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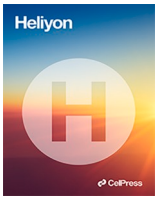

Research article

Effects of trace element dietary supplements on voice parameters and some physiological and psychological parameters related to stress

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ABSTRACT

Trace elements, often used as dietary supplements, are widely accessible without prescription at pharmacies. Pronutri has pioneered Nutripuncture®, a methodology that utilizes orally consumed trace elements to elicit a physiological response akin to that of acupuncture. Pronutri has empirically observed that the user's voice becomes deeper following an exclusive ingestion procedure. Given that alterations in vocal characteristics are often linked to stress, the Pronutri researchers postulated that the pills have the capacity to promptly alleviate stress upon ingestion. Nevertheless, there is a lack of scientific substantiation about the impact of these supplements on voice (or stress) indicators. The aim of this research was to determine whether there is a consistent impact of trace element ingestion on vocal characteristics, namely the fundamental frequency of the voice, as well as other physiological and psychological stress measurements.

In order to achieve this objective, we have devised a unique methodology to examine this hypothesis. This involves conducting a monocentric crossover, randomized, triple-blind, placebo-controlled trial with a sample size of 43 healthy individuals.

This study demonstrates that compared to placebo tablets, consuming 10 metal traces containing tablets at once is enough to cause noticeable changes in the vocal spectrum in the direction of an improvement of the voice timbre "richness", and a decrease in the occurrence of spontaneous electrodermal activity, suggesting a stress reduction. However, there were no significant changes observed in the other parameters that were tested. These parameters include vocal measures such as voice frequency F0, standard deviation from this frequency, jitter, and shimmer. Additionally, physiological measures such as respiratory rate, oxygenation and heart rate variability parameters, as well as psychological measures such as self-assessment analogic scales of anxiety, stress, muscle tension, and nervous tension, did not show any significant changes.

Ultimately, our research revealed that the ingestion of 10 trace elements pills may promptly elicit a targeted impact on both vocal spectrum and electrodermal activity. Despite the limited

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impact, these findings warrant more research to explore the long-term effects of trace elements on voice and stress reduction.

Abbreviations

EDA	electrodermal activity
PPG	photoplethysmogram

1. Introduction

The human voice is an essential vector of communication and is indispensable to certain professions where the quality of the voice has a significant impact on the public. This is the case for example for singers, teachers, politicians who have to make speeches, and journalists who speak in the media. The human voice depends on numerous factors such as vocal fold swellings, load-bearing vocal fold growths or lesions, vocal fold motion, and respiratory support acting either alone or in any combination that can cause modifications in the speed, stability, and completeness of glottal closure during the cycles of vocal fold vibrations.

Pronutri is an international company led by Dr. Patrick Véret that operates in the dietary supplements market. The company has been working on the development of trace element tablets for a number of years. They have also created a line of pills that contain dietary supplements based on metals and oligometals. The specific dosage and sequential order of assimilation of these supplements may potentially enhance voice quality. The tablets are currently available for purchase without a prescription and are made up of amino acids and trace metals, including copper, manganese, iron, zinc, and magnesium. Additionally, they contain small quantities of other metal compounds such as calcium carbonate, copper carbonate, iron citrate, zinc gluconate, tricalcium phosphates, copper gluconate, magnesium sulfate, and manganese carbonate. To ensure biocompatibility and bioavailability, these trace elements are consumed sequentially via the ingestion of 10 tablets. According to the designers, the intricate process of making the tablets based on a specific protocol enables the creation of synergistic effects among the various oligometals, hence maximizing their effectiveness in achieving the desired outcomes.

Trace elements are minerals that exist in our body in very small quantities and are crucial for the optimal functioning of our metabolism. Certain trace elements serve as co-factors in oxidation-reduction processes that include molecular oxygen (such as copper), stimulate T lymphocytes (like zinc), and eliminate hydrogen peroxide, which may generate free radicals (such as selenium) [1]. A deficiency in any of these trace elements may result in adverse consequences, including anemia, tiredness, and compromised immunological systems, which may ultimately lead to severe metabolic problems [2]. Ongoing research is being conducted on deficits and voice alterations. Vitamin A, C, and/or D deficiencies may cause dryness and heightened vulnerability to infections, thereby affecting voice health [3,4]. Voice tiredness and diminished voice performance may result from iron deficient anemia [5]. A deficiency of zinc might weaken the immune system, possibly influencing the well-being of the vocal cords [6]. Zinc sulfate treatment is used to treat vocal process granuloma [7].

Trace elements may mimic the effects of acupuncture by attaching to taste receptors on the tongue [8] and stimulating certain nerves that might impact distant target organs, similar to how an acupuncture needle would. This approach is referred to as Nutripuncture® [9].

Pronutri dietary supplements have been on the market for many years and preliminary observations involving perceptual judgments made by skilled listeners suggest an improvement of the voice similar to what can be observed secondary to throat relaxation, i.e. a deeper, richer voice [10–13]. Trace elements, by acting on metal ion receptors of the tongue may modify the activity of the afferent nerves and regulate the sympathetic tone, thus inducing changes in throat contraction parameters, which may affect the voice. However, no scientific study using quantitative measurements has appraised their effectiveness on voice parameters.

Voice parameters can be studied using many commercially available systems for acoustic analysis. These computer-interfaced units contain software designed to perform complex manipulations of voice signals via microphone connections. In general, values derived from all of these programs include: (1) fundamental frequency of voice (i.e., the patient's habitual pitch), (2) jitter (i.e., the degree of pitch instability or perturbations in the speed of vocal fold vibrations), (3) shimmer (i.e., the degree of loudness instability or perturbations in the amplitude of vocal fold vibrations), and (4) harmonics-to-noise ratio (i.e., the overall amount of noise in the voice signal). Using such software, several studies have shown that some voice parameters (particularly the mean frequency F0) are related to stress [10–13]. More advanced voice processing yields the vocal spectrum centroid, which contains information on the timbre and voice timbre "richness" [14,15].

The effects on the voice could thus be secondary to stress reduction acting on the sympathetic tone [16]. This is an important question because of the challenges imposed in our modern world, namely high competition between individuals, repeated distractions and because, sometimes in spite of ourselves, our poor adaptation to these external stimuli. Reducing stress has become a challenge that many professionals have tried to meet. Thus, the emergence of new therapies for stress, both conventional and/or complementary are needed [17]. The term "Stress" was introduced by Selye in 1936 and was defined by Cohen et al., in 1983 as "a process in which environmental demands tax or exceed the adaptive capacity of an organism resulting in psychological and biological changes" [18]. In

other words, it is an adaptive response to the environment and it manifests itself in the effect of different physiological, psychological and physical parameters on maintenance of the balance of our body's internal state in the face of events that challenge that balance. However, repetitive or prolonged response to stress can become deleterious and lead to anxiety, depression, burnout, low-grade inflammation and numerous disorders [19–22]. Stress reduction can be estimated quantitatively in a non-invasive way by measuring various physical and physiological parameters [23,24].

Pronutri has developed empirically tablets that they claim to have an effect on the voice, which could be related to stress reduction. This research aims to determine the potential impact of consuming trace elements on voice parameters, as well as other physiological and psychological stress metrics. The goal is to get a comprehensive understanding of the potential impacts of taking Pronutri tablets on reducing stress.

This study is groundbreaking in its area, since it is the first to examine the potential impact of Nutripuncture® on voice and on stress reduction. To our knowledge, no previous research has explored this topic.

2. Materials and methods

2.1. Ethic stratement

Our protocol was approved by French Ethics Committee 412 (Number: B412201524252) N/ref: 1494. Information was given to the ANSM and the protocol information was reported on Clinicaltrials.gov. The research study was sponsored by Pronutri Laboratories, but they had no direct involvement in it. The authors assert that they possess no conflict of interest in this work.

2.2. Study area

This study was performed in the CocoLab (MSHS Sud-Est - Université Côte d'Azur).

2.3. Participants

We included 46 participants and excluded one participant who revealed lactose intolerance on the day of the first session and two participants who failed to attend the session. Participant characteristics (43 participants, 33 women and 10 men) are presented in Table 1.

Participants declared no side effect following the ingestion of the pills. One participant complained of diarrhea, but thought that it may not be linked to the experiment.

2.4. Inclusion/exclusion criteria

Subject inclusion criteria were the following:

- Male or female over 18 years of age
- Healthy (healthy volunteers with no current treatment except contraception and no acute illness within 3 months prior)
- Affiliated to the French social security system
- Signed consent to participate

Non-inclusion criteria were the following:

- Voice disorders: dysphonia, aphonia …
- Communication disorders: aphasia, …
- Presence of a cardiac pacemaker
- Person intolerant to lactose or to any excipient present in the Pronutri product
- Person under legal protection or unable to give consent
- Minor
- Pregnant women or women who had given birth within 6 months or women of childbearing age without effective contraception
- Person deprived of liberty by a judicial or administrative decision or under psychiatric care.

Table 1
Characteristics of the participants (mean ± standard error of the mean).

Number of participants	Age (years)	Sex	Practice of sports	Meditation practice	Height (m)	Weight (kg)	BMI (kg/m^2)
43	37.30 ± 2.81	76.74 % female 23.26 % male	63.41%	43.90%	1.68 ± 0.09	64.00 ± 1.83	22.55 ± 0.51

Table 2
Composition of the oligo-element tablets for women and men and of the placebo tablets.

Women Tablets (for 40 KG)	Charging agent: Magnesium stearate	Oligo-elements Used	Weight (G) (Weighting tolerance: 0.1%)	Molecular weight g/mol	Oligo-elements Quantity (/50 mg tablet)
1	808.0 G	Calcium carbonate	1200	100.0	15 μmol
NUTRI 35		Copper gluconate	4.5	453.8	12.4 nmol
		Sodium iodine	0.3	149.9	2.5 nmol
		Tri magnesium phosphate	900	262.9	4.28 μmol
		Potassium gluconate	40.0	234.2	213 nmol
2	787.0 G	Copper gluconate	6	453.8	16.5 nmol
NUTRI 25		Manganese gluconate	10.5	445.2	19.5 nmol
		Zinc Sulfate	45.0	161.5	348 nmol
		Calcium carbonate	70	100.0	875 nmol
3	809 G	Potassium Gluconate	30	234.2	160 nmol
NUTRI 29		Sodium Chloride	20	58.4	428 nmol
		Magnesium Oxide	600	40.3	18.6 μmol
		Tri-magnesium phosphate	600	262.9	2.85 μmol
4	811 G	Copper carbonate	3	453.8	8.26 nmol
NUTRI 09		Zinc sulfate	45	161.5	348 nmol
		Tri-calcium phosphate	400	31.2	16 μmol
		Manganese carbonate	10.5	114.9	114 nmol
		Magnesium sulfate	900	120.4	9.34 μmol
		Copper sulfate	0.1	159.6	0.783 nmol
5	807.0 G	Calcium carbonate	200	120.4	2.08 μmol
NUTRI 24		Sodium carbonate	100	106.0	1.18 μmol
		Zinc Gluconate	45	455.7	123.4 nmol
		Manganese sulfate	10.5	151.0	86.92 nmol
		Tri calcium phosphate	800	310.2	3.22 μmol
6	788 G	Calcium carbonate	200	100.0	2.5 μmol
NUTRI 23		Copper gluconate	6	453.8	16.5 nmol
		Manganese carbonate	10.5	114.9	114.2 nmol
		Zinc sulfate	6	161.5	16.4 nmol
7	811 G	Copper carbonate	3	123.6	30.3 nmol
NUTRI 09		Zinc sulfate	45	161.5	348.3 nmol
		Tri-calcium phosphate	400	310.2	1.61 μmol
		Manganese carbonate	10.5	114.9	114.23 nmol
		Magnesium sulfate	900	120.4	9.34 μmol
		Copper sulfate	0.1	159.6	0.78 nmol
8	807.0 G	Calcium carbonate	200	100.0	2.50 μmol
NUTRI 24		Sodium carbonate	100	106.0	1.18 μmol
		Zinc Gluconate	45	455.7	123.4 nmol
		Manganese sulfate	10.5	151.0	86.92 nmol
		Tri calcium phosphate	800	310.2	3.22 μmol
9	802.0 G	Calcium carbonate	900	100.0	11.25 μmol
NUTRI 37		Copper gluconate	6	453.8	16.53 nmol
		Sodium iodine	0.5	149.9	4.17 nmol
10	819 G	Calcium carbonate	800	100.0	10.0 μmool
NUTRI 08		Manganese carbonate	10.5	114.9	114.23 nmol
		Manganese gluconate	10.5	445.24	29.48 nmol
		Tri magnesium Phosphate	900	262.9	4.28 μmol
		Potassium Gluconate	40	234.2	213.49 nmol
Men Tablets (Powder for 40 KG)	**Charging Agent: Magnesium stearate**	**Oligo-elements (Weighting tolerance: 0.1%)**	**Weight (G) (Weighting tolerance: 0.1%)**	**Molecular weight g/mol**	**Oligo-elements Quantity (/50 mg tablet)**
1	802.0 G	Calcium carbonate	900	100.0	11.25 μmol
NUTRI 36		Copper gluconate	6	453.8	16.53 nmol
		Sodium iodine	0.5	149.9	4.17 nmol
2	787.0 G	Copper gluconate	6	453.8	16.5 nmol
NUTRI 25		Manganese gluconate	10.5	445.2	19.5 nmol
		Zinc Sulfate	45	161.5	348 nmol
		Calcium carbonate	70	100.0	875 nmol
3	809 G	Potassium Gluconate	30	234.2	160 nmol
NUTRI 29		Sodium Chloride	20	58.4	428 nmol
		Magnesium Oxide	600	40.3	18.6 μmol
		Tri-magnesium phosphate	600	262.9	2.85 μmol
4	811 G	Copper carbonate	3	453.8	8.26 nmol
NUTRI 09		Zinc sulfate	45	161.5	348 nmol
		Tri-calcium phosphate	400	31.2	16 μmol
		Manganese carbonate	10.5	114.9	114 nmol

(*continued on next page*)

Table 2 (*continued*)

Women Tablets (for 40 KG)	Charging agent: Magnesium stearate	Oligo-elements Used	Weight (G) (Weighting tolerance: 0.1%)	Molecular weight g/mol	Oligo-elements Quantity (/50 mg tablet)
		Magnesium sulfate	900	120.4	9.34 μmol
		Copper sulfate	0.1	159.6	0.783 nmol
5	800.0 G	Calcium carbonate	600	100.0	7.5 μmol
NUTRI 14		Manganese carbonate	10.5	114.9	114.23 nmol
		Copper Gluconate	3	453.8	8.26 nmol
		Manganese sulfate	4	151.0	33.11 nmool
		Tri calcium phosphate	200	31.2	8 μmol
6	788 G	Calcium carbonate	200	100.0	2.5 μmol
NUTRI 23		Copper gluconate	6.0	453.8	16.5 nmol
		Manganese carbonate	10.5	114.9	114.2 nmol
		Zinc sulfate	6	161.5	16.4 nmol
7	811 G	Copper carbonate	3	453.8	8.26 nmol
NUTRI 09		Zinc sulfate	45	161.5	348 nmol
		Tri-calcium phosphate	400	31.2	16 μmol
		Manganese carbonate	10.5	114.9	114 nmol
		Magnesium sulfate	900	120.4	9.34 μM
		Copper sulfate	0.1	159.6	0.783 nmol
8	800.0 G	Calcium carbonate	600	100.0	7.5 μmol
NUTRI 14		Manganese carbonate	10.5	114.9	114.23 nmol
		Copper Gluconate	3	453.8	8.26 nmol
		Manganese sulfate	4	120.4	33.11 nmool
		Tri calcium phosphate	200	31.2	8 μmol
9	807.0 G	Magnesium carbonate	900	84.31	13.34 μmol
NUTRI 38		Copper gluconate	6	453.8	16.53 nmol
		Zinc gluconate	45	455.69	123.44 nmol
		Tri magnesium phosphate	200	262.9	950.9 nmol
10	819 G	Calcium carbonate	800	100.0	10.0 μmool
NUTRI 08		Manganese carbonate	10.5	114.9	114.23 nmol
		Manganese gluconate	10.5	445.24	29.48 nmol
		Tri magnesium Phosphate	900	262.9	4.28 μmol
		Potassium Gluconate	40	234.2	213.49 nmol
Placebo Tablets (for 40 KG)	Charging Agent: Magnesium stearate	Oligo-elements Used	Weight (G) (Weighting tolerance: 0.1%)	Molecular weight g/mol	Oligo-elements Quantity (/50 mg tablet)
Placebo	784 G	None	0		

2.5. Sample size

The sample size was calculated using power calculations or prior research to ensure that the study had sufficient statistical power to detect significant effects. A difference of about 15% on F0 is described in stressful conditions respective to normal conditions [12,25]. Based on our calculations using the GPower software, we determined that a sample size of approximately 40 participants would be adequate to detect a 15% difference between the two groups. This calculation assumes an effect size of 0.55, a standard deviation of the variation of the intergroup difference, an alpha risk of 0.05, and a power of at least 95%.

2.5.1. Study design

A triple blind study was conducted to examine the potential effects of Pronutri tablets compared to placebo tablets. The placebo tablets were designed to have a similar appearance, texture and taste and were composed of lactose, sorbitol, and magnesium stearate. In this study, neither the participants, nor the researchers conducting the experiments, nor the researchers analyzing the data were aware of the composition of the tablets labeled A and B. The effects were evaluated on a group of healthy participants who were not exposed to any stress-inducing factors. This decision was made in order to focus the investigation on the subtle stress experienced in daily life. During the first session (session 1), the subjects were given either the placebo or the trace elements tablets. Measurements were taken both before and after the administration of the pills. In the second session (session 2), which occurred at least one week later, the subject received the alternative treatment. Once again, the parameters were measured both before and after the person ingested the pills. The allocation of treatment A or B to session 1 or 2 was randomized, with 50% of participants starting with A followed by B, and the remaining 50% starting with B followed by A.

The full set of parameters measured is as follows:

The primary outcome was the change in voice frequency F0 before and after consuming the active product or its placebo. This parameter has been previously seen to differ between control and stress situations [10,17]. Participants were presumed to have come to the experiment with a moderate level of stress stemming from their everyday lives. We assessed the fundamental frequency (F0) by capturing the vocal recordings for a duration of 3.5 min immediately before and after the ingestion of the pill. This duration is sufficient to extract the main vocal characteristics from spoken language with Praat [26,27]. Secondary outcomes included other voice features,

such as jitter and shimmer, which were assessed by analyzing the fluctuations in basic frequency and amplitude on a cycle-to-cycle basis [26]. In addition, we conducted a spectral analysis and computed the mean, variance, kurtosis, and skewness of the spectrum centroid, which is a commonly used method in audio and music processing to quantify the brightness of a sound and assess the musical timbre of the voice [28].

- Physiological indicators associated with stress include blood pressure (systolic, diastolic, and pulse pressure), heart rate frequency and variability parameters [29], blood oxygenation, respiration rate, and electrodermal activity.
- The participants evaluated their stress, anxiety, muscular tension, and nerve tension using a self-assessment method. Each parameter was measured on a scale from 0 to 10 [24].

2.6. Tablets

To perform the experiment, we used a sample of a sequence of 10 tablets of trace elements and a sample of a sequence of 10 tablets of placebos for each session. The experiment was conducted according to a triple-blind procedure (the participants, the researchers, and the data analyst were blinded to the study group assignments). The samples were placed in sealed envelopes and named A or B by a person independent of the study who wrote down the correspondence between A and B and placebo or Pronutri tablets on a piece of paper placed in a sealed envelope kept in a safe place. The triple blind was removed at the end of the experiment: A Tablets were the placebo and B tablet were the Pronutri tablets.

There was an A and B sample sequence for women, and an A and B sample sequence for men, determined by Pronutri according to empirical criteria.

Each participant was asked to chew and swallow each tablet while standing up, in the order from 1 to 10 determined by the instructions from the company.

The composition in oligo-elements of the supplement tablets is shown Table 2. Tablets were prepared for 40 Kg using 29.2 KG DCL11 (Directly compressible Lactose) and 10 KG sorbitol (with a weighing tolerance of 1%). The 10 placebo tablets (A) were identically made with made by using 29,2 KG lactose, 10 KG sorbitol and 784 G magnesium stearate and contained no oligo-elements. Each tablet (A or B) weighted 0.2 G.

The composition of trace elements in the tablets differed slightly according to gender. The sequence for Woman B tablets was the following: Nutri 35, 25, 29, 09, 24, 23, 09, 24, 37 and 08. The sequence for Man tablets was the following: Nutri 36, 25, 29, 09, 14, 23, 09, 14, 38 and 08 (see Table 2).

2.7. Protocol sequence

The research consisted of a selection visit, followed by two 40-min measurement sessions at the Cocolab laboratory in Nice. These sessions took place on two separate days and included the evaluation of the participants' voice characteristics, as well as their physiological and psychological parameters. The evaluations were conducted both before and after the subjects took either a pill or a placebo. The washout time between the two measurement sessions was set at a minimum of one week based on information provided by the study sponsor, who indicated that the effect under investigation would not last for more than a few days and would definitely have dissipated by one week. However, in order to prevent any bias caused by a possible long-term effect of the tablets and to minimize the impact of the sequence in which the pills were taken, the timing of intake for tablets A and B was randomly counterbalanced among the participants.

In order to follow rigorously our sequence protocol (Fig. 1), we used Psychopy software. We coded the protocol to be followed step by step in order to have the same degree of regularity towards all participants and to automatize the data acquisition.

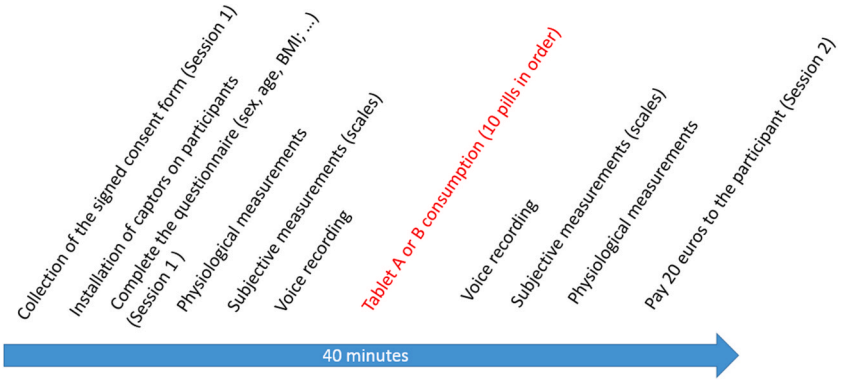

Fig. 1. Pre- and post-tablet intake protocol. The protocol was repeated twice at a one-week interval (each subject received one session with Tablet A and one session with Tablet B). To limit a potential effect of order, sessions for Tablet A or B were randomly counterbalanced across subjects. Actions specific to Session 1 or 2 are specified in the Figure.

After being informed of the study protocol in an information document, the participant was asked to give written consent to participate (Suppl. 1). For each measurement session, the participant seated on a chair, his or her waist measurement was taken, and we installed the various sensors used to measure physiological parameters. We then used PsychoPy, and a short questionnaire on information such as sporting activity, meditation, and medical information (medications taken, COVID vaccination) was completed. We measured blood pressure and then used The Observer software, which triggered simultaneously the CardioSensys and AcqKnowledge software programs, which simultaneously recorded the participant's physiological parameters for 10 min. The participant was asked to do nothing in particular, and to let his or her thoughts flow naturally while watching an hourglass display on the screen showing the passage of time programmed on PsychoPy.

At the end of the 10-min measurement, the participant was asked by Psychopy to self-assess his stress, anxiety, muscular tension and nervous tension on an analogic scale of 1–10.

The participant was then asked to stand up and face a recording microphone and to speak for 3.5 min continuously, answering various questions such as "What is the weather like today?", Repeat 3 times, "I am a man/woman", and to talk about what a series of neutral images (phone, book, globe, pens, paper, …), see Fig. 2a and Suppl 2. These measurements constitute the Pre-Ingestion measurements. Based on previous studies [26,27], we considered this 3.5-min duration to be a sufficient sample for vocal analysis, and used the whole sample for analysis.

Finally, at the end of the voice recording session, the participant was asked to chew and then swallow the 10 tablets one by one, sequentially, from 1 to 10. The protocol was then implemented in the reverse order, by recording the voice immediately after taking the tablets, then using the subjective scales, followed by the recording of physiological parameters by CardioSensys and AcqKnowledge, and ending with the blood pressure measurement.

These measurements will be referred to as the Post-Ingestion measurements.

2.8. Voice analysis

The voice parameters were captured using a Fuze microphone, which had a sampling rate of 44100 Hz and 16 bit quantization. The microphone was connected to the computer, and the sound was recorded directly using PsychoPy. The recordings were captured by positioning the recorder 20 cm away from the participant's mouth at a 45° angle. Prior to starting the primary recording sessions, the

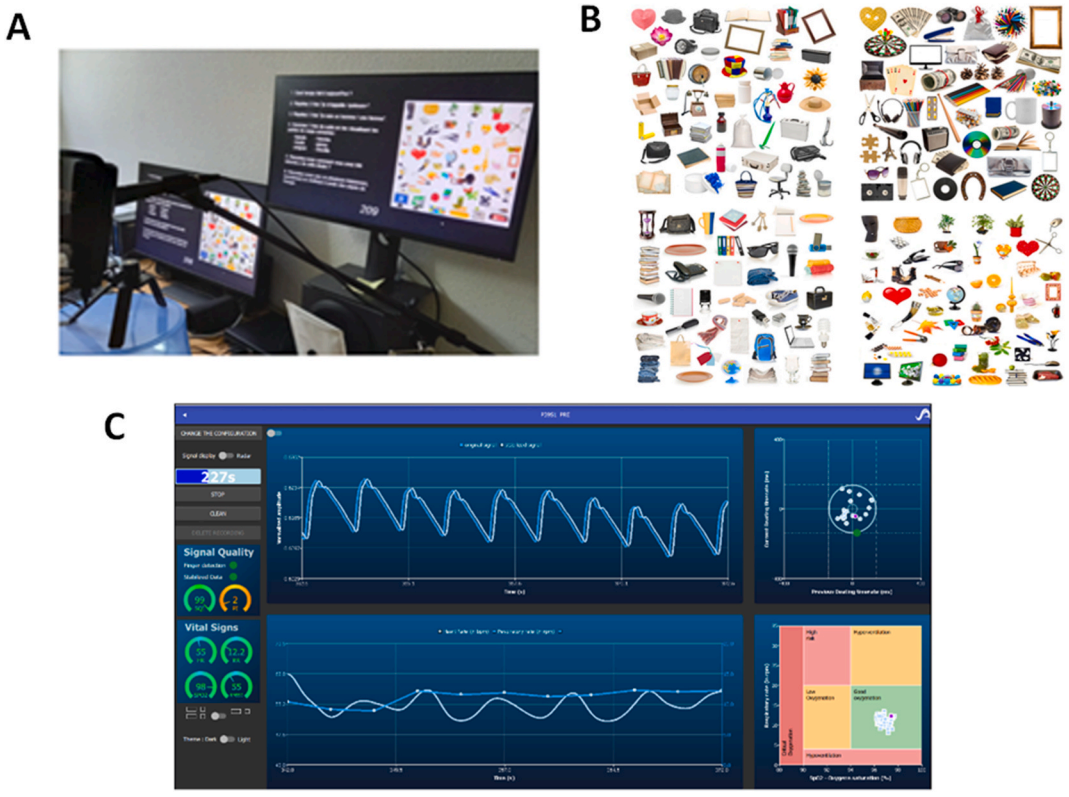

Fig. 2. Voice recording protocol and Cardiosensys software. A. Picture of voice recording session with a series of questions asked and a bank of images to assist the participant in speaking for the full 3.5 min. B. series of neutral images selected for evaluation sessions 1 and 2, Pre and Post Ingestion. C. Screen image captured showing CardioSensys software computing the oxygenation, the heart rate and the respiration rate in real time. (SQI: SignalQuality Index, the percentage of beats detected and used in the analysis of the arterial wave; Index perfusion >1%; IBI.txt: Inter Beat Interval).

examiners provided a detailed explanation of the task to each participant individually.

The participant recorded their voice twice during each session: once before ingesting the tablets (Pre-Ingestion session) and then again 1 min after ingesting the pill (Post-ingestion session). This was done since Pronutri claims that the impact on voice modulation is instantaneous.

The participants positioned themselves in front of the computer screen and were instructed to speak into the microphone for a duration of 3.5 min.

To mitigate emotional bias, participants were shown neutral pictures such as paper supplies, pens, binoculars, and fruits on the screen. They were then instructed to either create a narrative about each image, recall a personal experience, or describe the items until the time limit was up. Following the ingestion of the pill, we proceeded to replicate the same procedure for a second time, using the same set of inquiries and introducing novel visuals (Fig. 2b).

After gathering the audio recordings, we conducted a comprehensive analysis of the full recorded audio sample using the audio analysis program Praat [30]. The analysis included the average, median, standard deviation, maximum, and lowest values of the primary frequency F0 for each recording. Additionally, it included secondary metrics such as jitter, shimmer, harmonics-to-noise ratio, and the number of pulses or pauses. The Praat data parameters were transferred to an excel file for the purpose of organizing them for data analysis and calculating their statistics.

The Praat software, in addition to measuring the fundamental frequency F0, calculates jitter and shimmer, which are variations from cycle to cycle in fundamental frequency for jitter and in amplitude for shimmer respectively.

We also processed the audio signals and extracted the spectral centroid, a measure of the sound brightness, widely used in audio and music processing as a metric of musical timbre. The spectral centroid is calculated by taking the mean of the signal frequencies generated using the Fourier transform and weighted by their magnitudes. It corresponds to the barycenter of the spectrum.

We then calculated the mean, variance, kurtosis and skewness for the spectral centroid.

2.8.1. Physiological parameters

The participants' blood pressure and heart rate were assessed using a blood pressure monitor (Omron M3 V4 HEM-7154-E) both before and after the measurements, following the collection of other psychological and physiological data.

The Biopac module was used to measure the participants' various physiological data. The system consists of a breathing belt, an electrodermal activity (EDA) sensor attached to the palm of the hand, and a photoplethysmograph (PPG) positioned on the participant's earlobe. The sensors were straightaway connected to the Acqknowledge program via Bluetooth, enabling the recording of physiological data during the 10-min pre-ingestion and post-ingestion assessments. During these measurements, the participant stayed seated and focused on an hourglass displayed on a screen.

The blood oxygenation level, heart rate, and heart rate variability were calculated using a finger PPG sensor provided by Sensoria Analytics with the CardioSensys software. This was done by analyzing the signal contour of the PPG information [24], as shown in Fig. 2C. A PPG sensor is a device used to quantify the level of infrared light that is either absorbed or reflected by the blood. Pressure fluctuations in blood arteries throughout the cardiac cycle [31] are responsible for the variations in the blood volume. Cardiosensys, a software program, analyzed the PPG data and generated several parameters related to pulse wave analysis based on the recorded information from the PPG [24] including the heart rate variability parameters.

2.9. Subjective measurements

Each participant was asked to self-assess their state of stress, anxiety, muscle tension and nervous tension at the time of the measurement using four analog scales (from 0 to 10), one for each of these parameters, similar to those described in Gomes et al., 2020 [24], in order to maintain consistency with the procedures used in this previous study.

These four analog scales were completed during both pre-ingestion and post-ingestion measurement segments.

2.10. Statistical data analysis

Data and statistical analysis was performed using Sigmaplot 10.0, GraphPad Prism 8 and R software. Figures were created using GraphPad Prism 8 software. We used two-way repeated measures ANOVA (based on general linear models) in order to examine the influence of the two different categorical independent variables treatment (product or placebo) and time (before-after treatment) on the continuous dependent variable measured. As post-hoc tests, we used paired *t*-test when data were Gaussian-distributed, and otherwise the Wilcoxon test for paired series, corrected for multiple comparisons (Bonferroni correction). *t*-test was used when comparing the delta (post-pre) between tablets A and B. The significance level used for hypothesis testing (alpha level) was 0.05.

3. Results

3.1. Voice parameters

The vocal productions were quite consistent across participants. As expected, we found differences in certain voice parameters between men and women. For instance, in average F0 is lower in the population of men, who have a deeper voice than in the population of women (F0 = 157.42 ± 43.77 Hz for men, n = 10 and 199.81 ± 76.70 Hz for women n = 33, mean ± standard deviation (SD), *t*-test = 4.7 $p < 0.001$).

According to the data shown in Fig. 3 (A-I), there were no notable variations seen in the speech characteristics analyzed using the Praat program, both before and after ingestion, as well as between treatments A and B. The ANOVA testing yielded non-significant results.

Nevertheless, it was seen that many participants and the three researchers present during the recordings expressed the perception that Tablet A (placebo) had no discernible effect on the voice, but Tablet B (Pronutri tablets) enhanced the quality of the voice.

In order to conduct a more thorough analysis of the harmonics, we further conducted audio signal processing and retrieved the spectral centroid. This is a commonly used measure of musical timbre in the field of audio and music processing.

As can be seen in Fig. 4 (A-D), we observed a significant increase in the spectral centroid parameters (mean, centroid variance, skewness and kurtosis) with treatment B, whereas treatment-A did not modify these parameters significantly, suggesting that treatment B improves the richness of the vocal spectrum whereas treatment A has no effect.

4. Physiological parameters

4.1. Acute measurements

We found that participants with lower average arterial pressure and heart rate were (unsurprisingly) those who regularly practiced sport (and to a lesser extent practices such as meditation, yoga, tai chi, qi gong and sophrology) while the participants with a high BMI had in higher average values of blood pressure and cardiac rhythm.

As illustrated in Fig. 5 (A,C), ANOVA revealed a significant effect between pre-ingestion and post-ingestion measurements for systolic and pulse pressure measurements (Two-way ANOVA $F = 6.595$ $p = 0.01$ and $F = 5.372$ $p = 0.02$, respectively, $DFn = 1$, $DFd = 160$), but no effect for the measurements of diastolic blood pressure and heart rate frequency (Fig. 5 B, D). While this difference occurred during the course of a session, the statistical analysis did not reveal any significant difference between treatments A and B.

4.1.1. Heart rate variability measured from PPG

Similarly, we assessed the pre-ingestion and post-ingestion PPG levels after administering treatments A and B. Examinations of heart rate variability characteristics (for a further explanation of the parameters, refer to reference 17) indicated that there was no significant difference between treatments A and B. This was determined using a paired *t*-test with a sample size of 42 participants, accounting for one person with incomplete data (Fig. 6A–H).

We examined the changes (delta) in HRV parameters that are commonly associated with relaxation states, including a decrease in heart rate, an increase in its standard deviation, the activation of the parasympathetic system (indicated by an increase in rMSSD and

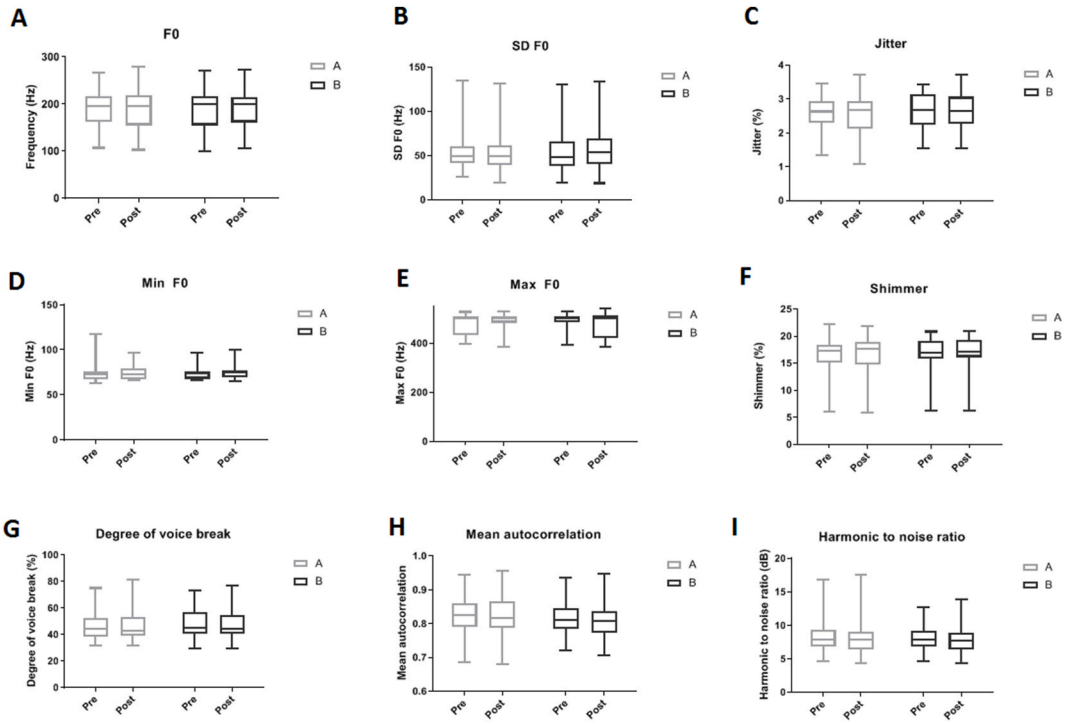

Fig. 3. Analysis of the voice parameters: A- F0, B- Sd F0, C- Jitter, D- Min F0, E– Max F0, F- Shimmer, G- Degree of voice breaks, G- Mean autocorrelation and I- Harmonics-to-noise ratio for N = 43 participants. Figures represent the median, minimum and maximum and quartiles before (Pre) and after (Post) taking the placebo (A) and trace element treatment (B).

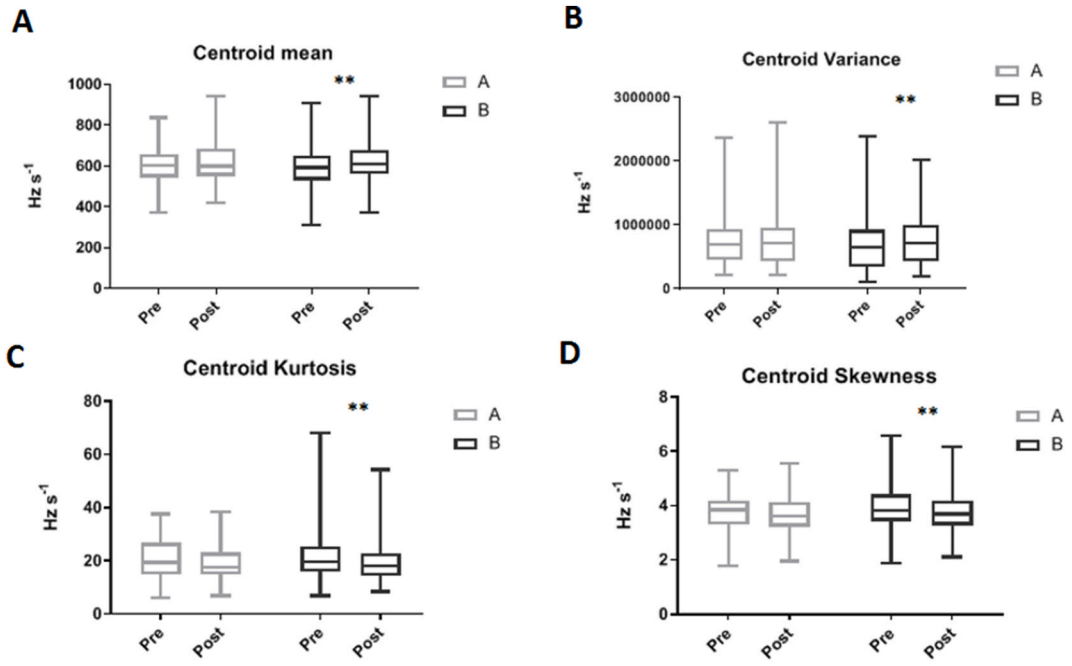

Fig. 4. Analysis of the voice parameters: A- Spectral centroid mean, B- Centroid variance, C- Centroid skewness and D- Centroid kurtosis for N = 43 participants. Figures represent the median, minimum and maximum and quartiles before (Pre) and after (Post) ingestion of placebo treatment (A) and trace element treatment (B), **p < 0.01, paired *t*-test after ANOVA.

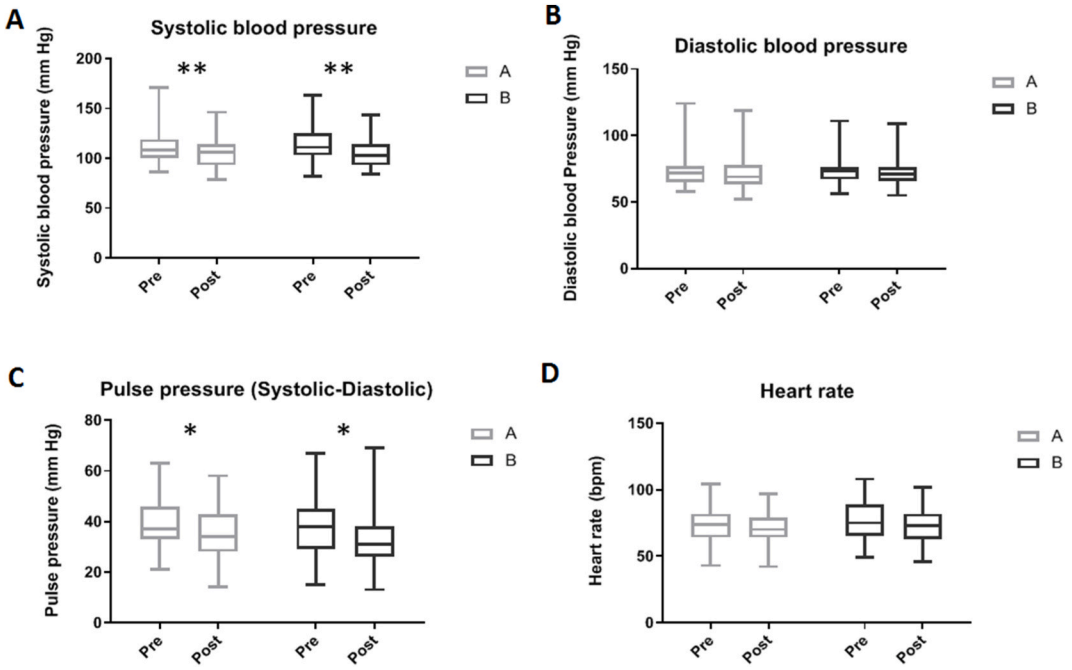

Fig. 5. Blood pressure and heart rate A. Analysis of Systolic blood pressure. B. Analysis of Diastolic blood Pressure. C. Analysis of Pulse pressure (systolic – diastolic) D. Analysis of Heart rate. *P < 0.05, **P < 0.02, paired *t*-test after ANOVA, N = 43. Graphs represent the median, minimum and maximum and quartiles before (pre-ingestion) and after (post-ingestion) taking the placebo treatment (A) and trace element treatment (B).

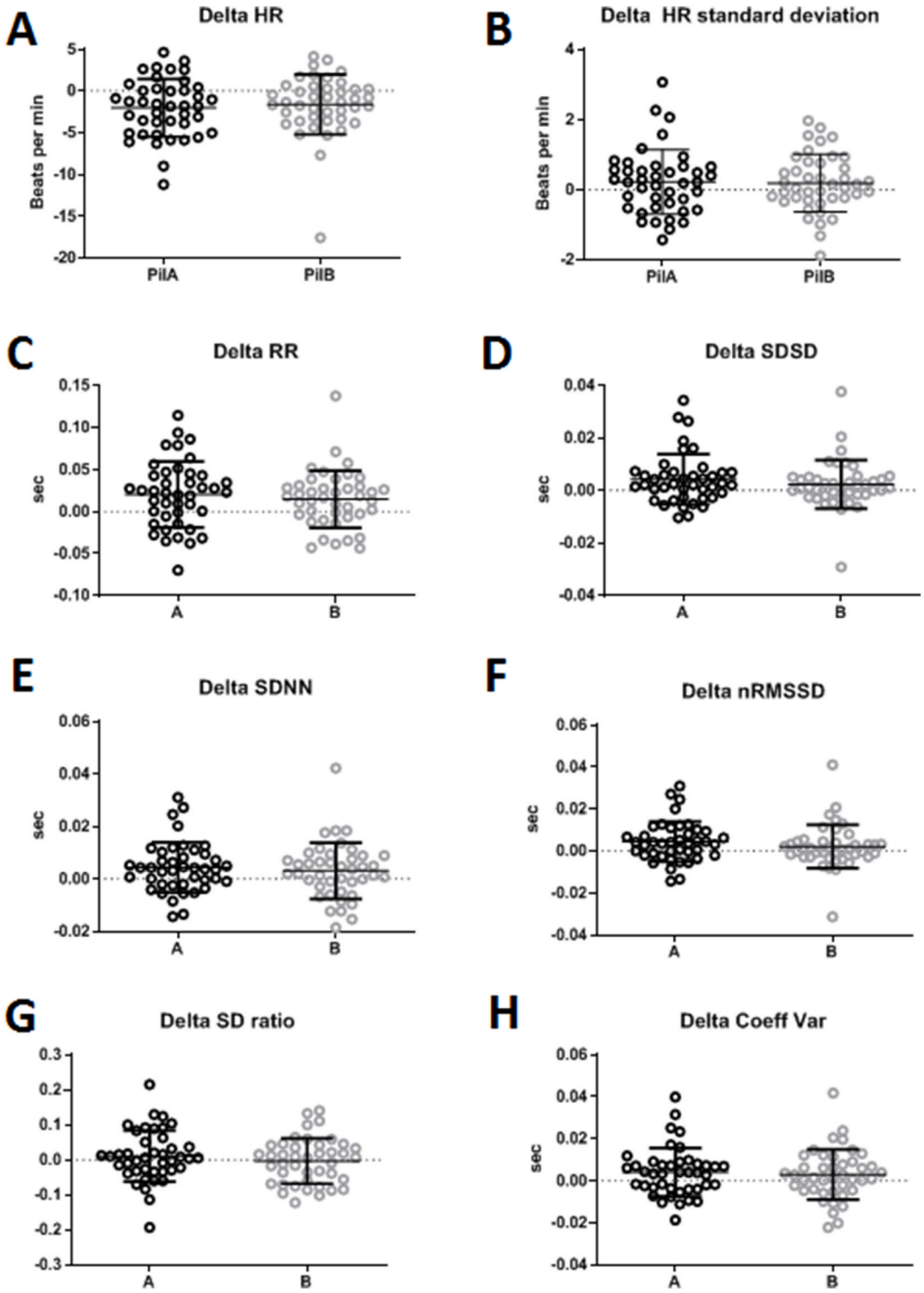

Fig. 6. PPG heart rate variability measurements during 10-min recordings. Delta Post-Pre of the different parameters indicated (A- HR, B- HR standard deviation, C- RR, D- SDSD, E– SDNN, F- nRMSSD, G- SD ratio and H- Coefficient of Variation). Each dot represents a participant. Mean ± SD is indicated by the bars. A: placebo treatment and B: trace element treatment.

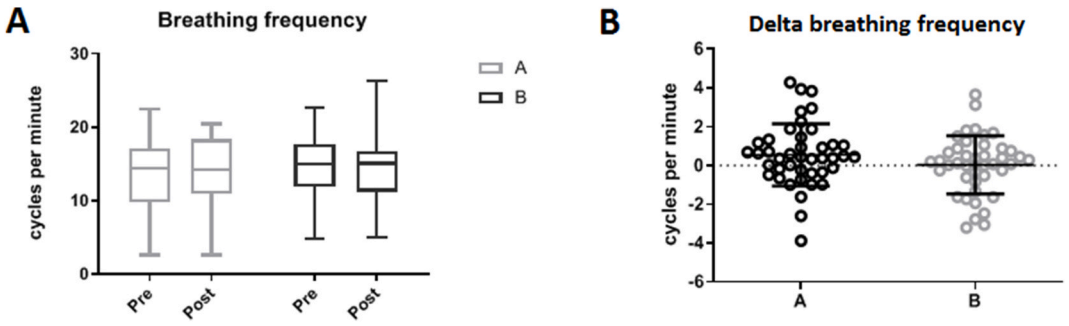

Fig. 7. Breathing frequency measurements during 10-min recordings. A: median, minimum and maximum and quartiles before (pre-ingestion) and after (post-ingestion) taking the placebo treatment (A) and trace element treatment (B). B: Delta (Post-Pre). Each dot represents a participant. Bars represent Mean ± SD. There was no significant difference between treatments A (placebo) and B (trace elements) (N = 42, missing data for one participant).

SDNN), and the deactivation of the sympathetic system (indicated by a decrease in SD ratio).

4.1.2. Respiration

As illustrated in Fig. 7 (A,B), there was no effect of treatment A or B on the breathing frequency and no post-/pre-treatment difference in breathing frequency between treatments A and B.

4.1.3. Electrodermal activity

Spontaneous electrodermal activity is described as more pronounced when people are stressed. We measured the frequency and amplitude of spontaneous skin conductance peaks for 10 min before and after taking treatment A or B. As illustrated in Fig. 8 (A-C), we found that there was on average a significant decrease in the frequency of skin conductance peaks between pre-ingestion and post-ingestion of treatment B while treatment A had no effect, with no change in the average amplitude of these events with either treatment.

4.1.4. Subjective scales

The results obtained on stress, anxiety, muscle and nervous tension subjective scales are presented in Fig. 9.

As illustrated in Fig. 9 (A-D), ANOVAs revealed no significant difference between treatments A and B or between pre-post sessions. However, when comparing the pre-post difference between treatments A and B on the anxiety subjective scale, we found a small but significant difference between the effects of treatments A and B: more subjective anxiety reduction with treatment A (placebo) than treatment B (trace elements) (paired *t*-test = −2.95 p = 0.005), although the size effect was small (Fig. 9A).

5. Discussion

We tested the effects of a single intake of 10 dietary supplement tablets believed to act on the voice and to reduce stress as evidenced in terms of voice parameters and stress-related physiological and psychological factors, versus placebo.

5.1. Effects on the voice

Our results show that there is no effect from a single intake of 10 Pronutri tablets on the common vocal parameters of voice frequency F0, the standard deviation around this frequency, jitter, and shimmer as analyzed by the Praat software. In particular, F0, which that we chose as the primary endpoint, was unaffected. F0 has been reported to change in stressful conditions. Here, the protocol was not designed to induce a particular stress in the participants and so the study conditions may not have been favorable for detection of an effect on F0. In addition, stress is a complex and multifaceted phenomenon, and its impact on F0 can vary widely among individuals and further research on stress-induced changes in voice parameters are needed. We find it important to publish these results as the publication of negative findings contributes to scientific transparency and helps prevent publication bias.

Interestingly, there was a modest but significant increase in the spectral centroid mean induced by the treatment that was not observed with the placebo, suggesting an increase in voice richness. This was in accordance with subjective perceptual judgments of several participants and of the three researchers present during the recordings, who felt that Tablet A (placebo) did not change the voice while Tablet B (Pronutri tablets) improved the timbre of the voice, making it deeper and richer, as would be reflected by an increase in the spectral centroid mean.

The effects on the voice were modest. However, it is important to mention that we recorded the voice immediately before and immediately after the tablet intake, because the study sponsor, Pronutri, informed us that the tablet acts instantaneously on variation of the voice. However, the ingestion of the 10 pills may not constitute conditions favorable to detection of a significant and lasting effect. Future studies might benefit from a different experimental design that allows for more extended monitoring of vocal changes.

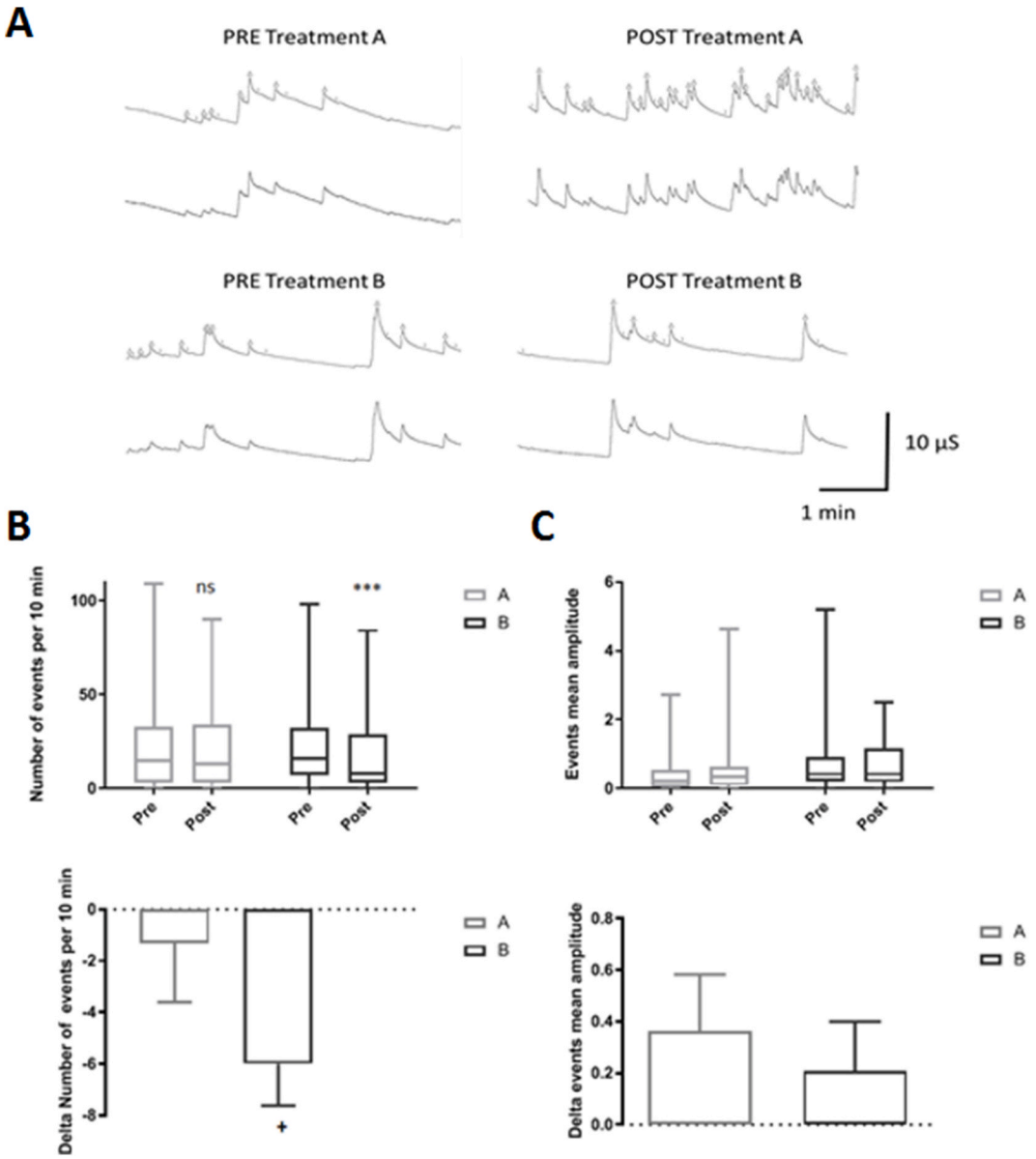

Fig. 8. Electrodermal activity. A. Examples of recordings obtained from one participant in the different conditions (bottom) and the detected peaks made with AcqKnowledge software (top). B- top: Number of skin conductance responses recorded in the 10-min period before (pre-ingestion) and after (post-ingestion) taking the treatment A or B, as indicated. Bottom: Delta between pre- and post-treatment of the number of events recorded for 10 min in both conditions. C- top: Amplitude of skin conductance responses recorded pre-ingestion and post-ingestion of treatment A or B as indicated. Bottom: Delta between pre-ingestion and post-ingestion of the amplitude of events recorded in both conditions. N = 43. ***$p < 0.001$ paired *t*-test after ANOVA Post vs Pre.+ $p < 0.05$ paired t when test comparing the delta Pre-Post for treatment A and B. A: placebo treatment, B: trace element treatment.

5.2. Effects on blood pressure, cardiac rhythm, oxygenation and respiration rate

In general, we observed no clear and significant impact of the treatment on the measured cardiac and respiratory parameters. This includes both immediate changes before and after the treatment (such as blood pressure and heart rate) and changes throughout a 10-min interval before and after ingestion (such as heart rate variability parameters and respiration rate). The observed disparities in systolic and pulse pressure between the pre-ingestion and post-ingestion states of tablets A and B can be attributed to the initial stress experienced by the participants, which was subsequently alleviated by the protocol that mandated them to remain seated for two periods of 10 min. No significant difference was noted between the treatments in terms of the reduction in Pre-Post measurements. Additional sophisticated study may be required to identify any subtle impacts of Pronutri therapy on physiological markers. Genetic

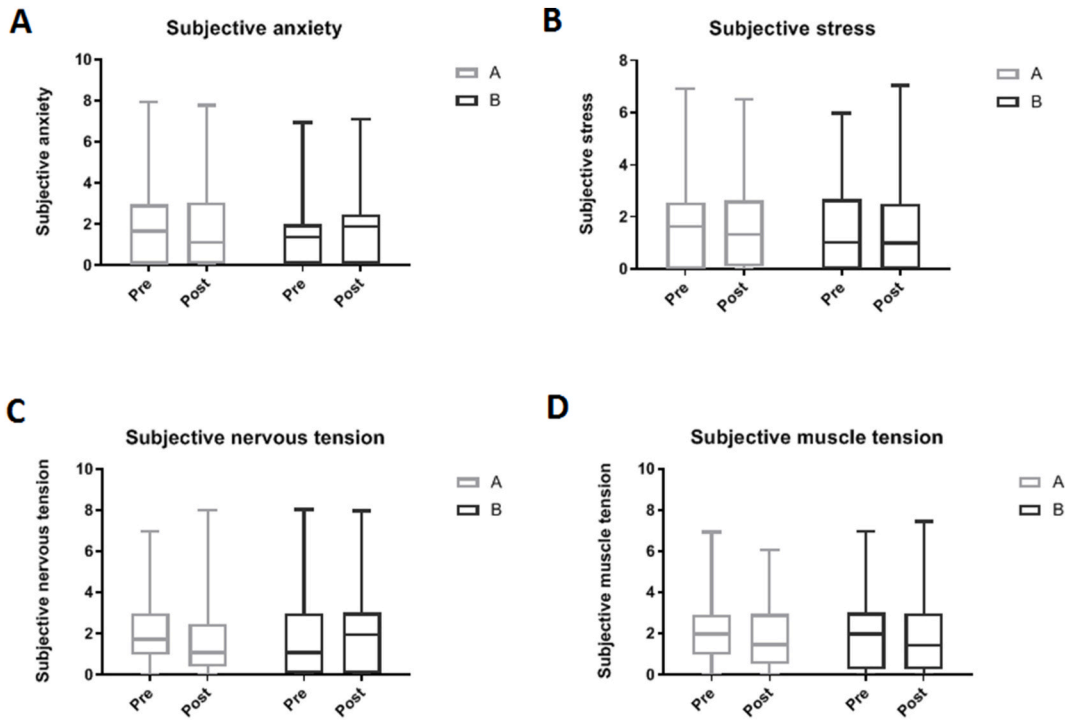

Fig. 9. Subjective scale analysis. A. Analysis of subjective anxiety for N = 41. B. Analysis of subjective stress for N = 38. C. Analysis of subjective muscle tension N = 41. D. Analysis of Subjective nervous tension for N = 37. Missing data in the subjective scale results are due to some participants clicking too quickly on the "continue" tab during the PsychoPy protocol. ANOVAs revealed no significant difference between treatments A and B or between Pre-Post sessions. A: placebo treatment, B: trace element treatment.

algorithms, for instance, may uncover subtle distinctions that cannot be detected using traditional statistical methods [32].

5.3. *Effects on electrodermal activity*

EDA is classically used to measure responses to external stress stimulation [33,34]. Here, we measured the spontaneous, internally driven skin conductance peaks when no stimulus was applied. Spontaneous fluctuations in skin conductance are often used to index sympathetic arousal and emotional states. They are caused by sudomotor nerve activity, which is a direct indicator of sympathetic arousal [35]. Interestingly, we found a significant decrease in the frequency of spontaneous skin conductance responses recorded after taking the Pronutri treatment compared to placebo, with no effect on the amplitude of these spontaneous events. The mean decrease in frequency of these events in the tested population could be an indicator of stress reduction, due to a least occurrence of stressful thoughts, for instance, and/or a lowered sympathetic tone [36]. The fact that the amplitude of the events was not modified suggests that the general mechanisms of sweat release are unaffected by the treatment. It would be interesting to test in a future study the effects of such treatment on skin conductance responses evoked by different stimuli.

5.3.1. *Subjective scales*

We measured the participants' self-assessed stress, anxiety, nervous and muscle tension states in order to have data on the psychological impact of the tablet on the participants. We realized afterwards that asking for the self-assessment immediately after the long voice recording may have skewed the results, as many participants reported feeling more stressed after having to speak 3.5 min into a microphone, while others reported to liking this and being more relaxed. The variability in participant reactions to the voice recording might have affected the psychological assessments. To limit these cognitive biases, a better timing for subjective scales assessments would have been after the 10 min of recording of physiological parameters in the post-treatment phase. More generally, timing of assessments may have influenced participant responses.

We found that there was no significant effect of the Pronutri treatment or the placebo treatment on any of the psychological parameters measured. Only when comparing the post-pre delta of the anxiety scale did we find a small but significant difference in subjective anxiety: Pronutri treatment slightly had a smaller reduction in anxiety observed than the placebo. These variations in subjective anxiety levels have to be considered with caution, as they are extremely small and are not correlated with the physiological data obtained. In addition, this difference was observed in only one out of 4 subjective parameters measured.

5.3.2. Putative mechanisms of action of Nutripuncture®

The effect of Pronutri tablets observed on the voice spectral centroid and the frequency of spontaneous EDA peaks occurred a few seconds after the participants took the tablets. This very rapid effect is unlikely to be due to a metabolic effect on the organism mediated through the digestive tract. Rather, it may result from a rapid action on the nervous system, particularly the sympathetic system. Trace elements may act on metal ion receptors of the tongue [8], thereby modifying the activity of the afferent nerves and regulating the sympathetic tone, thus inducing changes in both electrodermal spontaneous activity and spectral centroid voice parameters. In a recent double-blind randomized, placebo-controlled trial, Mares et al., 2020 [9] showed an effect of Nutripucture on endometriosis related pain. They suggest there is an effect at the level of the cellular membrane. Trace metals could modulate estrogen receptors and thus influence the tropism and the survival of cells involved in endometriosis. By a modulation of the antioxidant system, they might also interact with various parameters influencing tissue biochemistry, restoring a lost homeostasis and thus helping the body to recover its capacity for self-regulation [37].

In line with these hypotheses, trace metals in the mouth could interact with several taste receptors and activate selective neural pathways. Other authors have hypothesized that trace elements in contact with saliva may induce an electro-chemical reaction at particular receptors on the tongue located in taste buds and distributed throughout the oral cavity, and activate nerve afferents that project to the brainstem [38]. As there is a considerable divergence as well as convergence of information between multiple regions of the central nervous system that interact with the taste pathways, with reciprocal connections occurring between the involved regions, metabolic and visceral changes could occur following stimulation of some pathways with trace metals [39]. For instance, trace metals could affect salivation and swallowing, which may have an impact on the voice and a regulation of sweating could take place (like when eating chilli peppers for instance), which is directly linked to electrodermal activity [33]. More studies are needed to investigate these hypotheses.

5.4. Limitations of the study

Our study population was rather heterogeneous in age, varying from 18 to 84 years, with an average of 37 years, and in gender (33 women for 10 men), which may limit the generalizability of our findings for a specific age or gender group. A limitation of our study is the small sample size and the fact that the healthy volunteers were highly educated, and hence not representative of the general population. Voice analysis was performed on 3.5-min recording sequences while participants read questions on a computer screen, answered them, and invented stories inspired by images on the screen. The words spoken by the participants varied from one condition to another but the relatively long duration of the recording allowed the participants a great deal of freedom to express themselves as naturally as possible. Some participants said that they were stressed by speaking into the microphone, while others said they were relaxed after speaking and this may have interfered with the results. There was also a potential bias introduced by the timing of psychological assessments.

It was noted that the researchers reported differential subjective feelings on hearing voices after tablets A and B ingestion. It is possible that during the experiment this may have introduced a bias into the double blinding but the analyzed data blind shows no evidence of this bias.

It is also interesting to note that in our data we observed that people who practice meditation and sports have significantly better physiological parameters than those who do not: their heart rate and breathing were more stable, as was their oxygenation (data not shown). This could bias the data, as they would have a greater ability to manage their breathing and know how to reduce stress. Similarly, we calculated the BMI of each participant, and observed that it was very desirable in average with a low variance thus our results for now can be applied only to people with a good BMI. However, during the experiment, we observed that the few overweight participants (BMI above 25), had less stable cardiac parameters than other participants, which could affect the fluctuation of the results (data not shown).

Regarding the EDA, the initial results obtained from the Acqknowledge software were inconsistent due to variations in participants' hand moisture levels. Some participants had excessively dry hands while others had excessively moist hands. This led to contradictory physiological results regarding the actual stress level and also made it difficult to properly attach the electrode to the hand. In order to address these issues, we used a methodical approach by encircling the electrodes with rubber bands and applying more gel onto the electrodes.

There is a possibility that certain individuals may exhibit a higher level of responsiveness to the ProNutri therapy compared to others. This subject has the potential to be the focus of future research.

6. General conclusion

Our study demonstrates that consuming 10 Pronutri pills at once may cause noticeable changes in the speech spectral centroid and a reduction in the frequency of events in electrodermal activity (EDA). Using a conventional statistical analysis (ANOVA), we observed no impact on the heart rate variability, respiration, or other traditional voice metrics, such as voice frequency F0, standard deviation around this frequency, jitter, and shimmer. The experimental settings, which only included a single administration of tablets, may not have been favorable for proving an impact on these parameters. Additional research is required to comprehend the processes of Nutripuncture® and ascertain if the observed alterations in central spectroid parameters are associated with the reduction of stress. Conducting study with more challenging (stressfull) initial conditions would provide valuable insights into the impact of stress reduction. This would include longer periods of monitoring stress parameters changes and use brain imaging to evaluate the subject's reaction to Nutripuncture.

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Ethics approval

This study was performed in line with the principles of the Declaration of Helsinki. Our protocol was approved by French Ethics Committee 412 (Number: B412201524252) N/ref: 1494. Information was given to the ANSM and the protocol information was reported on Clinicaltrials.gov (Identifier: NCT05313867).

Consent to participate

Written informed consent was obtained from all individual participants included in the study.

Consent to publish

Not applicable.

Data availability

The datasets analyzed during the current study are available from the corresponding author on a reasonable request.

CRediT authorship contribution statement

Maxime Soula: Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Nour-Imène Messas:** Software, Methodology, Formal analysis, Data curation, Conceptualization. **Slah Aridhi:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Renaud Urbinelli:** Visualization, Validation, Methodology, Conceptualization. **Alice Guyon:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix ASupplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e29127.

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