

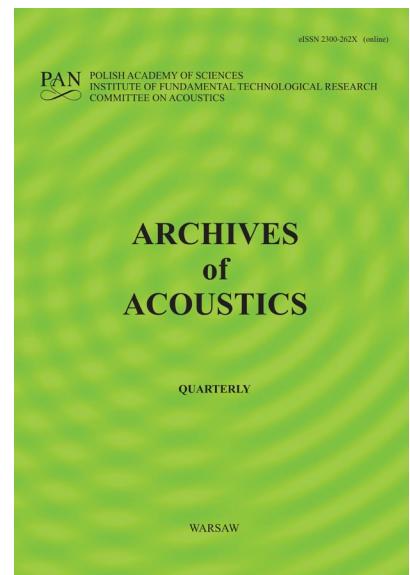
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## Masculinized or Feminized? Discriminant Analysis of Postmenopausal Women's Voices

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This study investigates the degree of vocal variation between men, pre- and postmenopausal women. The sample comprised 108 volunteers aged 18–66 y., divided into control and validation group. Each participant was subjected to voice recordings of five sustained vowels. Acoustic parameters were extracted using Praat software. The most significant parameters in intergroup correlation between canonical discriminant function and acoustic variables were: fundamental frequency (f0), shimmer, harmonics-to-noise ratio (HNR) and intensity. Premenopausal female voices were labeled with 97% correctness and male voices with 95.5% correctness. Interestingly, 65.5% of postmenopausal women were accurately classified as female voices and on average they had lower vocal pitches compared to premenopausal women. The differences in male and female voices are probably due to the difference in the size of the larynx and the length of the vocal cords. Hormonal changes during menopause may affect, but not significantly, the morphology of the laryngeal structures which develop during childhood and adolescence.

**Keywords:** acoustical analysis, aging, fundamental frequency, hormones, menopause.

## 1. Introduction

### 1.1. *Voice as a crucial biological trait*

Source-filter theory is a fundamental concept in understanding the production of speech in mammals. It posits that the generation of speech involves two primary components: the source, which refers to the sound produced by the vocal cords in the larynx, and the filter, which represents the shaping of this sound by the supralaryngeal vocal tract (SVT) (Fitch, 2000; Taylor & Reby, 2021; Tokuda, 2021). Both source and filter characteristics are essential for effective communication. In many species, variation of voice characteristics contributes to individual distinctiveness and seems to be especially crucial for kin recognition, specifically for mothers and their offspring (Taylor & Reby, 2010). Primary factors that contribute to acoustic characteristics and quality of voice are sex, age, body size/shape and health which are mainly associated with physiological changes in sex hormone levels (Leongómez *et al.*, 2021; Puts, 2005). Age-related changes are connected to morphological changes in childhood, puberty and elderly. With aging, there is reduced muscle tone, decreased elasticity and hydration of vocal fold tissues and alterations in vocal fold length and thickness (Sataloff *et al.*, 2017).

### 1.2. *Sexual dimorphism in voice parameters*

Sexual dimorphism, the biological differences between men and women of the same species, extends to various aspects of human physiology, including voice. The role of sex hormones is crucial in shaping sexual dimorphism in voice parameters, with a focus on vocal pitch, timbre and voice quality. Pitch stands as one of the most prominent markers of sexual dimorphism in the human voice (Rosenfield *et al.*, 2020). Men typically have longer vocal cords and vocal tracts compared to women, leading to a lower pitch and narrower spacing of formant frequencies in men. While the evolutionary explanations for these sex differences are not fully understood, some evidence suggests a role for intrasexual competition. Men's pitch is

approximately half as high as women's voices. This difference in pitch is largely attributed to men having vocal cords that are 60% longer than those of women, a much larger difference compared to the 7% disparity in height between both sexes (Puts *et al.*, 2007). The intersexual selection suggests correlations between female mate preferences and male voice characteristics, with women showing preferences for lower-pitched voices in potential mates. These preferences may reflect underlying genetic fitness or indicators of mate quality, such as physical size, health, and testosterone levels (Pisanski *et al.*, 2018). Sex hormones significantly affect the vocal folds due to the presence of receptors on both androgen and estrogen hormones on them (Abitbol *et al.*, 1999; Aufdemorte *et al.*, 1983; Kirgezen *et al.*, 2017; Newman *et al.*, 2000; Voelter *et al.*, 2008). Studies indicate that androgens play a crucial role in development, structure and function of the human larynx. Specifically, androgens induce the hypertrophy of thyroarytenoid muscles, leading to a deepening of the voice pitch (Damrose, 2009; Huang *et al.*, 2015). During puberty, testosterone levels rise in men, inducing elongation and thickening of the vocal folds, which subsequently leads to a lower voice pitch. Conversely, women typically exhibit shorter and thinner vocal folds, resulting in a higher pitch. This difference in length can be attributed to the secondary descent of the larynx, a feature specific to men that occurs during puberty (Markova *et al.*, 2016). The impact of sex hormones on voice parameters extends beyond puberty, with hormonal fluctuations throughout the menstrual cycle and pregnancy exerting notable effects on vocal function in women (Pisanski *et al.*, 2018). Variations in estrogen and progesterone levels during the menstrual cycle impact vocal fold tissue hydration and vascularization, leading to fluctuations in pitch and voice quality (Zamponi *et al.*, 2021).

### **1.3. *Voice characteristics in menopausal women***

Menopause is defined as the cessation of ovarian function and the decline in sex hormone levels, particularly estrogens, for at least 12 months (Lay *et al.*, 2020). Menopause and its

symptoms affecting voice characteristics, is still a relatively new area of research. The change in hormone levels due to menopause can significantly affect vocal mechanisms, resulting in lower fundamental frequency and changes in voice quality, but the findings are inconclusive (Damrose, 2009; Huang *et al.*, 2015; Markova *et al.*, 2016). Some studies indicate that voice pitch is a key parameter affected by menopause-related hormonal changes. In meta-analysis, Lă and Ardura (2022) stated that pitch is 0.94 semitones lower in post – as compared to premenopausal women. While notable, the extent of these declines falls below the threshold of perceptible difference and comfortably surpasses the threshold required to differentiate between female and male voices (Lă & Ardura, 2022). During menopause, decreased estrogen levels may contribute to vocal fold atrophy, stiffness, dryness and throat clearing (Hamdan *et al.*, 2017; Shankar *et al.*, 2021). On the other hand, Hamdan *et al.* (2017) found no significant difference in the acoustic parameters between the pre- and postmenopausal groups. Some research suggests noticeable variations in acoustic parameters in both pre- and postmenopausal women (Lă & Ardura, 2022) but others report inconclusive findings groups (Hamdan *et al.*, 2017). Both, male and female voices change over time – for example pitch (fundamental frequency, F0) in the case of men increase while in women – decrease (Tykalova *et al.*, 2021). It means that male and female vocal pitch become more similar with age. Thus, the main hypothesis of this study was to investigate whether postmenopausal women's voices are more similar to male or premenopausal female voices. The aim of the study was to apply discriminant function analysis for the classification of postmenopausal female voices to one of the groups: male or female voices and to establish the degree of method validity. What is more, it also identifies which acoustics parameters were fundamental for this assessment.

## 2. Methods

### 2.1. Participants

This study involved volunteers aged 18–65 years (mean = 31.7 y., sd = 11.8 y.). The material consisted of two groups:

- i) first group: 44 men (mean age = 37.45 y., sd = 13.45 y., range: 20.5-66.9 y.) and 35 premenopausal women (mean age = 32.42 y., sd = 11.52 y., range: 18.2-50.6 y.),
- ii) second group: 29 women in postmenopausal period (mean age = 57.18 y., sd = 4.51 y., range: 50.8-65 y.).

Research was conducted in accordance with the requirements of the declaration of Helsinki. The study was approved by the local ethical committee (Bioethics Committee at the Wroclaw Medical University, consent number: KB – 25/2021). All patients provided written consent prior to inclusion.

### 2.2. Preliminary questionnaire

Each participant of the study was firstly asked to complete a preliminary questionnaire containing inclusion and exclusion criteria. These were questions about factors which may impact on voice quality, especially: head/neck medical history of trauma and treatments, malocclusions, hearing and speech defects, being ill on the day of examination, cigarette smoking, drinking alcohol on the day prior to the day of examination, voice-over work (i.e., working as a teacher, singer, sales representative, instructor etc.), COVID-19 disease history, use of hormonal agents (i.e., oral contraceptive in women). Moreover, women from the first group were asked about the current phase of the menstrual cycle. None of the participants answered affirmatively to any of the questions regarding the presence of the aforementioned inclusion factors. From the first group 25 women were in the menstrual phase, 27 in the follicular one, 43 in luteal one and 9 of whom had ovulation. In the second group, for obvious

reasons, the phase of the menstrual cycle on the day of examination was not defined. All of them declared that they were post menopause.

### ***2.3. Anthropometric data***

Each participant had their height and weight measured. Body height was measured using an anthropometer with a range 0 cm to 200 cm and a precision to 0.1 cm. Body mass was measured using electronic scale InBody 270 to the nearest 0.1 kg. Finally, body mass index (BMI, kg/m<sup>2</sup>) was calculated using a following equation:  $BMI = \frac{\text{body mass [kg]}}{\text{body height [m]}^2}$ .

### ***2.4. Voice recording procedure and acoustic analysis***

The voices of the participants were recorded using the same equipment and equal acoustic conditions. The recording equipment consisted of a dynamic cardioid microphone Shure SM 58 SE with frequency response 50 Hz to 15 kHz situated on a tripod, an amplifier IMG Stageline MPA-202 with 45 dB sound amplification and low 60 Hz and a computer Dell Latitude E6400 with an integrated sound card. The distance between the tip of the mouth and microphone and an angle between midline of the face and microphone were the same for each participant and were 15 cm and 0°, respectively. The recording conditions were also the same for all participants - the silent room (acoustic background measured with a digital sound level meter Benetech GM1351 ~39 dB), sitting position, acoustic cabin Mozos Mshield (microphone inside), the same time of the day (9 - 12 AM) and season of the year (autumn). Each participant was asked to speak aloud five vowels /a:/, /ɛ:/, /i:/, /ɔ:/, /u:/ with sustained phonation for 3 s with 1 s break after each of them. All sound files were recorded with the sampling frequency of 44.1 kHz and 16-bit resolution as uncompressed (.wav) mono files.

All data was subsequently analyzed with Praat software v 6.0.56 (Boersma & Weenink, 2019) using a middle fragment of each vowel of equal length (0.2 s) to determine acoustic parameters. Those were mainly fundamental frequency (F0), formant frequencies (F1-Fn) and intensity. F0

is the perceived pitch of an individual voice, determined by the rate of vocal cord vibration and it varies among individuals based on factors such as age, sex, and health (Singh, 2019). Formant frequencies (formants) are resonant frequencies that shape the quality of vowel sounds in speech. They result from the acoustic filtering effects of the vocal tract on the sound produced by the larynx. Different vowels are characterized by distinct patterns of formant frequencies, which contributes to vowel identification (Pisanski *et al.*, 2016). Intensity refers to a pressure at which a sound is emitted, determining it as the loudness of the sound perceived by the listener (Zhang, 2016). Many studies prove that both pitch and timbre can indicate individual body size and shape among adult men and women. Besides timbre and mean pitch, certain voice parameters may also hint at differences in height, weight and body circumferences, such as minimum F0, maximum F0, and F0 variability (Pisanski, Fraccaro *et al.*, 2014; Pisanski, Jones *et al.*, 2016; Pawelec *et al.*, 2022; Teixeira *et al.*, 2013). In addition to these primary parameters, other factors such as jitter, shimmer, and harmonics-to-noise ratio (HNR) contribute to the acoustic characteristics of the voice were computed. Jitter and shimmer are measures of the variations in F0 and intensity. Jitter refers to the cycle to cycle variations in F0, while shimmer quantifies the cycle to cycle variations in amplitude (Teixeira *et al.*, 2013). HNR is a measure used to quantify the balance between harmonic components and noise in the speech. It reflects the degree to which the sound consists of harmonically related components, which are characteristic of voiced sounds produced by the vocal folds, versus non-harmonic noise components, which may arise from various sources such as turbulent airflow or vocal fold irregularities (Murphy *et al.*, 2008). All acoustic parameters were averaged using 5 vowels' values for each participant.

## **2.5. Statistical methods**

Basic descriptive statistics of physical and acoustics parameters were calculated (in the case of the first group separately for men and women) for both groups. To determine the discriminant

functions of voice acoustics parameters for men and women the first group was used as a control group. The sex of participants was taken as an independent (grouping) variable, and nine voice parameters: F0, F1-F4, jitter, shimmer, HNR, and intensity as dependent variables. The second group containing data of postmenopausal women was a validation group. The linear discriminant analysis (LDA) method was used. To assess the differences of premenopausal women, postmenopausal women and men vocal pitch the one-way analysis of covariance (ANCOVA) including age and BMI as confounding variables and Tukey's HSD post-hoc test for unequal counts were applied. The Statistica 13.5 software (1984-2017 TIBCO Software Inc. Palo Alto, California, USA) was applied for all analyses. The significance level set to  $p < 0.05$  was considered significant.

### 3. Results

#### 3.1. *Descriptive data*

Descriptive statistics presenting central tendency and dispersion measures of the sample were shown in Table 1.

**Table 1.** Descriptive data of a control and a validation group.

Trait	Control group (N= 79)								Validation group (N=29)			
	Premenopausal women (n = 35)				Men (n = 44)				Postmenopausal women			
	Mean	Sd	Min	Max	Mean	Sd	Min	Max	Mean	Sd	Min	Max
Age [years]	32.42	11.52	18.20	50.60	37.45	13.45	20.50	66.90	57.18	4.51	50.80	65.00
Body height [cm]	167.09	5.01	155.00	175.00	179.91	5.91	162.00	190.00	162.94	6.96	146.00	176.90
Body mass [kg]	67.14	12.90	40.00	106.70	85.12	17.18	57.00	135.00	71.49	11.90	54.30	103.10
BMI [kg/m <sup>2</sup> ]	24.02	4.36	16.65	38.70	26.23	4.81	18.40	38.20	26.97	4.42	20.86	34.97
F0 [Hz]	204.57	23.73	143.68	273.72	120.74	22.84	90.60	174.63	183.74	22.98	148.18	246.06

Jitter [%]	0.42	0.24	0.11	1.22	0.44	0.33	0.16	2.30	0.35	0.15	0.14	0.75
Shimmer [%]	3.90	2.77	1.20	13.16	4.18	2.37	1.17	11.84	3.40	1.98	0.77	10.54
HNR [dB]	22.66	5.12	10.12	34.49	18.58	3.65	10.42	25.88	25.81	4.95	14.45	35.05
intensity [dB]	72.99	8.05	55.88	89.21	76.54	8.97	61.95	90.26	73.36	15.66	61.86	149.05
F1 [Hz]	583.38	66.30	460.07	761.38	629.22	182.19	410.72	1113.27	575.09	62.02	469.42	777.23
F2 [Hz]	1570.71	223.54	1306.44	2636.22	1745.41	440.11	1283.67	3090.85	1538.51	205.95	1074.73	2302.56
F3 [Hz]	2883.25	280.79	2445.71	4171.51	2953.86	422.46	2530.88	4274.47	2879.53	219.68	2559.02	3728.81
F4 [Hz]	3990.10	351.97	3445.89	5706.09	4050.14	828.34	3387.96	6342.94	3945.70	395.36	3468.47	5736.74

### 3.2. Linear discriminant analysis (LDA)

The discrimination of the participants' sex based on selected voice parameters was highly significant (Wilks  $\lambda = 0.17$ ;  $F = 32.77, p < 0.001$ ). The significant acoustic characteristics for discriminant analysis were F0, shimmer, HNR, and intensity, therefore they were used for the next model. When these four variables were taking into account once again all of them remained significant (Wilks  $\lambda = 0.19$ ;  $F = 81.39, p < 0.001$  thus these variables were used for all subsequent analyses (Table 2).

**Table 2.** Voice characteristics and their meaning in discriminant function analysis.

Acoustic parameter	$\lambda$	partial $\lambda$	$F$	$p$
1st model				
F0	<b>0.64</b>	<b>0.27</b>	<b>189.33</b>	<b>&lt;0.001</b>
F1	0.17	1.00	0.17	0.685
F2	0.17	1.00	0.08	0.781
F3	0.18	0.95	3.41	0.069
F4	0.18	0.95	3.62	0.061
jitter	0.17	1.00	0.20	0.660
shimmer	<b>0.19</b>	<b>0.92</b>	<b>5.68</b>	<b>&lt;0.05</b>

HNR	<b>0.18</b>	<b>0.94</b>	<b>4.38</b>	<b>&lt;0.05</b>
intensity	<b>0.21</b>	<b>0.83</b>	<b>14.28</b>	<b>&lt;0.001</b>
Final model				
F0	<b>0.73</b>	<b>0.25</b>	<b>217.35</b>	<b>&lt;0.001</b>
shimmer	<b>0.20</b>	<b>0.94</b>	<b>5.14</b>	<b>&lt;0.05</b>
HNR	<b>0.20</b>	<b>0.92</b>	<b>6.03</b>	<b>&lt;0.05</b>
intensity	<b>0.23</b>	<b>0.81</b>	<b>17.40</b>	<b>&lt;0.001</b>

$\lambda$  – Wilk's lambda

There was only one canonical discriminant function which was statistically significant ( $\chi^2 = 126.47, p < 0.001$ . eigenvalue: 4.4) and its equation was as follow:

$$D1 = -0.57 - 0.05 F0 + 19.4 \text{ shimmer} + 0.12 \text{ HNR} + 0.07 \text{ intensity.} \quad (1)$$

The means of canonical discriminant function for men and women from a control group were presented in Table 3.

**Table 3.** Means of canonical discriminant function for both sexes – the control group.

Sex	Mean canonical discriminant function value
Men	<b>1.85</b>
Premenopausal women	<b>-2.32</b>

The highest significant intergroup correlation between canonical discriminant function and acoustic variables was found for fundamental frequency (F0) (Table 4).

**Table 4.** Intergroup correlations between canonical discriminant function and acoustics parameters.

Acoustic parameter	Canonical discriminant function
F0	-0.87
Shimmer	0.03
HNR	-0.22
Intensity	0.1

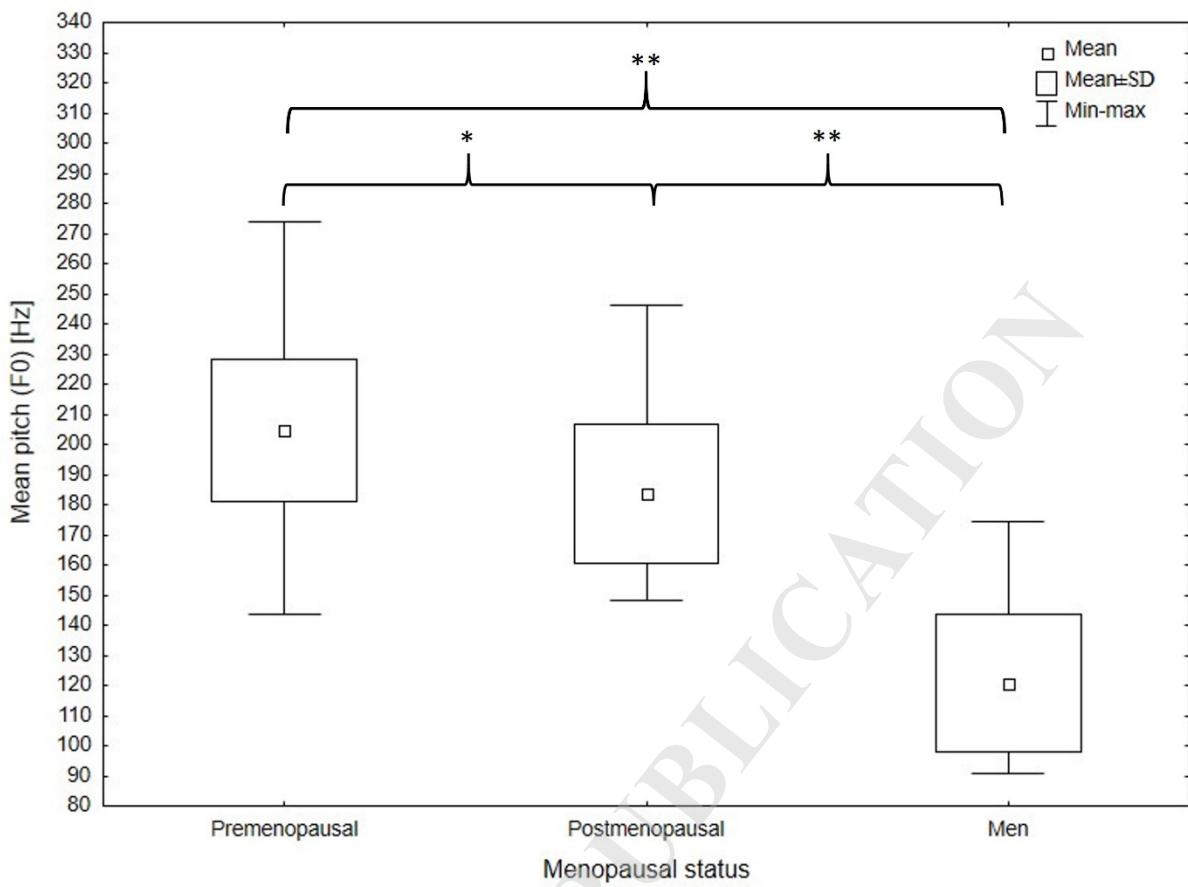
The classification matrix of training data (control group) was presented in Table 5. A priori classification probability for men was approximately 56% and in the case of women 44%. Women's voices were classified with a higher correctness (99%) than men's (93.8%).

Only one woman was classified as a man while 5 men were classified as women. Ten postmenopausal women were classified as men and 19 as women based on voice signal. The classification correctness was 65.5%. It means that acoustics parameters of postmenopausal women were more similar to premenopausal women than men's pitch, however the accuracy of classification was not 100% (Table 5).

**Table 5.** Classification matrix of men and women according to discriminant function.

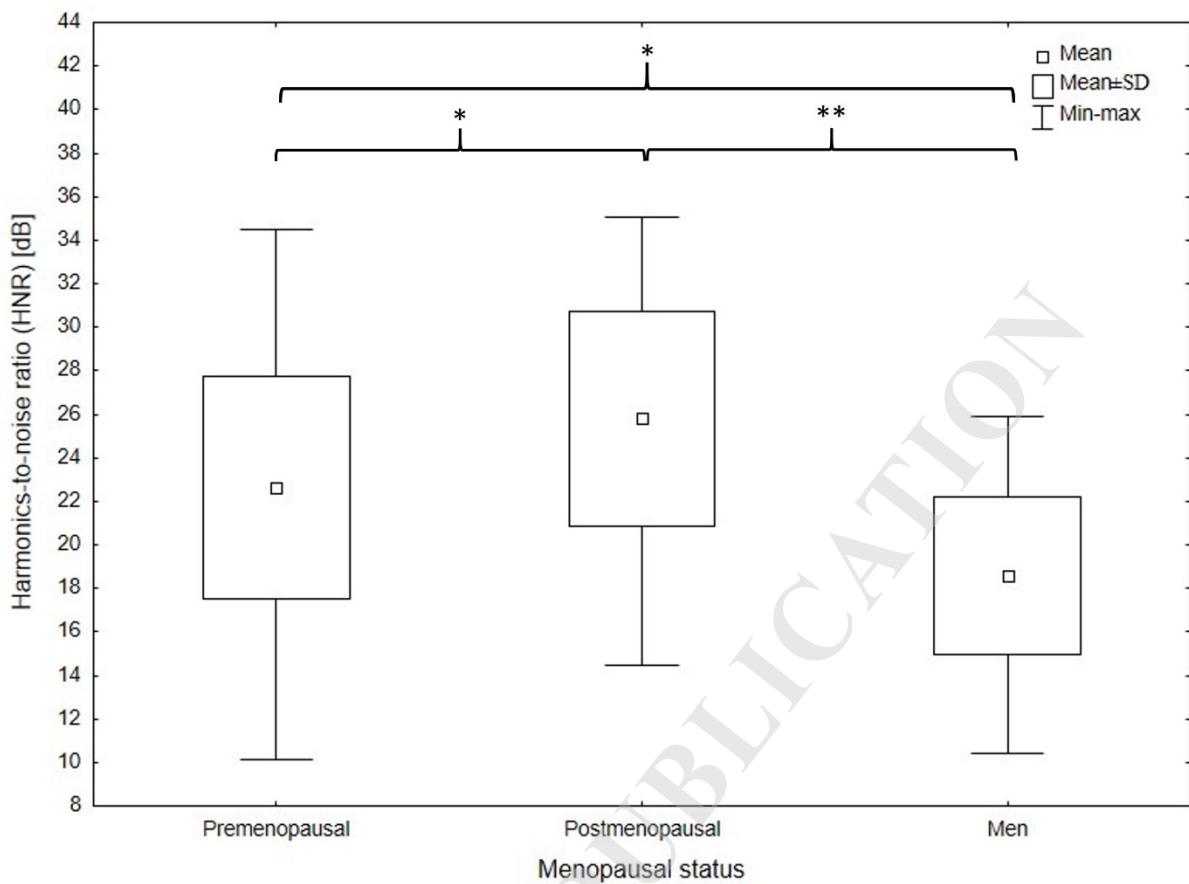
		Assessed as a	
		Assessed as a man	woman
Correct		classifications [%]	
			$p^* = 0.55696$
			$p = 0.44304$
	Men	95.5	42
Training group			2
(control)	Premenopausal women	97.1	1
			34
Validation group	Postmenopausal women	65.5	10
			19
	$\Sigma$	80.9	53
			55

\* *a priori* classification probability



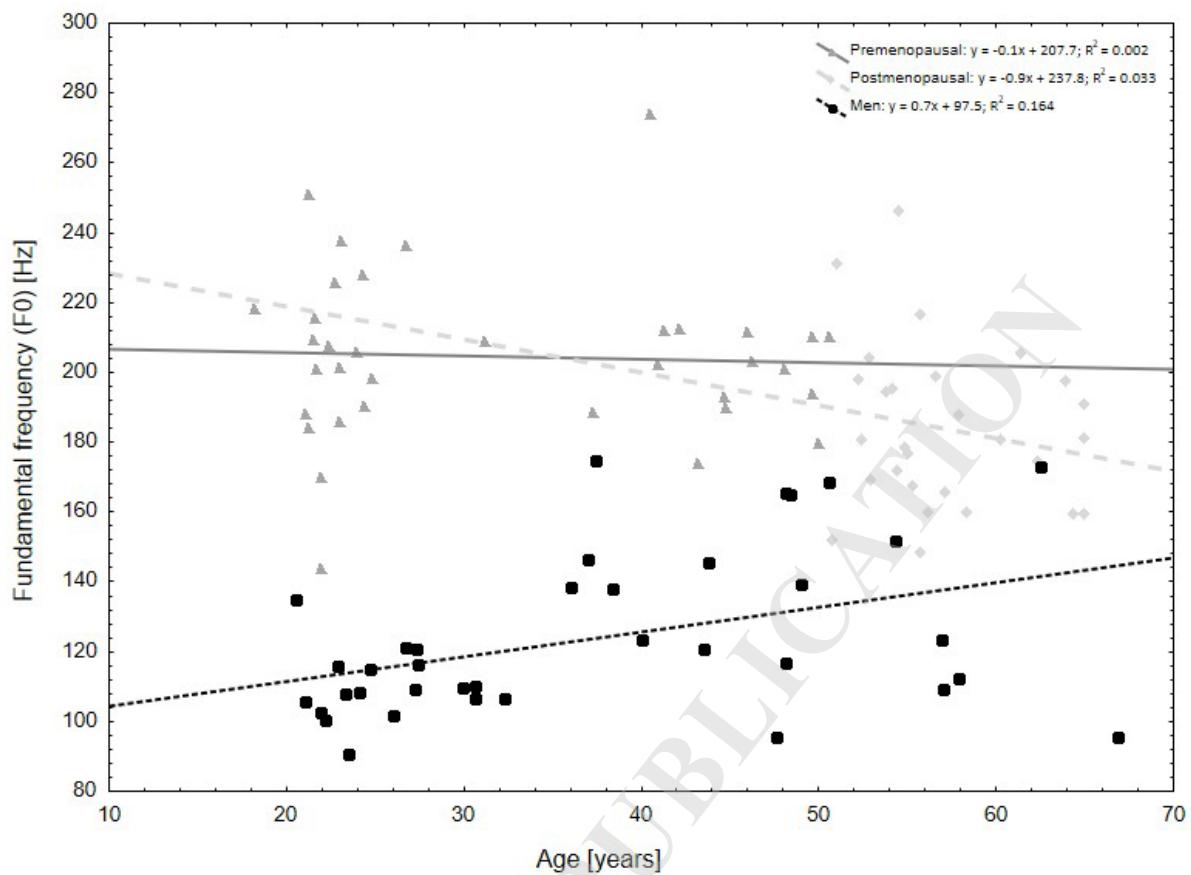
**Fig. 1.** Tukey's HSD post-hoc test for mean pitch (F0) differences between postmenopausal women and premenopausal women and men; \* $p < 0.01$ , \*\* $p < 0.001$ .

Those three groups differed from each other controlling age and BMI as confounding variables significantly in pitch ( $F = 114.16, p < 0.001$ ) and HNR ( $F = 11.55, p < 0.001$ ). Mean pitch of postmenopausal women was lower than that of premenopausal women ( $p = 0.0038$ ) but still significantly higher than men's ( $p < 0.001$ ; Fig. 1). HNR of postmenopausal women was higher than that of premenopausal women ( $p = 0.0072$ ) as well as that of men ( $p < 0.001$ ; Fig. 2).



**Fig. 2.** Tukey's HSD post-hoc test for HNR differences between postmenopausal women and premenopausal women and men;  $*p < 0.01$ ,  $**p < 0.001$ .

Fundamental frequency of postmenopausal women's voices was closer to that of premenopausal women, mostly separated from male voices. What is more, there were observed negative tendency of F0 and age for premenopausal female voices and positive tendency for male voices. Moreover, a negative trend was observed in postmenopausal women, as in premenopausal ones, but even stronger (Fig. 3).



**Fig. 3.** Linear fitting of age and mean pitch (F0) for postmenopausal women, premenopausal women and men. Linear regression equations and R-square for each group.

#### 4. Discussion

This study was undertaken to apply discriminant analysis for the classification of postmenopausal females voices to one of the groups: males or females voices and to establish the degree of method validity. It appeared that most voices which belonged to postmenopausal women were classified correctly based on discriminant function to women's group. Mean fundamental frequency (F0) of postmenopausal women's voices was significantly lower than mean F0 of premenopausal women's voices and higher than men's. Studies to date have shown consistent results: for men their pitch increases by up to 35 Hz and for menopausal women,

their pitch decreases by 10 Hz to 35 Hz (Singh, 2019). Awan (2006) in a study of middle-aged women (40–59 y.), who partly correspond to a group of postmenopausal women from this study, applying the discriminant analysis revealed that they were classified with 80% correctness to the middle-aged group. The most significant discriminators of classification were vital capacity (VC) and fundamental frequency standard deviation (called pitch sigma). The meta-analysis considered papers focusing on changes in vocal parameters after the menopause found that most studies had revealed after-menopause changes in speaking fundamental frequency, SFF ( $n = 8$ ) or fundamental frequency (F0) of sustained vowels ( $n = 10$ ). The weighted average absolute difference for SFF was 10.10 Hz and for F0 based on sustained vowel /a/ 13.41 Hz (Lă & Ardura, 2022). This finding confirms the current study results, in which it was found that discriminant analysis function was also built based on fundamental frequency of voice, among other acoustic parameters. The result of the current study is confirmed by the meaningful difference in pitch among male and female voices. The reasons for these discrepancies are the larynx size (~20% greater in men) and vocal cords' membranous length (~60% longer in men) which impact the voice properties of both sexes (Titze, 1989). The longer vocal folds, the lower fundamental frequency in men and women (Hollien & Moore, 1960). The voice differences revealed in this study come from the childhood and adolescence periods (first 20 years of life) when the larynx alongside vocal folds develop with various growth rates in men and women (Hirano, 1981). Those facts may support the hypothesis that, despite the altered hormonal profile in women during perimenopause, the anatomical differences in laryngeal structure created during progressive ontogeny are so strong that their voices are still categorized as female registers. It means that sex is a strong predictor of human voice pitch. Some evidence which enhances this statement is a study that examines event-related potentials (ERPs) of the brain as an answer to male/female voices. This findings revealed that i) participants were able to correctly discriminate a sex of the adult speaker based

on voice solely with 95% correctness; ii) the fastest brain responses were notices for low-pitched voices categorized as a man and for high-pitch categorized as a woman (Hirano, 1981). The authors stated that “These results showed that a person’s gender is in part derived from fundamental frequency (pitch) ...” (Latinus & Taylor, 2012, p. 200). On the other hand, there is known that changes in voice acoustic parameters, such as decrease of mean fundamental frequency and increase of shimmer, noise-harmonic ratio and voice turbulence index in female patient undergoing gender reassignment using testosterone, especially between the 3rd and the 4th month of therapy (Damrose, 2009). Moreover, another study indicated a significant difference in habitual pitch (HP) between menopausal women of comparable BMI who were on hormonal treatment (HT) or were not on HT. Higher value of HP was found in women on HT (Hamdan *et al.*, 2018). The authors explained those changes in voice quality by proliferative and hypertrophic estrogens’ effect on vocal folds mucosa with increase in mucus secretion and antiproliferative progesterone’s effect and decrease in glandular activity (Caruso *et al.*, 2000; D’Haeseleer *et al.*, 2012). D’Haeseleer *et al.* (2012) also found that in the case of postmenopausal women on HT had a significantly higher value of a speaking fundamental frequency (SFF) compared to those who were not on HT – the mean difference was approximately 14.2 Hz (D’Haeseleer *et al.*, 2012). In another study the same authors observed a significant positive correlation between BMI and pitch in postmenopausal women who were not undergoing hormone therapy. Conversely, no correlation was found in either the premenopausal group or the postmenopausal group receiving hormone therapy. The association between BMI and pitch in postmenopausal women not on hormone therapy suggests a potential link to heightened estrogen production in adipose tissue among individuals with elevated BMI (D’Haeseleer *et al.*, 2011). These findings show that the sex hormones impact on voice parameters is apparent, but even without a menopausal hormonal therapy (MHT) fundamental frequency is higher than average for man based on (Teixeira *et al.*, 2013).

#### ***4.1 Limitations and future directions***

This study has some limitations. First of all, the accurate determination of menopausal status of women is unknown as these women declared whether they were before or during/after menopause but the sex hormones' level in their blood or saliva was not determined. In the future research it would be essential to precisely define the menopausal status of participating women (premenopause/perimenopause/postmenopause). The second limitation is the lack of postmenopausal women using menopausal hormonal therapy (MHT) in our sample. According to other studies it seems to be important to compare both groups (MHT vs. not MHT) in each menopausal status (Caruso *et al.*, 2000; Hamdan *et al.*, 2018; Latinus & Taylor, 2012). The third limitation is the fact that non vocal apparatus imaging methods such as videolaryngoscopy, magnetic resonance imaging (MRI), or computed tomography (CT) were not performed. This step could help future researchers describe the quality of anatomical structures which take part in the speech production process (i.e. vocal folds and laryngeal cartilages, pharyngeal walls, soft palate, etc.). It would show what are the differences between these structures in men and in pre- and postmenopausal women. Another limitation of this study is the age of postmenopausal women (52-65 y.), which means that these women have recently gone through the menopause. One would expect that voices of women who would be older than those from our sample might be more similar to male voices. It would probably decrease the accuracy of discriminant analysis and as a result more difficulties with sex assessment. Finally, discriminant function analysis was the only method applied to differentiate voices. In the further consideration it would be helpful to use some subjective method, e.g., ask independent "judges" to try to guess to whom men/premenopause women/postmenopause women belongs each voice.

## 5. Conclusions

The current study revealed that though the voice parameters of postmenopausal women were significantly different from those of premenopausal ones; postmenopausal women voices were still assigned with 65.5% correctness to the women's group. The most discriminating voice parameter was f0, shimmer, HNR and intensity. Controlling both, age and BMI, pitch and HNR differed between three studied groups. Average postmenopausal female voice was significantly lower than a premenopausal woman but still higher than a man's. These significant differences in vocal pitch between above-mentioned groups are probably due to the anatomical variation between men's and women's vocal apparatus structures that originated in childhood and adolescence (Hollien & Moore, 1960). The influence of sex hormones on voice signal in those groups seems to be weaker but was not examined in the present study. Further research is needed to better understand the background of this phenomenon.

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## Conflict of interest

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

## Author's contribution

Maja Pietras provided portions of the manuscript. Łukasz P. Pawelec contributed to the concept and design of the study, acquisition of the data, as well as statistical analysis, interpretation of the results and writing of the manuscript. Monika Krzyżanowska contributed to the concept and design of the study, provided key edits and revised the manuscript. Anna Lipowicz contributed to the concept and design of the study, provided key edits and revised the

manuscript. All authors saw and approved the final version and no other person made a substantial contribution to the paper.

### **Ethical approval**

The study was approved by the local ethical committee (Bioethics Committee at the Wroclaw Medical University, consent number: KB – 25/2021). All patients provided written consent prior to inclusion.

### **Data availability statement**

There are no linked research data sets for this paper. The database for this study is available on request.

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