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Short-Term Variations in Phase Angle Following Frequency-Based Bioelectrical Treatment: An Exploratory Observational Study

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Abstract:

Objective

Phase angle (PhA), obtained from bioelectrical impedance analysis (BIA), is increasingly discussed as a parameter potentially related to cellular condition and hydration status. Despite its growing use, little is known about how rapidly it may vary over short periods of time, especially in relation to external physical stimuli. The aim of the present exploratory observational study was to evaluate whether frequency-based bioelectrical sessions were associated with short-term PhA variations under standardized measurement conditions.

Methods

The study included 126 subjects: 63 individuals undergoing frequency-based bioelectrical sessions and 63 observational controls. All measurements were performed under the same environmental and procedural conditions. In the bioelectrical session group, individualized electrical frequency patterns corresponding to previously identified frequency-response peaks were delivered through cutaneous electrodes during routine sessions. Phase angle was measured at baseline (T0) and after 5 minutes (T0+5′). The observational control group underwent the same measurement procedure during the same time interval without bioelectrical application.
Since data distribution was non-normal according to the Shapiro–Wilk test, non-parametric statistics were used. Within-group comparisons were performed using the Wilcoxon signed-rank test, whereas between-group differences in ΔPhA values were evaluated using the Mann–Whitney U test. Effect sizes were calculated using r ($Z/\sqrt{N}$) and Cliff's delta.

Results

Subjects undergoing frequency-based bioelectrical sessions showed an increase in PhA values between baseline and post-session measurements (Wilcoxon signed-rank test, $p = 0.0012$). The observed effect size was very large ($r = 0.97$). Median PhA increased from 4.8 to 5.5. In contrast, the observational control group remained substantially stable over the same time interval ($p = 0.3272$), with median values unchanged at both time points (5.1).
When ΔPhA values were compared, the increase observed in the bioelectrical session group was significantly greater than that observed in the observational control group (Mann–Whitney U test, $p < 0.0001$), with a large effect size (Cliff's delta = 0.98). A limited number of outliers was present but did not appear to substantially influence overall distribution patterns. No relevant differences emerged after sex stratification.

Conclusions

Within the observational setting adopted in this study, frequency-based bioelectrical sessions were associated with short-term increases in phase angle. These findings should be interpreted cautiously. The biological mechanisms potentially involved remain unclear, and no conclusions can currently be drawn regarding the persistence or clinical significance of the observed variations. Further observational and controlled studies will be necessary to better understand the physiological relevance of these findings.

Keywords: Phase angle; bioelectrical impedance analysis; bioelectronic medicine; frequency-based bioelectrical application; physiological measurement; cellular integrity; bioelectrical signals

Introduction:

Over the last few decades, bioelectrical impedance analysis (BIA) has become increasingly common in both clinical practice and research [47]. It is used not only in patients affected by established disease [37], but also in apparently healthy subjects [31]. Its diffusion is largely explained by practical aspects: the method is non-invasive, relatively simple to perform, and adaptable to different fields, including sports medicine, nutrition, epidemiological studies, hydration assessment, and oncology. BIA is based on the way biological tissues respond to a low-intensity alternating current. Through this principle, several body composition parameters can be estimated, including fat mass (FM), fat- free mass (FFM), and total body water (TBW). The impedance value (Z, Ω) depends on the interaction between the applied current and tissue composition [25,29]. In practical terms, impedance reflects the combined contribution of resistance (R, Ω), mainly influenced by fluid compartments, and reactance (Xc, Ω), which is related to the capacitive behavior of cell membranes [10,20,38].

Among BIA-derived parameters, phase angle (PhA) has received growing attention over recent years, although its interpretation remains controversial. It is generally considered an indirect indicator associated with membrane integrity and cellular condition [39]. Several studies have explored its possible prognostic value, especially in oncology and in disorders associated with chronic inflammation or oxidative stress [22,23,45,48]. At the same time, its routine clinical use is still limited. One explanation is the absence of universally accepted reference values. Another is the influence of variables such as age, sex, ethnicity, hydration status, and body composition [36].

A relationship between PhA, inflammation, and oxidative stress has been described in different clinical settings, although the biological mechanisms involved are still incompletely understood. Oxidative stress reflects an imbalance between pro-oxidant activity and antioxidant defenses, with consequent production of reactive oxygen species (ROS) [49]. These molecules are involved in inflammatory signaling and may contribute to cytokine activation and immune modulation [9,26]. Similar processes have been implicated in metabolic disease, cardiovascular disorders, neurodegeneration, aging, and cancer [15,33,35,51]. They may also influence body composition through progressive loss of lean mass and development of sarcopenia [14,17,43,46,55]. In some situations, pharmacological treatments themselves may further contribute to oxidative imbalance [12].

For these reasons, PhA has been proposed as a possible marker associated with cellular status and systemic conditions related to inflammation and oxidative stress [2,3,13,45,53]. Some preliminary reports have also suggested possible associations with circadian cortisol patterns, although the available evidence remains limited.

At present, it is not clear whether these associations reflect physiological processes capable of changing over short time intervals or whether they may be influenced by external physical stimuli. Despite the broad use of BIA, short-term PhA variations occurring after frequency-based bioelectrical sessions have received relatively little attention. In particular, the physiological meaning of acute PhA changes observed after frequency-modulated electrical applications remains poorly defined.

Within this context, the present exploratory observational study was designed to investigate whether electrical frequency patterns corresponding to subject-specific frequency-response peaks were associated with short-term variations in PhA measured under standardized conditions. An observational control group was included to evaluate measurement stability over the same time interval and to reduce the possible influence of variability related to the acquisition procedure.

Bioelectronic Approach

In recent years, increasing interest has emerged in the field of medical bioelectronics [28], a rapidly expanding area with potential applications across several clinical domains, including urological disorders [42], rheumatoid arthritis [19], inflammatory bowel diseases [5,50], dyslipidemia [41], and neurological and psychiatric conditions [16].

In several of these contexts, bioelectronic approaches are based on neuromodulation strategies aimed at influencing physiological processes through interaction with biological signaling pathways. Compared with some pharmacological interventions, these approaches may offer potential advantages in terms of selectivity and reduced systemic effects [40], although their mechanisms and clinical implications remain under investigation.

At the same time, implantable bioelectronic devices may be associated with local complications, including foreign body reaction (FBR), a well-documented inflammatory response to implanted materials [7]. For this reason, non-invasive approaches based on transcutaneous electrical applications are attracting growing interest, as they avoid several limitations associated with implantable systems.

In the present observational setting, electrical frequency patterns were delivered using a frequency generator (BioLife-Regen system) through cutaneous electrodes under standardized procedural conditions. The physiological significance of the observed short-term PhA variations remains unclear and may involve transient bioelectrical phenomena at the cellular or tissue level, although the underlying mechanisms have not yet been established.

Materials and Methods

Participants and study conditions

The study included a cohort of subjects undergoing bioelectrical impedance analysis (BIA) measurements under standardized conditions during routine frequency- based bioelectrical sessions. An observational control group was also included to assess measurement stability over time in the absence of bioelectrical application. All participants were evaluated according to the same

procedural protocol and under comparable environmental conditions.

A total of 126 subjects were included, with 63 in the observational control group (39 females, 24 males) and 63 in the bioelectrical session group (38 females, 25 males). Participants' age ranged from 18 to 73 years.

The present work represents a retrospective observational analysis of anonymized pre- and post- session physiological data collected during routine professional practice under standardized conditions.

Ethical Considerations. The study was conducted in accordance with the principles of the Declaration of Helsinki. All participants provided written informed consent for participation and for the anonymous use of their data for research purposes. Data collection and analysis were performed in anonymized form.

Measurement Conditions Standardization. Measurements were carried out under standardized environmental and procedural conditions to reduce external sources of variability. Subjects were positioned supine and instructed to remain still after a resting period [54].

Environmental conditions were maintained as stable as possible, with ambient temperature around 22°C, controlled lighting, minimal external disturbances, and consistent bed height.

The same examination bed was used for all assessments to ensure consistent subject positioning throughout the study. The BIA device was positioned at a fixed distance (~40 cm), and cables were arranged without tension.

To reduce operator-dependent variability, all measurements were performed by the same trained operator following an identical acquisition procedure.

Bioelectrical impedance analysis. Measurements were performed using a Bodystat 500 device operating at 50 kHz with a tetrapolar hand-to-foot configuration on the right side. Skin preparation ensured stable electrode contact. Disposable adhesive electrodes with an active conductive area of 2.1 × 2.1 cm were used. Electrode placement followed a standardized protocol using anatomical landmarks and proportional spacing based on the subject's hand width to ensure intra-subject consistency.

Measurement timing and stabilization. The 5-minute interval was introduced to allow stabilization of bioelectrical conditions in the supine position, reducing variability related to fluid redistribution and electrode–skin interface dynamics, in accordance with established BIA measurement recommendations [29,30].

Device and signal acquisition. The BioLife-Regen system consists of a frequency generator equipped with a biofeedback acquisition circuit and dedicated control software. The system enables real-time modulation of signal parameters such as frequency, amplitude, waveform, and duration, while simultaneously recording current responses associated with each emitted frequency.

The system operates within a frequency range of 100–600 kHz. During the scanning phase, current intensity (μA) was measured for each frequency, generating a frequency–current profile. Individual response curves were processed through comparison with a reference curve obtained by polynomial interpolation.

Peak identification and session protocol. Peaks were identified as deviations exceeding a predefined sensitivity threshold (SL), based on the device's internal calibration, from the interpolated reference curve. Each peak was defined as a frequency interval (Δ) centered on the peak value.

Peak detection relied on internal calibration parameters; however, identical acquisition settings and predefined sensitivity thresholds were consistently applied across all participants throughout the study.

For each identified peak, electrical frequency patterns were delivered across the corresponding frequency interval using the following parameters:

- waveform: square
- maximum voltage: 12 V
- frequency range: peak value ± 5000 Hz
- frequency step: 5 Hz
- burst: 10,000 cycles per frequency

Electrical frequency patterns were delivered through four separate adhesive conductive-gel electrodes (16 cm² conductive gel), positioned at the wrists and medial ankle regions.

Signal delivery was performed through two independent channels (CH1 and CH2), sequentially applied across predefined circuit configurations to ensure coverage of different current pathways throughout the body. The configurations included A–B, C–A, D–A, B–C, A–D for CH1, with corresponding complementary pathways for CH2 (D–C, B–D, C–B, A–C and C–D).

This configuration allowed signal propagation across multiple current pathways throughout the body. At the end of the session phase, a second scanning procedure was performed using the same acquisition parameters.

The circuit configurations used in this study were selected based on preliminary empirical observations conducted prior to study initiation, as they consistently showed greater reproducibility of short-term phase angle variations under standardized conditions. All participants underwent the same predefined circuit configuration.

Outcome measures. The primary outcome was the absolute and relative variation in PhA between T0 and T0+5′, evaluated both within and between groups.

Statistical analysis. The distribution of phase angle (PhA) values in both the observational control group and the bioelectrical session group was assessed using the Shapiro–Wilk test. As data were not normally distributed, non-parametric tests were applied. Within-group comparisons (pre vs post) were performed using the Wilcoxon signed-rank test. For between-group analysis, the change in phase angle (ΔPhA = post – pre) was calculated for each subject, and differences between groups were evaluated using the Mann–Whitney U test.

Effect sizes were calculated to complement hypothesis testing. For within-group comparisons, the effect size was estimated using r (Z/√N). For between-group comparisons (ΔPhA), Cliff's delta was calculated. Statistical significance was set at $p < 0.05$.

Results

PhA values increased in the bioelectrical session group after the session phase, with a clear difference between pre- and post-measurements (Wilcoxon signed-rank test, $p = 0.0012$) (Figure 1). The effect size was very large ($r = 0.97$). Median PhA increased from 4.8 to 5.5.

By contrast, values in the observational control group remained stable over the same time interval, without relevant variations between the two measurements (Figure 2). Median PhA values remained unchanged (5.1 at both time points).

Accordingly, the distribution of PhA values in the bioelectrical session group shifted towards higher values after the session phase, whereas no comparable change was observed in the observational control group.

When the change in PhA (ΔPhA = post – pre) was considered, the increase observed in the bioelectrical session group was greater than that observed in the observational control group (Figure 3). This difference was statistically significant (Mann–Whitney U test, $p < 0.0001$).

The effect size for the between-group comparison was large (Cliff's delta = 0.98). Outliers were present but had no relevant impact on the overall distribution.

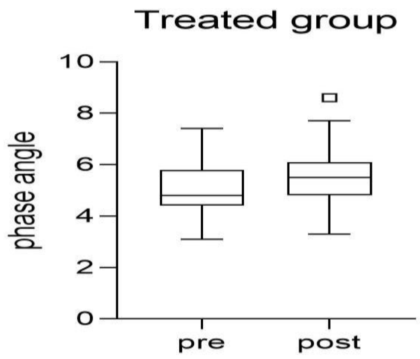

Figure 1. Phase angle values before (pre) and after (post) frequency-based bioelectrical sessions in the bioelectrical session group. Data are presented as boxplots (median, interquartile range, and range). A statistically significant increase in PhA was observed after the session phase (Wilcoxon signed-rank test, $p = 0.0012$). Sample size: $n = 63$.

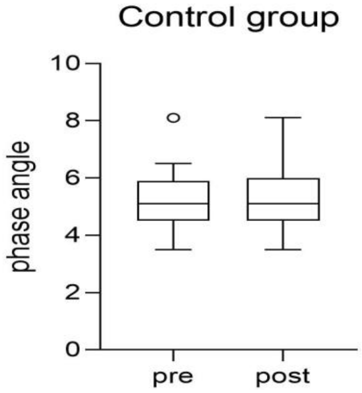

Figure 2. Phase angle values at baseline (pre) and after 5 minutes (post) in the observational control group under identical measurement conditions without bioelectrical application. No significant changes were observed between time points (Wilcoxon signed-rank test, $p = 0.3272$). Sample size: $n= 63$.

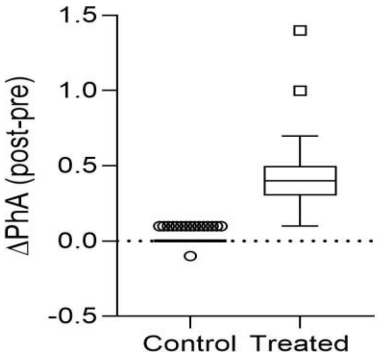

Figure 3. Change in phase angle (ΔPhA = post – pre) in the bioelectrical session group and observational control group. Data are presented as boxplots (median, interquartile range, and range). ΔPhA was significantly higher in the bioelectrical session group compared with the observational control group (Mann–Whitney U test, $p < 0.0001$), with a large effect size (Cliff's delta = 0.98). The dashed line indicates no change (ΔPhA = 0). Observational control group (n = 63), bioelectrical session group (n = 63).

Discussion

In the present observational study, short-term changes in phase angle (PhA) were observed in subjects undergoing frequency-based bioelectrical sessions, whereas no comparable variation emerged in the control group during the same observation interval.

Subjects undergoing bioelectrical sessions showed higher post-session PhA values compared with baseline measurements, while the control group remained essentially stable under identical procedural conditions.

This stability reduces the likelihood that the observed differences were simply related to measurement instability or to the acquisition procedure itself.

At the same time, interpretation of these findings remains difficult. PhA is influenced by several physiological factors, including hydration status, tissue electrical properties, membrane-related phenomena, and body composition.

For this reason, the present data do not allow identification of a specific biological mechanism underlying the observed variations. Nevertheless, the absence of meaningful variation in the control group supports the possibility that the changes observed after the bioelectrical sessions may reflect short-term physiological phenomena occurring under the conditions adopted in this study. The magnitude of the observed differences was also consistent with the calculated effect sizes.

These observations should however be interpreted cautiously. The study was limited to acute measurements obtained over a short time interval and was not designed to establish clinical efficacy or causal biological relationships. At present, the physiological meaning of these acute PhA changes is still uncertain.

Several explanations may be considered: transient bioelectrical phenomena, fluid redistribution, or short-term changes involving tissue electrical properties could potentially contribute to the observed variations. The mechanisms potentially involved in the observed phase angle variations remain unknown; although these phenomena may have played a role, the present study was not designed to investigate these aspects. Any biological interpretation should therefore be considered hypothetical. Some limitations should therefore be acknowledged. The observation window was restricted to five minutes, and no follow-up measurements were performed. In addition, the observational design limits mechanistic interpretation. Although environmental and procedural conditions were carefully standardized, the influence of unrecognized confounding factors cannot be entirely ruled out.

Further observational and controlled studies will be necessary to better clarify the biological basis, reproducibility, temporal persistence, and possible physiological relevance of these findings.

Conclusion

Under the observational conditions adopted in the present study, frequency-based bioelectrical sessions were associated with short-term increases in phase angle (PhA).

These findings should be interpreted cautiously, as the underlying biological mechanisms remain incompletely understood, while the temporal persistence, reproducibility, and potential clinical significance of the observed variations are currently unknown. Further observational and controlled investigations are required to better characterize the physiological relevance of these findings.

Institutional Review Board Statement

The present work consisted of a retrospective observational analysis of anonymized physiological data collected during routine professional functional assessments performed under standardized conditions. The evaluated procedures and bioelectrical measurements were part of the authors' standard professional practice and were not introduced specifically for research purposes. All participants received information regarding the anonymous scientific use of physiological data collected during routine sessions, and written informed consent was obtained from all subjects prior to data collection. All procedures were non-invasive and conducted in accordance with the principles of the Declaration of Helsinki.

According to local regulations and considering the retrospective, observational, and non- interventional nature of the analysis, formal approval by an Institutional Review Board or Ethics Committee was not required.

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