What Voice-Related Metrics Change With Menopause? A Systematic Review and Meta-Analysis Study

*Filipa M.B. Lã, and †Diego Ardura, *†*Spain*

Summary: Voice complaints associated with menopause have been reported by a substantial number of studies. However, to assess the clinical relevance of menopause to voice is still difficult as the extent to which menopausal symptoms are reflected on voice metrics remains unclear. A comprehensive review and meta-analysis were carried out to identify voice-related metrics that change with menopause and to quantify the magnitude of those changes. Academic Search Premier, Medline, SciELO, Scopus, PubMed, and Web of Science were searched without restriction of publication year until January 2020. Cross-sectional studies comparing voice-related metrics between pre- and post-menopausal women were included. Studies assessing effects of hormonal-replacement therapy were excluded. Datasets with more than one publication were also disregarded. Methodological quality of included studies was assessed applying the Newcastle-Ottawa Scale for cross-sectional studies. Given the heterogeneous nature of the primary studies, random-effects models were applied to pool the estimates. Eight articles were considered eligible for meta-analyses, assessing the effects of menopause on 6 voice metrics: mean fundamental frequency (f_o), extracted from (1) speech and (2) from sustained vowel /a/; frequency perturbation measures (3) jitter, (4) shimmer and (5) noise-to-harmonics ratio; and (6) maximum phonation time. Both speech fundamental frequency and f_o for sustained vowel /a/ were found to be 0.94 and 1.18 semitones lower in post- as compared to pre-menopausal women, respectively. Although significant, the magnitude of these decreases is below the just noticeable interval difference and well above the cutting point for distinguishing female from male voices. No significant differences were found for jitter, shimmer, noise-to-harmonics ratio, and maximum phonation time. The evaluation of acoustic metrics that reflect a single aspect of voice production at a time may conceal the effects of hormonal shifts during menopause. In addition, several variables interplay during voice production and acoustical measures may constitute weak predictors of vocal folds' status, where changes associated to sex steroid hormones are most likely to occur.

Key Words: Sex steroid hormones−Menopause−Voice-related metrics−Meta-analysis.

INTRODUCTION

Sex steroid hormones - estrogens (E), progesterone (P), and testosterone (T) - are crucial to the definition of sex-specific physical characteristics. These same hormones are also implicated on the development of sex-dependent voice characteristics. Different sizes between male and female's lungs, vocal folds, laryngeal cartilages and vocal tracts, triggered by the production of sex steroid hormones at puberty,[1−5] add to the growing body of evidence that substantiate the voice as an organ of sex hormonal influence.[6] Due to the complexity of the female's endocrine reproductive system, the female voice is notoriously more affected by variations in sex hormones across lifespan. Besides puberty, the female voice may change in response to the sex steroid hormonal variations across the menstrual cycle.[7−9] Such response is attenuated when the cyclical hormonal fluctuations during the menstrual cycle are dampened as, for example, during the use of oral contraceptive pills.[10−14] Significant hormonal shifts are also experienced at the end of a female's reproductive life, starting at menopause.[15] Once again, changes in sex steroid hormonal concentrations seem to be associated with vocal symptoms.[7,16] It is expected that, with increasing numbers of longer-lived population, a greater number of menopausal women would seek for maintaining high levels of effective communication skills, notwithstanding vocal disturbances that may be associated with it. Thus, the current investigation focuses on the female voice and on how and much it changes with respect to menopause.

Menopause is defined as the last episode of menstrual bleeding (without pathology or a surgery).[15] It usually occurs around 51 years old, with 3 to 4 years interval deviation.[17,18] Until then, menstrual cycles become increasingly irregular and unpredictable. This stage of menopausal transition - perimenopause or climacterium - is characterized by significant changes in concentrations of sex steroid hormones.[19]

The significant increase in concentrations of gonadotropins (ie, follicle stimulating hormone and luteinizing hormone) during climacterium leads to a reduction in serum concentrations of all three sex steroid hormones, but at different rates. Serum concentrations of E (the most common estrogen), fall approximately 72 %, whereas concentrations of P and T decrease about 63 % and 22 %, respectively.[20] The markedly higher decrease in concentrations of both E

FUNDING STATEMENT
Great appreciation is given to the Program for Research Talent Attraction of the Comunidad de Madrid, Spain (*Atracción de Talento Investigador C. de Madrid / Proyecto 2018-T1/HUM-12172*) for the financial support of the current investigation.
Declarations of interest: None.

From the *Faculty of Education, Department of Didactics, School Organization and Special Didactics, The National Distance Education University (UNED), Madrid, Spain; and the †Faculty of Education, Department of Research Methods and Diagnoses in Education I, The National Distance Education University (UNED), Madrid, Spain.
Address correspondence and reprint requests to Filipa M.B. Lã, National Distance Education University (UNED), Faculty of Education, Department of Didactics, School Organization and Special Didactics, Calle Juan del Rosal 14, 28040 Madrid, Spain. E-mail: filipa.la@edu.uned.es

Journal of Voice, Vol. 36, No. 3, pp. 438.e1−438.e17
0892-1997

https://doi.org/10.1016/j.jvoice.2020.06.012

and P as compared to T has consequences in the female body, the larynx being no exception.[21]

A possible explanation on how T could affect the female voice during climacterium concerns the upsurge of sex-steroid hormone receptors in the mucosa of the vocal folds that became free to connect to T (instead of E or P). Receptors for sex steroid hormones have been identified in several subunits of the vocal folds,[22–24] despite previous failed attempts to prove their existence.[25] In both female and male vocal folds, receptors for E and androgens (among which, T is the most common) seem to be more abundant.[26] Hypothesizing that the distribution of these receptors remain constant during climacteric years, the greater fall in concentrations of E and P as compared to T leave the receptors that were to be specifically connected to E and P free to bound to a similar hormone that became more abundant (ie, T). In addition, a rise in free T is also possible during climacterium, as the decreased concentrations of E have been associated with a reduction in sex hormone binding globulin.[27] Both phenomena will account for an enhanced effect of T on female laryngeal tissues. However, if T concentrations seem to influence males f_o continuously during adult years,[28,29] for female voices, such effect is still debated. If some studies have found that an increase in concentrations of T through life causes a lowering of f_o in women,[30] others have failed to replicate such findings.[28,29] Similarly, controversy is found with respect to the impacts of T on acoustical properties of the voice during menopause, despite reports of perceived vocal changes among professional and nonprofessional female voice users.[16,31]

A second possible explanation for the effects of sex-steroid hormonal shifts at menopause on voice concerns changes observed in vocal folds mucosa. Histological similarities between the mucosa of the vocal folds and the mucosa that covers the cervix (ie, the neck of the uterus), have been found. More specifically, the percentage of cells that constitute the mucosa of female vocal folds change cyclically with phases of the menstrual cycle, mimicking the changes found in the cervical mucosa in response to variations in sex steroid hormones during the menstrual cycle. In male vocal folds, mucosal changes are not observed: the percentage of cells that constitute vocal folds mucosa remain constant throughout the whole month.[8,32] Changes in vocal folds observed during the menstrual cycle, which have been related to menstrual vocal symptoms,[8,32] seem to be even more notorious during climacterium.[21] The similarity between epithelial smears of both vagina and larynx during menopause is such that a distinction between them is almost impossible in about 90% of menopausal women not using hormonal replacement therapy (HRT).[21] During menopause, both vocal folds and vaginal mucosae present signs of cell degeneration and weakness. These may well contribute to the reported menopausal vocal and gynecological symptoms.[33] However, it is worth mentioning that there is a great inter-subject variability among these observations.[33] Thus, the extent to which female larynxes may change with sex steroid hormones during climacterium seems to be highly individual.[21]

Besides this great intra-subject variability, it is also unclear how changes in vocal fold's mucosa during menopause may be reflected on acoustical properties of the voice. Mendes-Laureano and associates (2006) compared f_o of sustained vowels (/i/ and /e/) between pre- and post-menopausal women, the latter group divided into HRT users and nonusers.[34] Comparisons of harmonics-to-noise ratio (HNR) were also made. No significant differences between groups were found for both f_o and HNR. Additionally, no changes in the vocal folds were observed when video laryngoscopies were made. Meurer et al (2004) also compared pre- and post-menopausal voices, looking at f_o, intensity, formants, and verbal diadochokinesis.[35] No significant differences between the two groups could be found for any of these parameters. Contrary to these studies, others have found a relationship between menopause and changes in the acoustical properties of the voice.[36] Lindholm et al (1997) found a reduction in mean f_o and in sound pressure level (SPL) for sustained vowels for post- as compared to pre-menopausal voices.[27] These findings were further corroborated by Linville's study (1987) and D'haeseleer (2011), which reported a lowering of speaking fundamental frequency (SFF) in post- as compared to pre-menopausal voices.[37] D'haeseleer et al (2012) also found differences in SFF between these two groups. The magnitude of these changes was around 14 Hz for non HRT users and 13 Hz for HRT users.[38] Post-menopausal women with a high body mass index (BMI) also seem to reveal a nonsignificant decrease in SFF when compared with pre-menopausal women. The authors speculated that this was because reduced concentrations of E are mitigated when BMI values are high.[39,40]

The type of vocal symptom reported to be associated with menopause and its prevalence are also variable. Elite professional voice users, such as singers, report to lose high notes and complaint about huskiness, reduced suppleness of the vocal folds and timbral changes during menopause.[16] These type of voice changes were reported by 77 % of singers.[16] However, the response rate to this questionnaire was rather low (18 %), which in turn may well have contributed to an overestimation of the prevalence of vocal distress associated with menopause.[16,41] In non-singers, complaints of menopause-related vocal changes also exist. Decreased pitch range, lowering of speaking voice, vocal fatigue, difficulties with vocal control, lack of intensity and hoarseness, have been some of the most commonly reported perceived symptoms.[8,38,39,42] Once again, the prevalence of different complaints is quite varied. Dysphonia has been reported to affect about 17 % of menopausal women,[8] whereas vocal changes and vocal discomfort have been reported to affect about 46 % and 33 %, respectively.[43] Video laryngoscopic and stroboscopic observations of the vocal folds revealed at least one of the following cases: mucosal viscosity, mucosal swelling, Reinke's edema, or edema of the free vibrating edges of the vocal folds. Similar observations could not be found when observing the vocal folds of menopausal women who had no history of menopausal-related voice complaints.[43]

The controversial and incomplete nature of these previous reports substantiates further research on effects of menopause on the voice. Here, a systematic review and meta-analyses are proposed to: 1) identify studies inspecting menopause-related voice characteristics; 2) categorize voice-related metrics that change with menopause; and 3) investigate the magnitude of menopausal changes for each voice-related metric.

METHODS

Data Sources and Searches

Studies included in the systematic literature review and in the meta-analyses were identified by conducting an electronic search in bibliographic databases which cover the interdisciplinary nature of menopause-related voice characteristics: PubMed, Web of Science, Scopus, Academic Search Complete, Medline, and Scielo. The bibliographic search was performed without restriction of year of publication, from November 1, 2019 up to January 1, 2020. A combination of the following keywords was used: “voice and menopause”, “voice and climacterium”, “voice quality and menopause”, “dysphonia and menopause”, “effects of menopause on the voice”, “fundamental frequency and menopause”, and “acoustic voice characteristics and menopause”.

Eligible Criteria

Studies were selected based on the following inclusive criteria: 1) to be a full article published in English in a peer-reviewed journal; 2) to contain detailed information on main attributes of the study, namely participants’ characteristics (eg, age, reproductive stage), sample size, study design, and voice-related metrics; 3) to be a cross-sectional study. Cross-sectional studies that assessed the effects of HRT were excluded, except when results for both pre- and post-menopausal non HRT users were also provided; 4) to report mean and standard deviations for comparable voice metrics (ie, obtained from similar methods of data acquisition and analysis).

Studies meeting the above criteria were included for further analysis. If information on mean standard deviations and p values of specific voice metrics were missing, an email was sent to the corresponding author asking for clarifications. Studies with more than one article published on the same dataset were excluded, unless data concerned voice metrics that have not yet been reported. Studies reporting video stroboscopic observations of the vocal folds, voice symptoms obtained from nonvalidated questionnaires, case reports, case-control studies, guidelines, reviews, and letters to the editor were also excluded.

Data Extraction and Synthesis

An independent search for references was carried out by both authors. Titles and abstracts were first screened for relevance as to what concerns the menopause. Non-related works were immediately excluded. From the remaining studies, those who were duplicates were eliminated. Relevant information was summarized in an excel sheet for all considered studies, with respect to: (i) sample characteristics; (ii) study design; (iii) vocal tasks; (iv) methods of data analysis; (v) voice metrics; and (vi) results and their statistical relevance.

Methodological Quality

The methodological quality of the included studies was assessed using a set of criteria previously established in an adaptation of the Newcastle-Ottawa Scale for assessing quality of cross-sectional studies.[44]

Statistical Analyses

In order to investigate the existence and the magnitude of voice-related menopausal changes, a meta-analysis was performed for each of the voice-related metrics, compared between pre- and post-menopausal women. This was possible only when the same voice metric was assessed in three or more studies. The effect size used was the standardized mean difference defined as the difference between the two means divided by a pool within-group standard deviation.[46] A random-effects model was chosen, the rationale being two-fold: 1) the small number of primary studies; and 2) being a more realistic model than the fixed-effects model for most scenarios.[46,47] Heterogeneity among size effects was tested using the Cochran’s Q statistic[45] and quantified through the I^2 index.[48] Statistical analyses were carried out using the metaphor package in R.[49]

RESULTS

Identification of Studies and Voice Metrics

About 1430 references were found during the initial search in the selected electronic databases. After inspection of their titles and abstracts, 1217 studies were excluded as they were not directly related to the effects of menopause on vocal characteristics. Thus, only 213 references were left for further scrutiny. A total of 117 found to be duplicates and therefore excluded. From the remaining 96 studies, 78 failed to meet the inclusive criteria and where disregarded. The 18 studies that remained were analyzed with respect to sample size and characteristics, methods for data collection and analysis.Table 1 represents a summary of these studies, with respect to: (i) study identification; (ii) sample characteristics; (iii) data collection; (iv) data analysis; (iv) main outcomes. Based on this information, a further screening was made to assess inclusion/ exclusion from meta-analyses.

As the same voice measurement could be reported by several studies, and a minimum of three studies were required for selecting a given voice metric to be meta-analyzed, studies were organized according to the voice metric assessed. Twenty eight types of voice measurements were found, as summarized in Figure 1. Twenty of them concerned acoustical properties of the voice; three were related to aspects of voice production, such as phonatory-breathing capacity and glottal parameters; three assessed prevalence of self-

TABLE 1.
Summary of the Main Attributes of Studies Scrutinized for Possible Meta-Analysis Inclusion

Study identification	Sample characteristics	Data collection	Data analysis	Main outcomes
Hamdan et al, 2018. JVoice[50]	Menopausal non hormonal replacement therapy (HRT) users (n = 34; mean age 53.5 ± 5.57); Menopausal HRT users (n = 19; mean age 53 ± 6).	Audio recordings of (i) a sustained vowel /a/ at comfortable pitch and loudness and (ii) a count to 10, using Visi-Pitch IV (by Pentax) and microphone at 10 cm from mouth. Self-reports: (i) symptoms questionnaire; (ii) voice handicap index, short (VHI-10).	Acoustic: (i) mean fundamental frequency (f_o); (ii) habitual pitch; (iii) shimmer; (iv) relative average perturbation; (v) HNR; (vi) VTI. Self-reports: (i) symptoms prevalence; (ii) VHI-10 mean scores.	Acoustic: habitual pitch and jitter higher for HRT users as compared to non HRT users. No differences between groups for all remaining parameters.
Hamdan et al 2017. JMM.[51]	Pre-menopausal (n = 35; mean age 46.69 ± 5.97); Post-menopausal (n = 34; mean age 53.5 ± 5.57) women. Latter group divided into low and high body mass index (BMI).	Audio recordings of (i) sustained vowel /a/ at comfortable pitch and loudness and (ii) a count to 10, using a microphone placed at 10 cm from the mouth and real-time pitch module in VisiPitch IV. Self-reports: (i) symptoms questionnaire; (ii) VHI-10.	Acoustic: (i) f_o; (ii) habitual pitch; (iii) shimmer; (iv) relative average perturbation; (v) harmonics-to-noise ratio (HNR); (vi) voice turbulent index (VTI); Self-reports: (i) symptoms prevalence; (ii) VHI-10 mean scores.	Self-reports: higher vocal fatigue and throat dryness in post- as compared to pre-menopause. No differences between groups for remaining parameters.
Basilio et al 2016. CEFAC[42]	Pre-menopausal women (n = 21; mean age 42.47); Post-menopausal (n = 21; mean age 53.6) non HRT users.	Self-reports: (i) Voice Scale Symptom (VoiSS); (ii) voice handicap index (VHI); (iii) Voice-related quality of life questionnaire (V-RQoL)	Total summation of scores for both VoiSS and VHI. Algorithmic calculation of scores for V-RQoL.	Lower total score in VoiSS for post-as compared to pre-menopause. Higher functional VHI score for post- as compared to pre-menopause. Lower socio-emotional V-RQoL score for post- as compared to pre-menopause.
D'haeseleer et al, 2014. Folia Phoniat Logop[40]	Pre-menopausal women with low and high BMI (n = 22, mean age 48.5 ± 2.3; n = 13; mean age 48.1 ± 2.3, respectively); Post-menopausal non HRT users with low and high BMI (n = 28; mean age 58.5 ± 5.5; n = 12; mean age 59.4 ± 5.4, respectively); Post-menopausal HRT users with low and high BMI (n = 35, mean age 57.5 ± 5; n = 19, mean age 56.7 ± 4.1, respectively).	Audio recordings of a reading text at a habitual pitch and loudness using Computerized Speech Lab (Kay Elemetrics).	Acoustic: SFF. Body mass index (BMI)	Only low BMI and non HRT users' groups presented differences between pre- and post-menopause: SFF was low in post-menopausal as compared to pre-menopause

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TABLE 1. (*Continued*)

Study identification	Sample characteristics	Data collection	Data analysis	Main outcomes
Ferraz et al, 2013. JVoice[52]	Pre-menopausal women (n = 43; mean age 29.1 ± 8.5); Post-menopausal (n = 63; mean age 64.3 ± 9.4) non HRT users.	Audio recordings of (i) a sustained vowel /a/ in habitual loudness and pitch and (ii) consonants /s/ and /z/ using an unidirectional microphone, a computer sound card and modules "voice quality" and "voice analysis" from VoxMetria 2.7h software. Self-reports: V-RQoL. Perceptual evaluations: (i) self-reported quality assessment; (ii) GRBASI.	Acoustic: (i) mean f_o; (ii) f_o mode; (iii) minimum f_o; (iv) maximum f_o; and (v) f_o variation. Phonatory: (i) maximum phonation time (MPT) of sustained consonants /s/ and /z/ and the vowel /a/, measured with stopwatch as the mean of 3 consecutive moments; (ii) glottal-to-noise excitation ratio (GNE). Self-reports: total scores. Perceptual: (i) occurrence; (ii) distribution of the mode of GRBASI parameters.	Acoustic: mean f_o, mode, and maximum and minimum f_o lower and f_o variation and standard variation higher for post- group as compared to pre-menopause . Phonatory: lower MPT in consonant /s/ for post-as compared to pre-menopause. No differences in GNE between groups. Self-reports: higher occurrence of pleasant, low pitched and rough for post- as compared to pre-menopause. No differences in total and itemized VoQL scores. Perceptual: mild degree of roughness, strain and instability in both post- and pre-menopausal groups.
D'haeseleer *et al.*, 2012. *JVoice*[38]	Post-menopausal HRT users (n=59; mean age 57.6±4.5); Post-menopausal non HRT users (n=46; mean age 58.5± 5.1).	Audio recordings of (i) a sustained vowel /a/ in habitual pitch and loudness after a maximal inspiration, minimal pitch (F_{low}), minimal intensity (I_{low}), maximal pitch (F_{high}) and maximal intensity (I_{high}); and (ii) reading text using the Multidimensional Voice Program from CSL (Kay Elemetrics). Perceptual evaluations: (i) GRBASI; (ii) VHI. Videostroboscopic evaluation.	Acoustic: (i) dysphonia severity index (DSI); (ii) voice range profile; (iii) mean f_o; (iv) f_o variation ; (v) f_o-tremor intensity index (F_{tri}); (vi) amplitude tremor intensity (A_{tri}); (vii) jitter; (viii) shimmer; (ix) HNR; (x) speaking fundamental frequency (SFF). Phonatory: Mean of three repetitions of (i) MPT with a chronometer while sustaining the vowel /a/ in comfortable pitch and loudness; (ii) vital capacity (VC) using a spirometer; and (iii) phonation quotient = MPT/VC.	Lower minimum f_o and SFF for HRT users as compared to non-users. No significant differences between groups for remaining parameters.
D'haeseleer et al, 2012. LPV[53]	Pre-menopausal women (n = 42; mean age 48 ± 2.3); Post-menopause non HRT users (n = 42; mean age 59 ± 4.8); Post-menopausal women using sequential HRT	Audio recordings of (i) sustained vowels /a, i, u/, (ii) consonant /m/ at habitual pitch and loudness, (iii) three reading texts with different degrees of nasalization	Nasal resonance scores (%): ratio of nasal to nasal plus oral acoustic energy multiplied by 100.	No differences between groups for all isolated sounds and three reading texts.

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TABLE 1. (*Continued*)

Study identification	Sample characteristics	Data collection	Data analysis	Main outcomes
	(n = 63; mean age 57 ± 5.4); Post-menopausal with estrogen only hormonal therapy (ET) (n = 20; mean age 58 ± 4.2); Post-menopausal with a combination of estrogen and progesterone therapy (E-PT) (n = 43; mean age 57 ± 5.1).	(oronasal, oral, nasal texts) using a nasometer.		
D'haeseleer et al, 2011. Menopause[39]	Pre-menopausal women (n = 41; mean age 48.1, 45-54); Post-menopausal non HRT users (n = 26;mean age 55.5, 50-59); Post-menopausal HRT users (n = 38; mean age 54.8, 44-59).	Audio recordings of a reading text (at a habitual pitch and loudness using Computerized Speech Lab (Kay Elemetrics).	Acoustic: SFF of a standard. BMI measurements.	Positive correlation between BMI and SFF for post-menopausal women non HRT users.
D'haeseleer et al, 2011. Menopause[54]	Pre-menopausal women (n = 34; mean age 48; 45-54); Post-menopause non HRT users (n = 38; mean age 58, 50-69).	Audio recordings of (i) sustained vowel /a/ as long as possible at habitual pitch and loudness, (ii) sustained vowel /a/ for 2 seconds at habitual pitch, minimal pitch (F_{low}) and maximal pitch (F_{high}) with minimal (I_{min}) and maximal intensity (I_{max}), and (iii) reading a text using multidimensional voice program and real-time pitch program from CSL and electroglottograph (EGG) (Kay Elemetrics). Self-reports: VHI. Perceptual evaluations: (i) GRBASI; (ii) videostroboscopic evaluations.	Acoustic: (i) highest and lowest f_o; (ii) highest and lowest intensity; (iii) men f_o; (iv) f_o variation; (v) F_{tri}; (vi) A_{tri}; (vii) jitter; (viii) shimmer; (ix) NHR; (x) SFF; (xi) EGG open quotient (OQ) measured in sustained vowel /a/; (xii) dysphonia severity index (DSI). Phonatory: (i) MPT measured with a chronometer; (ii) vital capacity (VC) with a dry spirometer; (iii) phonation quotient (PQ) as the ratio of VC to MPT. Self-reports: (i) mean VHI scores. Perceptual evaluations: (i) mean of GRBASI scores; (ii) prevalence.	Acoustic: lower VC, PQ, F_{low} and SFF for post- as compared to pre-menopause. Higher f_o variation, F_{tri} and A_{tri} for post- as compared to pre-menopause. Perceptual: higher roughness and strain and lower breathiness for post- as compared to pre-menopause. No differences between groups for remaining parameters.
D'haeseleer et al, 2011. JVoice[36]	Pre-menopause young group (n = 22; mean age 22, range 20-28); Pre-menopause middle age group (n = 22; mean age 48, range 46-52).	Audio recordings of (i) a sustained vowel /a/ in habitual loudness and pitch, (ii) sustained vowel /a/ for 2 seconds at habitual pitch, minimal (F_{low}) and maximal pitch (F_{high}) with minimal (I_{min}) and maximal intensity (I_{max}), and (iii) reading a text using	Acoustic: (i) F_{high} and F_{low}; (ii) I_{min} and I_{max}; (iii) mean f_o; (iv) f_o variation; (v) F_{tri}; (vi) A_{tri}; (vii) jitter; (viii) shimmer; (ix) NHR; (x) SFF; (xi) EGG open quotient (OQ) measured in sustained vowel /a/; (xii) dysphonia severity index (DSI). Phonatory: (i) MPT measured	Acoustic: lower F_{low}, F_{high} and I_{max} for pre-middle age as compared to the pre-younger age group. Lower average f_o and SFF for pre- middle age as compared to pre-younger group. Phonatory: soft phonation index higher in pre-middle age as compared to

(*Continued*)

TABLE 1. (*Continued*)

Study identification	Sample characteristics	Data collection	Data analysis	Main outcomes
		multi-dimensional voice program, real-time pitch program from CSL and Voice Range Profile (VRP) (Kay Elemetrics).	with a chronometer; (ii) vital capacity (VC) with a dry spirometer; (iii) phonation quotient (PQ) as the ratio of VC to MPT. Self-reports: (i) mean VHI scores. Perceptual evaluations: (i) mean of GRBASI scores; (ii) prevalence.	pre-younger age group. No differences between groups for remaining parameters.
Sovani & Mukunan, 2010. SAJCD[55]	Pre-menopausal women (n = 52; mean age 30-35); Post-menopausal (n = 40; minimum age 45 and 1-5 yrs. since menopause) non HRT users. Women were divided into level II (teachers) and level IV (clerks) professional voice users.	Audio recordings of (i) sustained vowels /a, i u/ at comfortable pitch and loudness, (ii) first 3 lines of reading text and (iii) spontaneous speech using Sony ICD-P620 digital voice recorder and Multidimensional voice profile from VisiPitch III software. Phonatory: sustain vowel /a/ and consonants /s/ and /z/ as long as possible. Self-reports: VHI.	Acoustical: (i) mean f_o; (ii) relative average perturbation (RAP); (iii) noise-to-harmonic ratio (NHR); (iv) shimmer; (v) VTI; (vi) SFF (for both reading and spontaneous speech). Phonatory: MPT. Self-reports: total and subscales of VHI.	Acoustic: f_o and SFF for both reading and spontaneous speech lower and both NHR and VTI higher for post-menopause as compared to pre-menopause. Self-reports: lower VHI scores in post-menopause as compared to pre-menopause. No differences between groups for remaining parameters.
Raj et al, 2010. JVoice[56]	Pre-menopausal women (n = 35; age 20-30); Post-menopause (n = 20; with more than 5 years of menopause) non HRT users.	Audio recordings of five phrases using a speech recorder and VAUGHMI software.	Acoustic: (i) fo; (ii) frequency range (FR); (iii) optimal fo; (iv) HNR; (v) nasalence; (vi) jitter; (ix) shimmer Phonatory: (i) ratio between phonation time of /s/ and /z/ (S/Z); (ii) maximum phonation duration (MPD).	Acoustic: lower fo, FR and MPD for post- as compared to pre-menopause. Phonatory: S/Z higher for post- as compared to pre-menopause. No differences between groups for remaining parameters.
Mendes-Laureano et al , 2009. JVoice[57]	Pre-menopausal women (n = 15; mean age 30.3, range 20-40); Pre-menopause non HRT users (n = 15, mean age 56.5, range 45-60); Pre-menopause HRT users (n = 15; mean age 54.5, range 45-60).	Audio recordings of sustained vowels /e/ and /i/ at habitual pitch and intensity, using a dynamic unidirectional microphone at 4 cm from the mouth and Dr. Speech.	Acoustic: (i) jitter; and (ii) shimmer.	No differences between groups for all parameters.
Mendes-Laureano et al, 2006. Maturitas[34]	Pre-menopausal women (n = 15; mean age 30.3, range 20-40); Pre-menopause non HRT users (n = 15, mean age 56.5, range 45-60); Pre-menopause HRT users (n = 15; mean age 54.5, range 45-60).	Audio recordings of sustained vowels /e/ and /i/ at habitual pitch and intensity, using a dynamic unidirectional microphone at 4 cm from the mouth and Dr. Speech.	Acoustic: mean fo	No differences between groups. Differences between vowels for all groups.

(*Continued*)

TABLE 1. (*Continued*)

Study identification	Sample characteristics	Data collection	Data analysis	Main outcomes
Meurer et al, 2004. JVoice[35]	Pre-menopausal women (n = 45; mean age 35.61 ± 3.25); Post-menopausal non HRT users (n = 45; mean age 62 ± 7.42).	Audio recordings of (i) sustained vowel /a/ for 1.5.seconds, (ii) five repetitions of vowel combination /iu/ and (iii) five productions of the diadochokinesis (DDK) /pa ta ka/ using a digital tape recorder minidisc with polar and unidirectional microphone at 10 cm from the mouth and CSL by Kay Elemetrics.	Acoustic: (a) for the sustained vowel /a/: (i) mean f_o; (ii) lowest f_o (F_{low}); (iii) highest f_o (F_{hi}); (iv) f_o standard deviation (f_o variation); (v) pitch; (b) for the combination /iu/: (i) second formant frequency (F2); (ii) magnitude of F2 variation (F2magn); (iii) minimum F2 (F2min); (iv) maximum formant (F2max). For speech DKK: (i) rhythm pattern; (ii) speed; (iii) variation; (iv) intensity.	Acoustic: higher f_o variation for post- as compared to pre-menopause. No differences between groups were found in remaining parameters.
Meurer et al, 2004. Maturitas[41]	Pre-menopausal women (n = 45; mean age 35.61 ± 3.25) + Post-menopausal women (n = 45; mean age 62 ± 7.42).	Audio recordings of (i) meaningful and (ii) meaningless phrases, produced with six intonation variations - neutral, exclamation, interrogative, angry sadness and happiness - using a digital tape recorder minidisc with polar and unidirectional microphone at 10 cm from the mouth and CSL by Kay Elemetrics.	Acoustic: (i) SFF; (ii) pitch; (iii) f_o maximum; (iv) f_o minimum; (v) f_o standard deviation.	Lower SFF in interrogative, exclamative and happy productions of meaningful phrase for post- as compared to pre-menopause. Higher pitch in interrogative, exclamative and happy productions for post- as compared to pre-menopause. Lower f_o minimum in exclamative, sad and happy productions for post- as compared with pre-menopause. No differences found in remaining parameters.
Lindholm et al, 1997. Maturitas[27]	Menopausal non HRT users (n = 13); Menopausal E-HRT users (n = 14); Menopausal EP-HRT users (n = 15).	Audio recordings of (i) spontaneous speech, (ii) reading a text, (iii) sustained vowel /a/ and (iv) five repetitions of /paappa/ syllables using comfortable level, loud level and whispering level using a digital recorder and a measurement microphone at 30 cm from the mouth. Self-reports: (i) questionnaire assessing subjective symptoms.	Acoustic: (i) mean f_o; (ii) mean SPL. Self-reports: total occurrence of each subjective symptom.	Acoustic: menopausal non HRT users presented the highest decrease in f_o for spontaneous speech and reading when compared to the other groups. Menopausal non HRT users presented the highest decrease in SPL for spontaneous speech, reading and normal phonation when compared with the other two groups. Self-reports: negative changes in self-assessments

(*Continued*)

TABLE 1. (Continued)

Study identification	Sample characteristics	Data collection	Data analysis	Main outcomes
				of production of speech in 44 menopausal non HRT users, as compared to 38 menopausal E-HRT users and 65 menopausal EP-HRT users.
Linville, 1987. Folia Phoniat.[37]	Young (n = 24; age range 25-35); Middle age (n = 20; age range 45-55); Elderly (n = 23; age range 70-80).	Audio recordings of (i) a descending scale up-to the lowest note possible excluding vocal fry sung in /a/ and (ii) ascending scale up to the highest note, falsetto included sung in /a/.	Acoustic: (i) highest pitch produced; (ii) lowest pitch produced; (iii) total number of semitones in range	Elderly group differentiated from the other groups by the highest pitch and total number of semitones in range (both reduced for the elderly group). Middle age group (includes menopausal women) differentiated from the other groups by the lowest pitch produced (significantly lower lowest pitch for middle age group).

reported symptoms by means of validated questionnaires; and two were focused on aspects of voice perception with clinical relevance.

Despite the great number of acoustic voice metrics found in these studies, not all could be considered for a meta-analysis because of: (1) compared groups failed to include pre- and post-menopausal women, the latter being non HRT users; (2) methods of data extraction were not comparable for all metrics; (3) metrics assessed in a comparable way were not assessed by a sufficient number of studies to allow a meta-analysis (ie, minimum of three studies). For example, from the 10 studies assessing mean f_o from a sustained vowel, only five used the same sustained vowel (in this case, the vowel /a/). The same applied for jitter: one study reported values for the vowels /i/ and /e/ [57] and thus could not be included in the meta-analysis. The metric HNR was also extracted from different vocal tasks, leaving an insufficient number of studies to allow for a meta-analysis. In addition, from the eight studies found to extract mean f_o from speech (SFF), only four have used the same vocal task for that extraction, ie, reading a text. The other studies reported to extract SFF from reading one phrase or from spontaneous speech. Metrics such as voice turbulent index (VTI) and relative average perturbation also could not be included in the meta-analysis because two of the three studies[50,51] concerned the same sample population. The voice handicap index (VHI) could be considered for a meta-analysis, given the higher number of studies reporting on this measure. However, once again, methods of voice analysis were too heterogeneous to allow a meta-analysis. Jacobson and associates (1997)[58] summed up the scores of all items, whereas Hamdan and associates (2017)[51] and Sovani & Mukundan (2010)[55] reported the mean of the scores of all items. Thus, from the initial 18 studies, only nine could be considered for investigating the effects of the menopause on six different voice metrics using separate meta-analyses for each of these metrics. From these nine studies, two needed further clarifications with respect to data distribution and dispersion characteristics. An email was sent to the authors requesting this information; only one replied. This yielded a total of only eight studies to be included in the meta-analyses, as shown in the flowchart of Figure 2.

These primary studies were all cross-sectional non-randomized investigations. All data were collected using validated measurement tools and all clearly characterized pre- and post-menopausal women. Given the nature of this research, a limited number of respondents were justified. The overall quality of studies rendered a scoring range in the Newcastle-Ottawa Scale between six and eight points.

The percentage of agreement during the selection process was about 78%. Consensus was applied to solve discrepancies as to what considering a study inclusion or exclusion of the six meta-analyses that were carried out per voice metric.

The voice metrics assessed in these eight studies were: i) SFF; ii) mean f_o extracted from the sustained vowel /a/; iii) jitter; iv) shimmer; v) noise-to-harmonics ratio (NHR); and vi) maximum phonation time (MPT). These were compared between pre- and post-menopausal groups of non-professional female voice users. Figure 3 summarizes the number of studies included in these meta-analyses per voice metric.

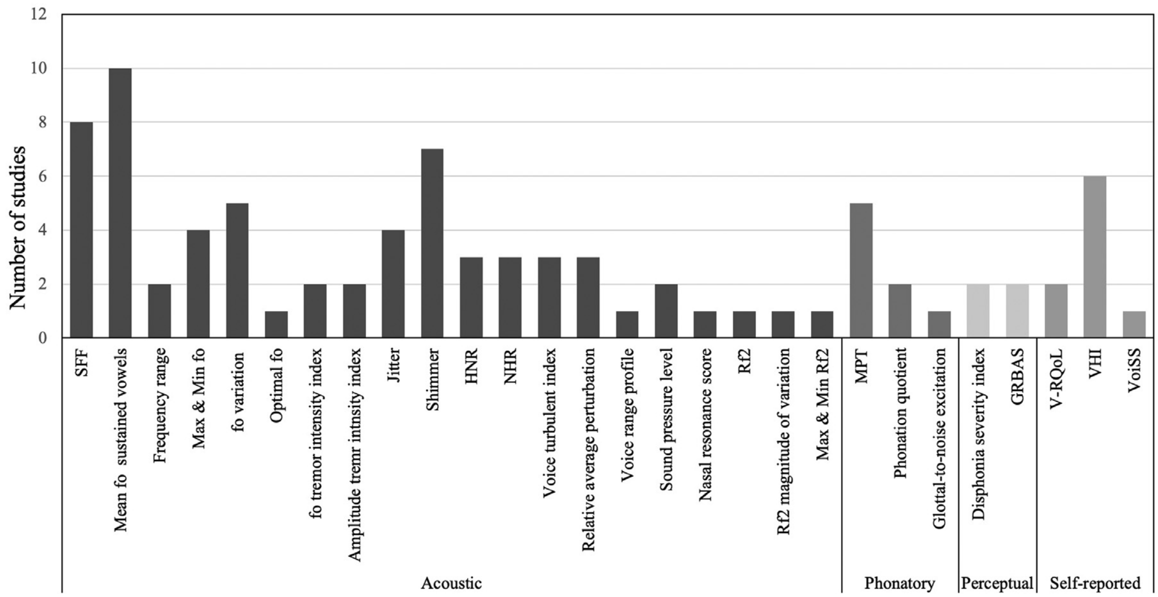

FIGURE 1. Number of studies reporting voice-related measures. Note: SFF = speaking fundamental frequency; f_o = mean fundamental frequency; MPT = maximum phonation time; V-RQoL = voice-related quality of life questionnaire; GRBAS = grade of roughness, breathiness, asthenic and strained scale; VHI = voice handicap index; VoiSS = voice symptoms scale questionnaire; Max & Min f_o = maximum and minimum fundamental frequency; HNR = harmonics-to-noise ratio; NHR = noise-to-harmonics ratio; SPL = sound pressure level; Rf_2 = frequency of the second resonance of the vocal tract; Max & Min Rf_2 = maximum and minimum Rf_2.

SFF

Table 2 displays the sample size, the mean and the standard deviations for the 6 studies included in the meta-analysis concerning SFF. Overall, the meta-analysis indicated a significant effect of menopause on female's SFF. The post-menopause condition presents a lowering of SFF of about 10.1 Hz (95 % Confidence Interval [CI] = [-14.14 Hz; -6.07 Hz]) as compared to pre-menopause, corresponding to a reduction of about 0.94 semitones (ST). The Q test rendered a nonsignificant heterogeneity, Q(5) = 6.73 (P = 0.241). The I^2 index was 25.8 % (95 % CI: [0.0 %; 68.9 %]).

Four of the primary studies included in the meta-analysis presented individual significant effects of menopause on SFF. D'haeseleer et al (2011a)[39] was the most contributing study, with a weight of 27.0 % on the final model, whereas the work by Sovani and Mukundan (2010)[55] displayed the lowest weight (7.9%) on the final model. In turn, two studies, Meurer et al (2004)[41] and Hamdan et al (2017)[51] reported non-significant individual effects and weighted 20.0 % and 9.0 %, respectively. Figure 4 displays the corresponding forest plot.

Mean Fundamental Frequency (f_o) for the Vowel /a/

Table 3 displays the sample size, the mean and the standard deviations for all the studies included in the meta-analysis carried out for the metric mean f_o extracted from the sustained vowel /a/. Our computations uncovered a decrease in mean f_o from 204.00 Hz in pre-menopause to 190.59 Hz in post-menopause (1.18 ST interval difference). Among the 6 primary studies, three reported a non-significant effect with mean differences ranging from 1.49 Hz (0.13 ST) in the D'haesseleer et al (2011)[54] to 4.66 Hz (0.40 ST) in the Meurer et al (2004) study[35]. The studies by Sovani & Mukundan (2010),[55] Raj et al (2010),[43] and Ferraz et al (2012) [53] found significant differences between pre- and post-menopausal groups. These differences were within the magnitude of 29.31 Hz (2.44 ST), 27.00 Hz (2.15 ST), and 17.41Hz (about 1.56 ST), respectively. The weight of the six studies on the total effect varied from 11.0 %[55] to 24.5 %[52] (see Figure 5).

A significant heterogeneity was found in the sample of primary studies: Q(5) = 21.46 (P < 0.001), I^2 = 76.7% (95 % CI: [48.0 %; 89.6 %]). In addition, it is worth noting that the standard deviations in the study by Ferraz et al (2012)[52] were, on average, more than six times smaller than those in the rest of studies.

Jitter

With respect to the effect of the menopause on jitter, the sample size, the mean and the standard deviations for all primary studies included in the meta-analysis are displayed in Table 4.

None of the studies rendered significant differences between pre- and post-menopausal voices. The contribution of each study is uneven as the study from Raj and associates (2010)[56] has a contribution to the model of 73.6 % whereas the study by D'haeseleer et al (2011)[54] only contributes with 6.7% (see Figure 6). The overall effect when the three studies are meta-analyzed is null, with a 95 % confidence interval for the mean difference of [-0.10%; 0.07%]. The Q test proved the non-significant heterogeneity of the sample of studies, Q(2) = 1.40 (P = 0.496). The I^2 index was 0 % (95 % CI: [0 %; 85.2 %]).

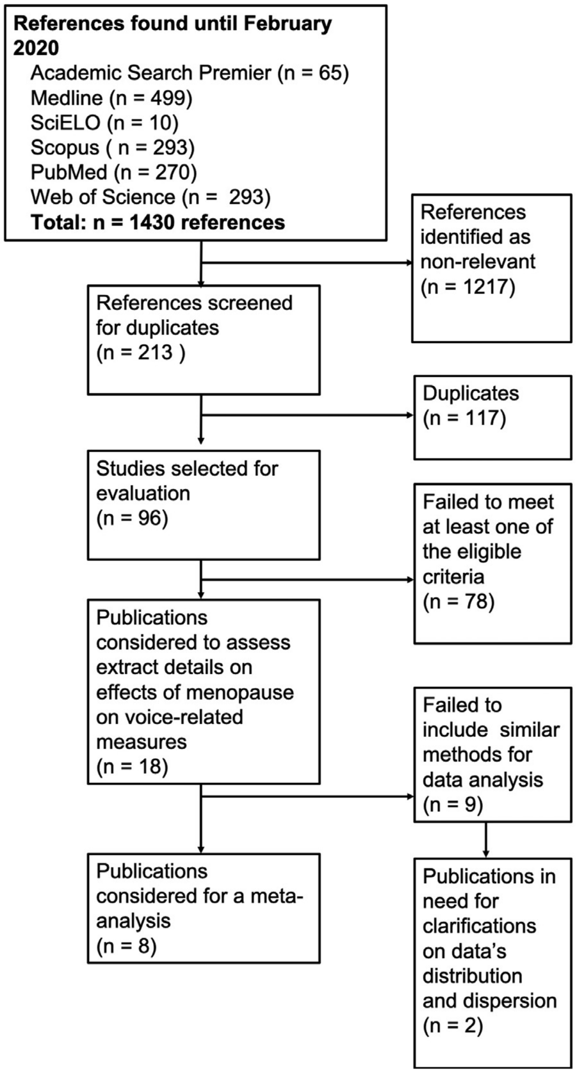

FIGURE 2. Flowchart summarizing the selection of studies included in the meta-analyses carried out per voice metric.

Shimmer

Table 5 presents the sample size, the means, and the standard deviations for the studies included in the meta-analysis concerning shimmer. None of the three studies found significant differences between pre- and post-menopausal voices.

Consequently, the overall effect of the menopause was nonsignificant (see Figure 7). It is interesting to note that the weights of the primary studies on the final model were uneven. The one by D'haeseleer et al (2011)[54] was the most contributing study to the final model (70.6 % of the total weight) while the one by Hamdan et al (2017)[51] was the least contributing with 13.0%. The Q test rendered a nonsignificant heterogeneity, Q(2) = 0.62 (P = 0.732), with an I^2 index of 0 % (95% CI: [0 %; 66.6 %]).

NHR

Concerning the effect of menopause on NHR, the meta-analysis also rendered a nonsignificant overall effect of the menopause (see Table 6).

The Q test found a nonsignificant heterogeneity, Q (2) = 2.32 (P =.313) and the I^2 index was 14.0% (95 % CI: [0.0 %; 91.1 %]). The most contributing primary study to the total effect was the study by Hamdan et al (2017) (75.9%) (see Figure 8).

MPT

Table 7 displays the sample size, the mean and the standard deviations for the three studies included in the meta-analysis concerning MPT. Onge again, a non-significant effect of menopause was found, being the 95% mean difference confidence interval [-6.28%; 1.74%].

As shown in the forest plot of Figure 9, only the study by Raj and associates (2010)[56] reported a significant effect of menopause on MPT. This study contributed to the model with a weight of 37.9%. In turn, the two other studies, by Sovani & Mukundan (2010)[55] and by D'haeseleer et al (2011),[54] found non-significant effects with weights to the model of 32.2 % and 29.9 %, respectively. A significant

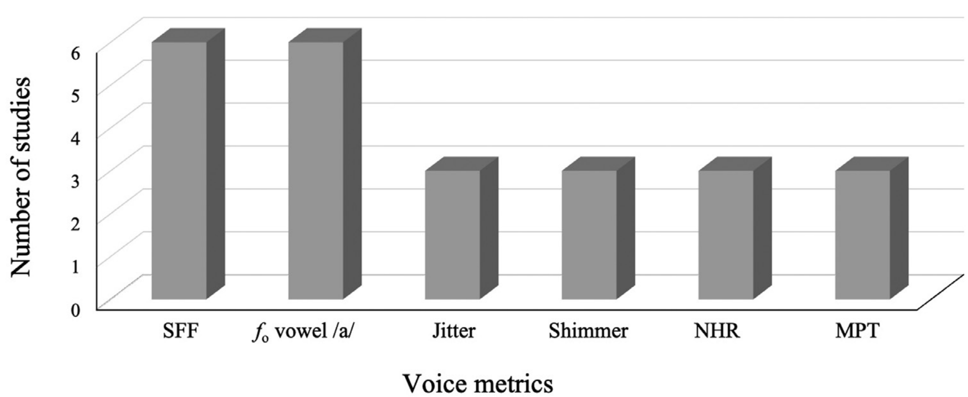

FIGURE 3. Number of studies per voice metric meta-analysed.

N.B.: SFF = speaking fundamental frequency; f_o = fundamental frequency; NHR = noise-to-harmonics ratio; MPT = maximum phonation time.

TABLE 2.
Sample Size (n), Mean and Standard Deviations (SD) for the Studies Included in the Meta-Analysis Concerning Speaking Fundamental Frequency (SFF)

Study identification	Pre-menopause			Post-menopause		
	n	Mean [Hz]	SD [Hz]	n	Mean [Hz]	SD [Hz]
Meurer et al, 2004[41]	45	195.53	23.74	45	188.38	18.17
Sovani & Mukundan, 2010[55]	20	226.37	21.56	20	201.7	21.7
D'haeseleer et al, 2012[53]	42	188	15.8	42	178	19.9
D'haeseleer et al, 2011a[39]	41	185.8	17.99	26	178.93	12.12
D'haeseleer et al, 2011b[54]	34	189.15	16.18	38	176.42	16.60
Hamdan et al, 2017[51]	35	173.71	21.88	34	165.6	26.5
Weighted average [Hz]		190.88			180.78	

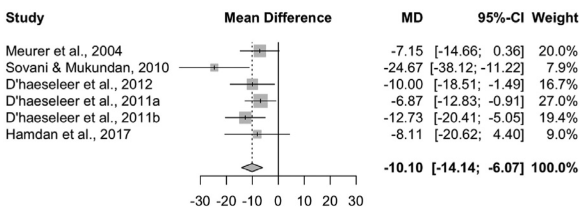

FIGURE 4. Forest plot displaying the effect size of menopausal changes on speaking fundamental frequency (SFF), using a random effect model.

heterogeneity was found, $Q(2) = 11.95$ ($P < 0.001$) with an I^2 index of 83.3% (95 % CI: [49.2 %, 94.5 %]).

DISCUSSION

Menopause corresponds to the end of a female's reproductive life[15] and constitutes an example of a developmental stage during which voice complaints have been described by a substantial number of studies.[8,16,43] However, it is difficult to assess the clinical relevance of the menopause to voice: the extent to which vocal symptoms may be reflected on voice-related metrics is still unclear. Previous results are controversial and have not yet been systematized and integrated. Thus, a systematic review of previous literature followed by meta-analyses on comparable voice metrics assessed with respect to menopausal effects were carried out. The aims were to identify which voice metrics were likely to change and to investigate the magnitude of those changes. The choice for meta-analyses seems appropriate: besides the controversial nature of previous results, 28 different methods of voice assessment were identified in only 18 studies comparing pre- with post-menopausal women.[59]

The great number of voice-related measures found in primary studies could have contributed to a broader understanding of the effects of the menopause on several aspects of voice production when carrying out meta-analyses for each of these measures. However, this was not the case. The heterogeneity found in the primary studies and the variety of methods used in data collection and analysis confined the meta-analyses to few studies and few voice-related metrics. SFF was found to be assessed from spontaneous speech, reading a phrase or reading a passage. However, it is known that f_o values extracted from reading a passage are higher as compared to those extracted from conversational speech.[60] This vocal task-dependency is also observed when extracting f_o from different sustained vowels.[60] Thus, only studies using the same method of f_o analysis were considered for meta-analyses, resuming the inclusion to a total of 6 primary studies for both SFF and mean f_o for the vowel /a/.

TABLE 3.
Sample Size (n), Mean and Standard Deviations (SD) for the Studies Included in the Meta-Analysis Concerning Mean f_o for the Sustained Vowel /a/

Study identification	Pre-menopause			Post-menopause		
	n	Mean [Hz]	SD [Hz]	n	Mean [Hz]	SD [Hz]
Meurer et al, 2004[35]	45	206.58	19.43	45	201.92	29.4
Raj et al, 2010[56]	35	231.09	24.10	20	204.09	22.83
Sovani & Mukundan, 2010[55]	20	222.99	20.27	20	193.68	29.74
D'haeseleer et al, 2011b[54]	34	192.34	21.95	38	190.85	20.38
Ferraz et al, 2012[52]	43	202.48	3.02	63	185.07	4.46
Hamdan et al, 2017[51]	35	175.15	19.97	34	170.62	31
Weighted average [Hz]		204.00			190.59	

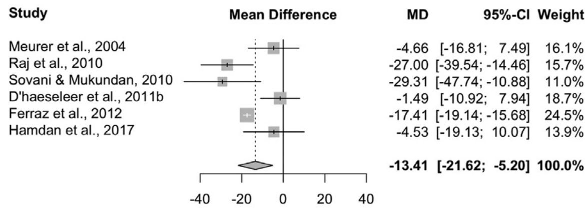

FIGURE 5. Forest plot displaying the effect size of menopausal changes on mean f_o extracted from the sustained vowel /a/, using a random effect model.

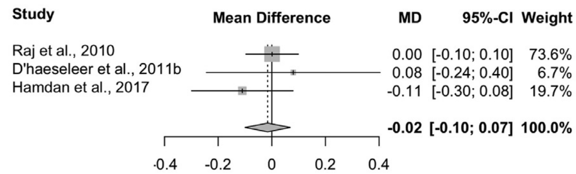

FIGURE 6. Forest plot displaying the effect size of menopausal changes on jitter, using a random effect model.

The heterogeneous nature of primary studies also concerned other measures, such as VHI. In some studies, VHI data were assessed as the mean of the scores [51,55] despite the use of total sum of the scores in its validation study.[58] Such disparity in methods of data analysis challenges the possibility of performing meta-analyses with these studies, compelling for their exclusion. Meta-analyses of a higher number of voice-related metrics was also not possible due to the small numbers of primary studies investigating some of the metrics (eg, SPL, voice range profile, glottal-to-noise excitation, and phonation quotient).

For voice metrics that were meta-analyzed (ie, SFF,f_o for sustained vowel /a/, jitter, shimmer, NHR and MPT), a possible limitation of the outcomes could have been the inclusion of a small number of primary studies (between 3 and 6 per voice metric). However, this limitation was circumscribed by using a random-effects model. The use of this model provides more realistic results even when a small number of studies are considered.[46] In addition, publication bias is not expected as primary studies did not apply any intervention on woman's voices and there was no privileged direction in the results. Nevertheless, the overall effects found in the present investigation must be interpreted carefully.

From all meta-analyses carried out, only the metrics SFF and mean f_o for the sustained vowel /a/ were found to significantly change with menopause. For post-menopausal groups, SFF was found to be 10.1 Hz lower and f_o for the sustained vowel /a/ was 13.41 Hz. Although these observations corroborate previous self-reported perceptions of a drop in pitch with menopause,[8,16,27,31,41] the effects of such decrease seem to be small. Our findings revealed that SFF and mean f_o for the sustained vowel /a/ were about 0.94 and 1.18 ST lower in post- as compared to pre-menopausal women, respectively. These values fall below the 2 ST just noticeable interval difference reported for both sinus tones and for voices produced within the range of 160 to 200 Hz.[61] It is therefore possible that the effects of menopause on voice may be imperceptible in continuous speech, at least to a non-trained listener.[61] In addition, the SFF and mean f_o for the vowel /a/ reported in this study for post-menopausal voices were 180.78 Hz and 190.59 Hz, respectively. Both values are well above the cutting point for distinguishing female from male voices (ie, 165 Hz).[62] Thus, one may argue that the impacts of menopause in females f_o seem not to reach a point of misleading the listener to whether the voice that is being heard belongs to a female or male speaker. This, however, does not mean that the speaker may not perceive such mild differences, especially for the case of being a singer. For those whose work, career and livelihood entirely depend on voice quality, voice changes, even if minor, may not be trifling.[63]

Our findings substantiate the f_o dependence on methods of data collection and extraction described in previous investigations.[61,62,64] The decrease in mean f_o observed in post-menopausal voices was higher when f_o was extracted from the sustained vowel /a/ as compared to when it was extracted from reading. Despite the provision of more complete acoustic information when extracted from connected speech,[65] this result seems to corroborate previous claims that f_o extracted from sustained vowels seem to be more sensitive to the effects of sex hormones.[66] However, this assumption should be interpreted with caution. Mean f_o in sustained vowels may depend more on methods of collection rather than on effects of hormonal variations. For example, Ma & Love's study (2010) compared f_o values extracted from electrolaryngographic signals between young (25 years. ± 3.16) and older (69.73 years.± 3.69) women when

TABLE 4.
Sample Size (n), Mean and Standard Deviations (SD) for the Studies Included in the Meta-Analysis Concerning Jitter

Study identification	Pre-menopause			Post-menopause		
	N	Mean [%]	SD [%]	n	Mean [%]	SD [%]
Raj et al, 2010[56]	35	0.35	0.18	20	0.35	0.18
D'haesseler et al, 2011b[54]	34	0.89	0.42	38	0.97	0.70
Hamdan et al, 2017[51]	35	0.88	0.44	34	0.77	0.40
Weighted average [%]		0.49			0.47	

TABLE 5.
Sample Size (n), Mean and Standard Deviations (SD) for the Studies Included in the Meta-Analysis Concerning Shimmer

Study identification	Pre-menopause			Post-menopause		
	n	Mean [%]	SD [%]	n	Mean [%]	SD [%]
Raj et al, 2010[56]	35	3.58	1.33	20	3.7	1.38
D'haeseleer et al, 2011b[54]	34	1.92	0.83	38	1.94	0.79
Hamdan et al, 2017[51]	35	3.32	1.35	34	3.71	1.81
Weighted average [%]		2.37			2.46	

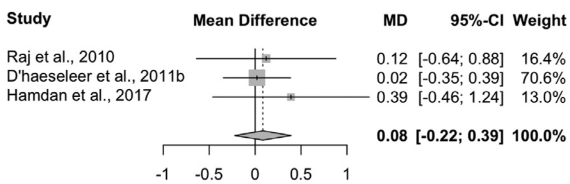

FIGURE 7. Forest plot displaying the effect size of menopausal changes on shimmer, using a random effect model.

TABLE 6.
Sample Size (n), Mean and Standard Deviations (SD) for the Studies Included in the Meta-Analysis Concerning NHR

Study identification	Pre-menopause			Post-menopause		
	n	Mean	SD	n	Mean	SD
Sovani & Mukundan, 2010[55]	20	0.14	0.03	20	0.17	0.06
D'haeseleer et al, 2011b[54]	34	0.11	0.03	38	0.12	0.11
Hamdan et al, 2017[51]	35	0.13	0.033	34	0.13	0.026
Weighted average		0.13			0.14	

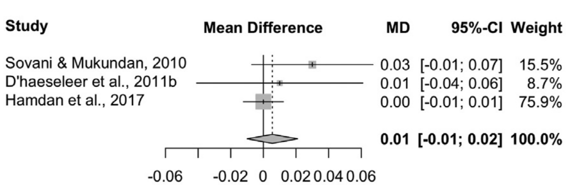

FIGURE 8. Forest plot displaying the effect size of menopausal changes on HNR, using a random effect model.

sustaining a vowel, reading a phrase and reading a passage.[64] Their results showed that f_o were always higher when sustaining a vowel (205.87 ± 33.22 Hz) as compared to reading a phrase (176.46 ± 22.75 Hz) or a passage (184.73 ± 20.16 Hz). In addition, the great heterogeneity of the primary studies meta-analyzed substantiate the argument that f_o differences between pre- and post-menopausal women could be related to other effects, such as the f_o dependency on methods of collection and extraction, rather than only the sex hormonal shifts that characterize the menopause. This is further substantiated by the fact that a great variability of standard deviations was found in primary studies. For example, Ferraz et al (2012)[52] reported on average more than six times smaller SD for f_o extracted by means of the sustained vowel /a/ as compared to the remaining studies.

The same line of thought may be applied to the results obtained for jitter, shimmer, HNR, and MPT. Differences in these metrics between pre- and post-menopausal voices were nonsignificant. Such result was unexpected given the significant effects of sex steroid hormonal variations across the menstrual cycle on vowel spectral noise and jitter.[10,11,67,68] It is possible that the effects of the menopause on these metrics have been concealed due to the high sensitiveness of methods of professional-grade data acquisition (DA) that were used in primary studies. Discretization errors for f_o, jitter and shimmer measurements have been associated with different DA environments and their dynamic ranges.[69]

Other equally important considerations when interpreting the results of these meta-analyses is that differences between pre- and post-menopausal voices could have been of greater magnitude if post-menopausal women included in the primary studies were selected depending on self-reported menopausal vocal symptoms. In addition, effects of menopause on voice metrics meta-analyzed may have been concealed due to the age difference between pre- and post-menopausal women. Female's f_o decreases not only because of hormonal changes associated with the menopause but also because of ageing.[21,70] It is known that, for non-pathological voices, middle-aged women (around 40s) have lower voices when compared to younger ones (around 20s).[60] In some of the primary studies included in these meta-analyses,[41,52] the age range reported for the middle-aged groups was between 30 and 47 years old. This seems a rather sparse interval for controlling effects of age. According to the World Health Organization (WHO) recommendations, middle-aged women with a restricted age range should be compared with younger and post-menopausal women if the aim is to investigate menopausal symptoms (WHO, 1996).[71] Although the majority of primary studies relied on age as inclusive criteria

TABLE 7.
Sample Size (n), Mean and Standard Deviations (SD) for the Studies Included in the Meta-Analysis Concerning MPT

Study identification	Pre-menopause			Post-menopause		
	n	Mean [s]	SD [s]	n	Mean [s]	SD [s]
Raj et al, 2010[56]	35	14.29	4.41	20	8.74	3.08
Sovani & Mukundan, 2010[55]	20	13.45	4.47	20	12.8	5.20
D'haeseleer et al, 2011[54]	34	20.34	6.89	38	20.48	8.16
Weighted average [s]		15.83			13.65	

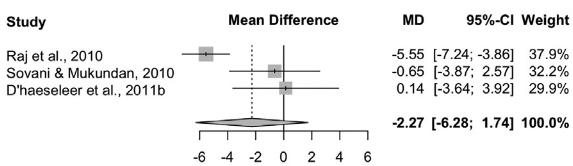

FIGURE 9. Forest plot displaying the effect size of menopausal changes on MPT, using a random effect model.

for distributing participants among pre- and post-menopausal groups, the use of hormonal and gynecological features to guide participant's inclusion in these groups seems to be preferable, so that age range can be reduced.

Finally, the results from the meta-analyses carried out in this study address the need for re-thinking methods of data collection and analysis so that they can suit best the aim of investigating the effects of menopause on the voice. The primary studies used in the meta-analyses assessed several aspects of voice production, but taking one dimension of voice production at a time (eg, f_o for a sustained vowel in a comfortable pitch, jitter, shimmer, NNR). Other studies including metrics that reflect multidimensional aspects of voice production were not found in a sufficient number of studies to allow a meta-analysis. Voice production is a rather complex phenomenon, varying within several degrees of freedom.[72] For example, f_o reflects concomitant adjustments of subglottal pressure, glottal adduction, and vocal fold tension.[73] The interdependency on physiological and aerodynamical aspects of voice production naturally challenges the understanding of how the vocal folds react to hormonal shifts based solely on acoustical analysis of a vowel produced at a comfortable pitch and loudness.[72] Moreover, previous hypothesis state that sex steroid hormonal variations impact on the histological properties of the vocal folds.[7] It is therefore expected that the study of metrics related to voice source and vibratory patterns of the vocal folds would best reflect effects of menopausal hormonal shifts on the voice as compared to acoustical measures. The latter reflect both source (ie, pulsating transglottal airflow) and filter (ie, vocal tract resonances) events; thus, the interpretation of acoustical changes associated with the menopause cannot be directly interpreted as reflecting changes at the level of the vocal folds.

CONCLUSIONS

The menopause is associated with a decrease in f_o within the magnitude of 10.1 to 13.4 Hz, depending on whether the vocal task is reading or sustaining the vowel /a/. This decrease is well below the 2 ST just noticeable interval difference reported for both sinus tones and for voices produced within the range of 160 to 200 Hz. Thus, f_o reduction may affect women to a different extent, depending on whether they are elite performers or other professional voice users. No significant effects of the menopause were found for measures of frequency perturbation (ie, jitter, shimmer, HNR), nor for MPT. The heterogeneity of primary studies, including methods of data collection and analysis, address the need for changing the current voice research paradigm as to what concerns the assessment of effects of menopause on the voice. Voice metrics that reflect more than one dimension as to what concerns acoustical, physiological and aerodynamic aspects of voice production possibly will suit best the understanding of the complex relationships underlying effects of sex hormones during the menopause on voice.

ACKNOWLEDGMENTS

We would like to thank the authors of primary studies who have contributed to the clarification of information on their studies.

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