2 (4.2)	2 (4.2)	0.185		
Voice breaks	0 (0)	0 (0)	2 (4.2)	1 (2.1)	0.930		
Hot flushes (4/5)	0 (0)	1 (2.1)	0 (0)	5 (10.4)	7.86(.005)	0.49 (.483)	2.50 (0.114)
Term pregnancies	0.5 (2)	2 (1)	1 (2)	2 (2)	7.90(.005)	0.32 (.574)	0.66 (0.418)

Results of Robust Anova with median method effects (Q) for discrete variables are also presented. Fisher's exact test was computed for categorical variables. Abbreviations: IQR, interquartile range.

TABLE 2.**Participants' Hormonal Concentrations According to Reproductive Status (Premenopause and Postmenopause) and Professional Voice Use Occupation (Levels 1 and 2)**

Hormones	Median (IQR)				Q ($p_{\text{menopause}}$)	Q ($p_{\text{professional}}$)	Q ($p_{\text{interaction}}$)
	Level 1		Level 2				
	Pre	Post	Pre	Post			
	($n = 10$)	($n = 10$)	($n = 13$)	($n = 15$)			
FSH [UI/L]	8.7 (10.10)	82.15 (32.1)	7 (4.3)	71.5 (25.8)	46.73 (<0.001)	0.37 (0.541)	0.84 (0.360)
LH [UI/L]	7.45 (16.4)	32.35 (12.7)	5.2 (5.8)	32.5 (14.3)	16.83 (<0.001)	0.03 (0.869)	2.43 (0.119)
E2 [pg/mL]	111.65 (142)	14.99 (0)	102.2 (64.7)	14.99 (0)	9.18 (0.002)	0.02 (0.876)	-
P [nmol/L]	6.76 (6.47)	0.69 (0.64)	4.42 (22.48)	0.33 (0.29)	1.18 (0.277)	0.08 (0.883)	0.05 (0.828)
T [nmol/L]	1.05 (0.80)	0.65 (0.71)	0.8 (0.3)	0.6 (0.71)	1.01 (0.314)	0.25 (0.615)	0.13 (0.721)
AMH [ng/mL]	0.71 (1.32)	0.019 (0)	0.53 (2.66)	0.019 (0)	0.86 (0.353)	0.02 (0.889)	0.02 (0.889)
A [ng/mL]	0.65 (1.81)	0.4 (0.31)	0.8 (0.7)	0.29 (0.11)	0.80 (0.370)	0.00 (0.962)	0.10 (0.752)
DHEAS [μ mol/L]	2.8 (2.8)	1.95 (1.4)	2.7 (1.4)	2.4 (2.6)	0.44 (0.507)	0.04 (0.840)	0.15 (0.702)
SHBG [nmol/L]	81.9 (0.80)	60.1 (61.1)	79.8 (20.20)	58.6 (17.9)	1.81 (0.179)	0.01 (0.910)	0.24 (0.623)
FAI	2.05 (2.4)	0.9 (0.8)	0.9 (1)	0.9 (1.3)	0.83 (0.361)	0.83 (0.361)	0.85 (0.356)

Results of Robust Anova with median method effects (Q) are provided. *Abbreviations:* IQR, interquartile range; FSH, follicle-stimulating hormone; LH, luteinizing hormone; E2, estradiol; P, progesterone; T, testosterone; AMH, antimüllerian hormone; A, androstenedione; DHEAS, dehydroepiandrosterone sulfate; SHBG, sex-hormone-binding globulin; FAI, free androgen index.

folds, such as increased thickness and dryness. As mucosal dryness tends to increase tissue's viscosity, pressure thresholds that set the vocal folds into vibration and into collision (PTP and CTP, respectively), may increase with menopause.¹⁵ To test this hypothesis, PTP and CTP were compared between premenopausal and postmenopausal FPVUs with high vocal demands and at pitches below and above the female speaking range.

To our knowledge, this has been the first investigation on the effects of menopause on vocal fold's vibration that measures concentrations of hormones to ensure correct allocation of participants in premenopausal and postmenopausal groups and possible relationships between voice parameters and specific hormonal concentrations. The results confirmed that postmenopausal FPVUs in both occupational levels showed significantly higher concentrations of FSH and LH, while concentrations of E2 were significantly reduced as compared to premenopausal professionals. This corroborates previous studies indicating

this hormonal profile as a criterium for differentiating premenopausal from postmenopausal women when follicle counting is not available.¹ Moreover, participants' hormonal concentrations fell within the normal ranges reported for premenopausal and postmenopausal women.⁴⁴ As to what concerns concentrations of T, DHEAS, and FAI, the results indicated no significant differences between the four groups. This result follows previous cross-sectional studies in which no significant differences in concentrations of androgenic hormones were found between premenopausal and postmenopausal women.^{42,45} In addition, the results confirm that participants in the postmenopausal group were at early stages of postmenopause: a significant decrease in concentrations of both preandrogens and T is more often observed in association with later than with earlier stages of postmenopause.^{46,47} Besides hormonal concentrations, there were significant differences between groups with respect to days of amenorrhea and menopause-related symptoms, namely hot flushes.

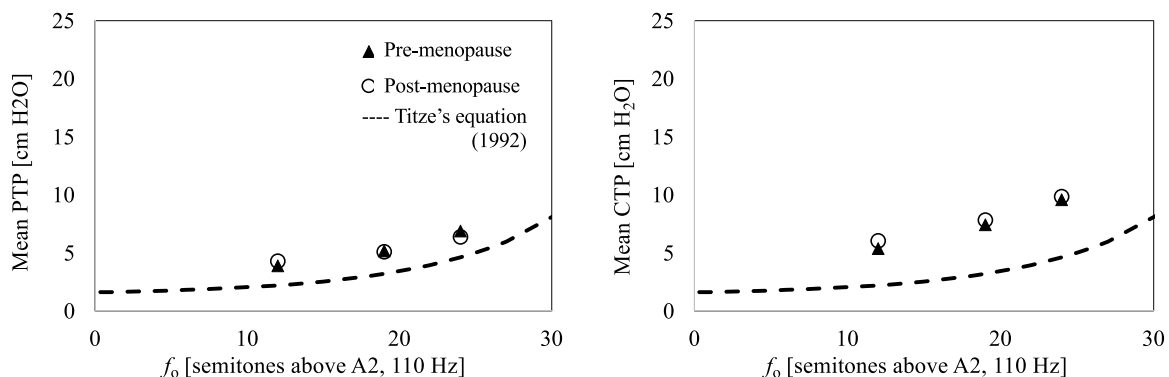


FIGURE 3. Mean phonation threshold pressure (PTP) (left) and mean collision threshold pressure (CTP) (right) as a function of fundamental frequency (f_0). The dashed line represents PTP values for female nonsingers, calculated from Titze's equation.¹⁹

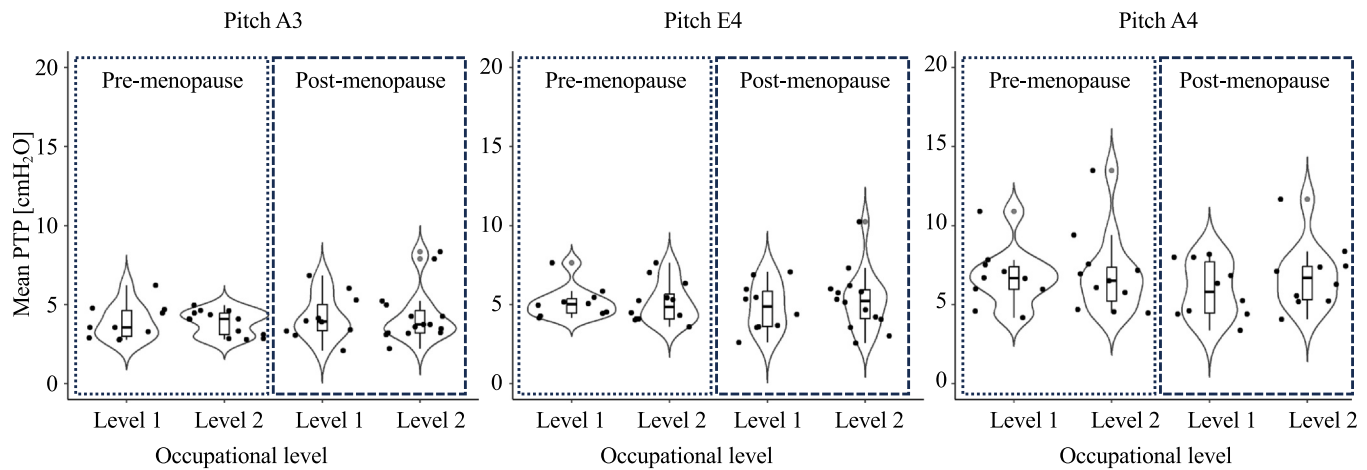


FIGURE 4. Violin plots of phonation threshold pressure (PTP) for the three analyzed pitches (A3, E4, and A4), and for both reproductive status (premenopause and postmenopause) and occupational level (1 and 2). The violin plot represents the distribution of the data and its probability density. The horizontal line crossing the interior of the box represents the median, and the whiskers outside the box the smallest and largest values that are not extreme values nor outliers. The latter are indicated by gray dots.

A correlation between hormonal concentrations and both PTP and CTP could not be found. These results seem to support previous investigations showing no correlations between variations in concentrations of E2 and P and variations in acoustic parameters (eg, jitter, shimmer, harmonics-to-noise ratio, cepstral peak prominence, and maximum phonation time) between premenopausal and postmenopausal women.^{48,49} One may argue that such correlations are difficult to find because bodily responses to low E2 associated with menopause are highly individual. In addition, the extent to which female tissues respond to low levels of E2 is varied.⁴⁷ In the present study, the absence of a correlation between hormonal concentrations and voice metrics could also be associated with the fact that premenopausal FPVUs were recorded at both follicular and luteal phases of the menstrual cycle and, consequently, presented varying concentrations of E2. In future cross-sectional studies, it will be recommended to record premenopausal females during the ovulatory phase of the menstrual cycle, when E2 concentrations are at their highest value. With this respect, Shankar and associates⁴⁹ reported to record women at days 12 to 14 of their

menstrual cycles; however, a peak of E2 during this phase might not occur if the duration of the menstrual cycle is longer than 28 days.⁵⁰ The use of ovulatory phase detection tests seems therefore more appropriate.

One may argue that a confounding effect of age could conceal effects of menopause on vocal folds' vibratory characteristics; postmenopausal FPVUs were approximately 10 years older, and this may have impacted on the results for both PTP and CTP. However, postmenopausal participants presented no evidence of endocrinological aging given that significant differences in DHEAS between premenopausal and postmenopausal groups were not found.¹ In addition, despite the significant age difference between premenopausal and postmenopausal groups, PTP and CTP showed no correlation with biological age. Previous studies comparing adolescent female voices (between 4 and 17 years) with young female adults (between 18 and 24 years) showed a lower PTP in the first group. However, it is important to highlight that these differences were found only for the mechanical method of PTP data acquisition. When a comparable PTP data collection to the one used in this study was applied (ie, labial method), the

TABLE 3.

Participants' Phonation Threshold Pressure for Pitches A3, E4, and A4, Distributed According to Reproductive Status (Premenopause and Postmenopause) and Professional Voice Use Occupation (Levels 1 and 2)

Pitch	Median (IQR)				Q ($p_{\text{menopause}}$)	Q ($p_{\text{professional}}$)	Q ($p_{\text{interaction}}$)
	Level 1		Level 2				
	Pre	Post	Pre	Post			
	($n = 10$)	($n = 10$)	($n = 13$)	($n = 15$)			
A3	3.55 (1.79)	3.94 (1.95)	4.09 (1.37)	3.74 (1.77)	0.00 (0.978)	0.08 (0.784)	0.35 (0.555)
E4	5.02 (1)	4.88 (2.37)	4.87 (1.8)	5.24 (1.91)	0.03 (0.870)	0.02 (0.876)	0.15 (0.698)
A4	6.68 (1.54)	5.80 (3.57)	6.51 (2.87)	6.69 (2.21)	0.26 (0.616)	0.07 (0.799)	0.47 (0.503)

Results of Robust Anova with median method effects (*Q*) are also presented. *Abbreviation:* IQR, interquartile range.

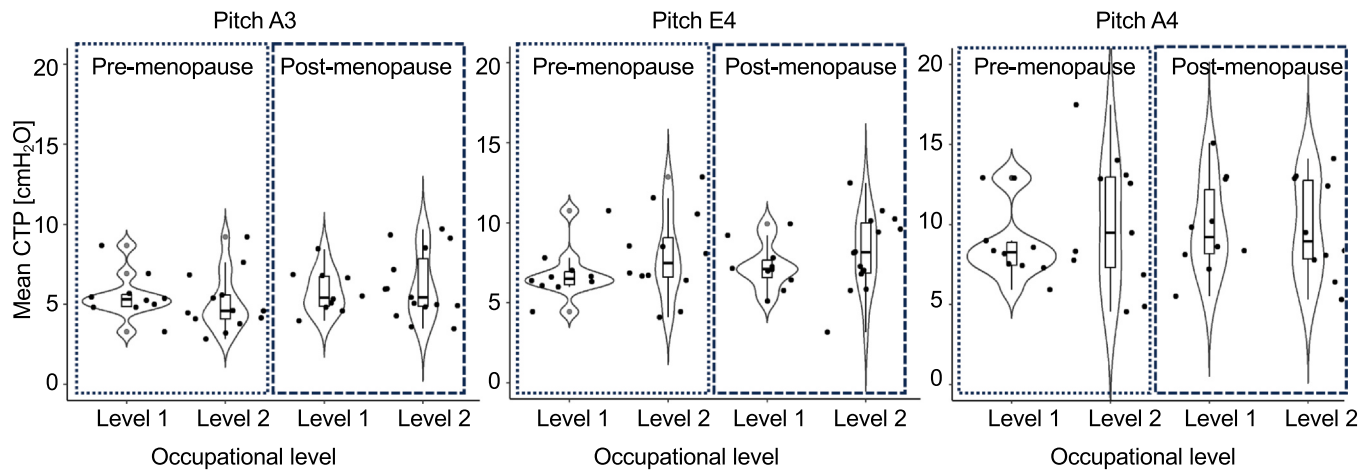


FIGURE 5. Violin plots of collision threshold pressure (CTP) for the three analyzed pitches (A3, E4, and A4), and for both reproductive status (premenopause and postmenopause) and occupational level (1 and 2). The violin plot represents the distribution of the data and its probability density. The horizontal line crossing the interior of the box represents the median, and the whiskers outside the box the smallest and largest values that are not extreme values nor outliers. The latter are indicated by gray dots.

results failed to show a significant difference between age-related groups.⁵¹ Also, one could argue that, to eliminate age as a confounding factor on sole effects of menopause on voice metrics, premenopausal and postmenopausal females matched by age should be compared. However, although possible, this is very hard, for example, only 5% of females aged between 40 and 45 years reach menopause.⁵² Moreover, the inclusion of aged-matched males as controls would be rather questionable; both males' vocal apparatus and endocrinological profiles are not comparable to the ones of females.

In the present investigation, only FPVUs with similar BMI were included in both premenopausal and postmenopausal groups. E2 is mainly produced by the ovaries but it can also be metabolized through cholesterol.⁵³ Thus, women with a high BMI are expected to have a higher baseline E2, a fact that may result in less severe menopause-related symptoms.⁵⁴ Therefore, in cross-sectional studies, comparisons between premenopausal and postmenopausal females with similar concentrations of BMI are important, particularly with respect to the effects of menopause on the voice. For example, a lowering of the mean speaking f_o was

found in postmenopausal women with low but not with high BMI scores.⁵⁵

The results of the current investigation suggest that there were no significant differences in both PTP and CTP between groups, nor for the interaction between reproductive status and professional level for any of the analyzed pitches. In addition, no significant correlations between voice metrics and hormonal concentrations differentiating premenopausal from postmenopausal groups were observed. The present results thus seem to contradict previous results suggesting a higher onset/offset time and increased vocal folds' stiffness in postmenopausal females as compared with younger reproductive females at the ischemic phase of their menstrual cycle.¹⁴ However, a restricted age range between premenopausal and postmenopausal women was not considered, despite recommendations of the World Health Organization for cross-sectional studies investigating the effects of menopause.⁵⁶ In the present investigation, this recommendation was followed. Moreover, a younger women's group was not included because around 40 years old, the female voice changes significantly.⁵⁷

TABLE 4.

Participants' Collision Threshold Pressure for Pitches A3, E4, and A4, Distributed According to Reproductive Status (Premenopause and Postmenopause) and Professional Voice Use Occupation (Levels 1 and 2)

Pitch	Median (IQR)				Q ($p_{\text{menopause}}$)	Q ($p_{\text{professional}}$)	Q ($p_{\text{interaction}}$)
	Level 1		Level 2				
	Pre	Post	Pre	Post			
	($n = 10$)	($n = 10$)	($n = 13$)	($n = 15$)			
A3	5.31 (0.85)	5.42 (1.98)	4.58 (1.48)	5.43 (3.69)	0.27 (0.606)	0.15 (0.700)	0.17 (0.682)
E4	6.50 (0.94)	7.12 (1.37)	7.47 (3.02)	8.15 (3.32)	0.33 (0.563)	0.80 (0.370)	0.01 (0.903)
A4	8.27 (1.45)	9.22 (4.72)	9.47 (6.22)	8.93 (5.09)	0.01 (0.904)	0.07 (0.785)	0.20 (0.654)

Information on statistical significance between groups is also provided, based on Robust Anova with median method effects (Q). IQR, interquartile range.

TABLE 5.

Spearman Correlation Tests Between Phonation and Collision Threshold Pressures (PTP and CTP, Respectively), Age, and Hormonal Concentrations Differentiating Premenopausal and Postmenopausal Female Professional Voice Users for the Three Analyzed Pitches (A3, E4, and A4)

	PTP						CTP					
	Pre			Post			Pre			Post		
	A3	E4	A4	A3	E4	A4	A3	E4	A4	A3	E4	A4
Age	-0.10	-0.002	-0.15	-0.19	-0.19	0.08	0.676	0.740	0.873	0.975	0.130	0.924
FSH [UI/L]				0.30	-0.90	-0.60				0.30	-0.80	-0.80
LH [UI/L]	0.02	0.03	-0.03	0.34	0.23	0.21	-0.12	-0.11	-0.14	0.06	-0.06	-0.24
E2 [pg/mL]	-0.15	-0.14	-0.01	-0.08	-0.25	-0.23	0.14	0.06	0.25	-0.15	-0.15	0-.15

Abbreviations: IQR, interquartile range; FSH, follicle-stimulating hormone; LH, luteinizing hormone; E2, estradiol; r_s = correlation coefficient.

On the face of the unexpected lack of differences in vocal fold's oscillation and collision between premenopausal and postmenopausal FPVUs, another relevant question is whether these results could reflect the study's limitations. For example, it is arguable that PTP and CTP, being extracted from intraoral estimates of subglottal pressure, may not result sufficiently robust to allow for observable effects of sex steroids on vibratory characteristics of the vocal folds. In such case, a direct observation of vocal folds' mucosal waves would be preferable, such as those obtained from stroboscopic, high-speed imaging or cine-MRI techniques. Nevertheless, as previous investigations have not carried out, objective quantifications of premenopausal and postmenopausal FPVUs' vocal folds vibratory characteristics and laryngoscopic observations are always invasive and more expensive methods of voice analysis, it seemed naturally to, as a first step, investigate possible effects using noninvasive methods. PTP and CTP have been reported as metrics that provide reliable information of vibratory characteristics of vocal folds with clinical use.

Other possible limitations of this study include the small sample size in each occupational group could have concealed effects of menopause. Indeed, although previous investigations found effects of sex steroid hormonal variations during the menstrual cycle on small numbers of premenopausal professional singers,⁵⁸ an effect of menopause might not be depicted if the number of participants is small. Only about 17% postmenopausal women seem to report menopause-related vocal folds' abnormalities.¹² One could also argue that changes in vocal fold's vibration related to menopause can only be expected when FPVUs complain of moderate-to-severe vocal symptoms. In the present investigation, voice symptoms differed with respect to occupation: schoolteachers perceived a "weaker" voice as compared with singers. This result is not surprising; there is a general lack of coping strategies among these professionals.⁵⁹ However, with respect to reproductive status, differences in vocal symptoms were not found between groups. Thus, in future investigations, it seems worthwhile to include also FPVUs with high scores for self-perceived menopause-related voice impairment. These

cases could be screened by including also validated patient outcome measures, such as the Menopause Voice-Related Work Limitation scale⁶⁰ and the Voice Handicap Index.⁶¹

Another possible limitation of the present study could have been the choice of recorded pitches. The lowest one, A3 (220 Hz), is within the vicinity of the mean average speaking f_0 for female voices, ie, around 200 Hz.⁶² The pitch E4 lies within the first transitional region of the female voice, or, in accordance with the terminology used in classical singing education, the first *passaggio*.⁶³ Within this region, alterations in the pattern of vibration of the vocal folds are likely to occur.⁶⁴ The highest pitch, A4, is closed to the end of the female's middle voice and, for lower voices, it may constitute the beginning of substantial changes in vocal folds' vibratory modes to allow further stretching and thinning when increasing f_0 .⁶⁵ In level 2 FPVUs' group, most participants had no current voice education. This had a direct impact when testing the recording protocol: the choice of pitches, although arguably not representative of the female's physiological vocal range, seemed an adequate compromise between task's demand and possibility to detect effects of menopause on vocal fold's oscillation. However, it is possible that these pitches concealed the effects of menopause, particularly if mild. Voice production is a complex phenomenon that requires many degrees of freedom; thus, depicting alterations in vocal folds' vibratory modes may require a wider range of frequencies and intensities, performed outside the boundaries of habitual and comfortable phonation ranges.^{66,67} However, choosing vocal tasks that could be performed in the same f_0 by all participants seemed important, particularly because the aim was to explore the effects of menopause on vocal folds' vibratory characteristics across different voice professionals. To do this, the whole recording protocol was tested with four untrained FPVUs. In these pilot recordings, performing high pitches resulted vocally taxing or even impossible. Thus, a compromise between pitches that were representative of different degrees of vocal effort and performance ability was required. In future investigations, another approach to be considered may be a choice of pitches based on self-reported relative degree of vocal effort or discomfort.

Also, the difficulty of the vocal task to measure PTP seems worthwhile to discuss. To phonate as softly as possible while keeping f_o constant is difficult, also for trained voices, particularly during /p/ occlusion.²¹ In addition, measuring PTP from intraoral pressure peaks requires peaks that are good estimates of P_{sub} . The latter exist when nasalization is avoided, as well as excessive glottal adduction, saliva accumulation in the tube, and insufficient number of repetitions.²⁰ Thus, participants were trained to perform *diminuendo* sequences prior to the recordings. Also, during the recordings, several repetitions were made (between 5 and 7 takes), and shapes of the pressure peaks were visually inspected in an oscilloscope (Data Mordax Systems, USA). Graphical representations of f_o and SPL (voice maps *xx* and *yy* axis, respectively) were made visible in FonaDyn software to both participants and researchers.³³ To ensure that participants varied SPL without changing f_o , the cursor, driven by the participant's voice, was kept within the same column in the voice map, so that only SPL (and not f_o) varied during each *diminuendo* sequence. This methodological approach seemed important and should be taken into consideration in future investigations targeting nonsingers. The real-time visual feedback of vocal tasks guarantees that even nontrained subjects correctly perform vocal tasks outside their habitual use of the voice.

Care was also taken when measuring pressure peaks; those not completely flat were disregarded from analysis. The trustworthiness of PTP and CTP values was further ensured by comparing values collected in the current investigation with those reported elsewhere for female voices; an increase in PTP with increasing f_o , a relatively higher CTP as compared with PTP and a strong correlation between these two measures was also found.^{18,21}

Another worth mention limitation was the timing of data collection, which was over a period of approximately 6 consecutive months after the end of COVID-19 lockdown. Thus, one may argue that, for singers, the usual number of professional engagements was not yet achieved, and, for schoolteachers, exposure to vocal demands associated with poor acoustic conditions of classrooms and related to high numbers of students in face-to-face classes just started.^{68,69} In future investigations, measures of PTP and CTP for premenopausal and postmenopausal FPVUs should be taken also under more habitual working conditions. To conclude, the results of this investigation and the derived methodological considerations seem to point at the direction of further research designs targeting larger samples of premenopausal and postmenopausal FPVUs and the inclusion of postmenopausal FPVUs of different occupational groups who complain of moderate-to-severe menopause-related voice symptoms.

Declaration of Competing Interest

The authors report there are no competing interests to declare.

Acknowledgments

The authors would like to acknowledge all participants who so willingly have dedicated their time to participate in this study; the research assistants who helped with recruitment and data collection; the nurses responsible for collecting blood samples; and Sten Ternström, for consultancy on recording equipment and procedure. Also, this study was not possible without the financial support given by the following grants and entities: (i) Programa de Atracción de Talento Investigador a la Comunidad de Madrid 2018-T1/HUM-12172; (ii) Ayudas para Contratos Predoctorales para el Desarrollo de Tesis Doctorales UNED-Santander 2022 and 2023; (iii) Ayudas de Movilidad Internacional del Banco Santander—UNED; and (iv) Theramex Spain, supporting blood sample analysis.

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