Original Article

Displacement of bioelectrical impedance vector analysis after an intervention with whey protein, vitamin C and vitamin E in patients on haemodialysis: a pilot, double-blind randomized controlled clinical trial

Deslocamento do vetor da impedância bioelétrica após intervenção com proteínas do soro do leite, vitamina C e vitamina E em pacientes em hemodiálise: ensaio clínico piloto randomizado, duplo–cego e placebo-controlado

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ABSTRACT
Introduction: Nutritional assessments are fundamental for monitoring nutritional status in haemodialysis (HD) patients. Bioelectrical impedance vector analysis (BIVA) combines the evaluation of aspects of mass and cellular health and hydration. The present study aimed to verify the effects of a nutritional supplementation protocol on BIVA in HD patients. **Method:** The study protocol was previously registered in the Brazilian Clinical Trials Registry. This was a double-blind randomised, controlled clinical trial. Patients were randomised into two groups: the supplementation group received 20 g of whey protein, 250 mg of vitamin C, and 600 IU of vitamin E. The placebo group received 20 g of rice flour and microcrystalline cellulose capsules. Both groups received the interventions immediately after the HD, 3 times a week, for 8 weeks. BIA evaluation was carried out at the beginning of the study and after 8 weeks. **Results:** Initially, 29 patients were included. However, there were six losses, so 15 women and 8 men completed the study, with an average age of 54.3±13.1 years. After the intervention, the mean vector of the BIVA in the supplementation group shifted to the central region within the 50% ellipse and the mean vector of the placebo group shifted from the 95% to the 75% ellipse. Regardless of the intervention, there were significant increases over time in the outcomes fat mass (kg) (p=0.020), % fat mass (p=0.034), Xc (p=0.008), Xc/H (p=0.014), R (p=0.040), and Z (p=0.037). In addition, regardless of time, significant differences were observed in the outcomes Xc (p=0.039) and Xc/H (p=0.046) between the supplemented and placebo groups. **Conclusion:** Displacement of BIVA vectors was observed in both intervention groups, suggesting positive changes in body cell mass and hydration status.

RESUMO
Introdução: A avaliação nutricional é fundamental para monitorar o estado nutricional de pacientes em hemodiálise (HD). A análise vetorial da impedância bioelétrica (BIVA) combina a avaliação de aspectos da massa e saúde celular e da hidratação. O objetivo foi verificar os efeitos de um protocolo de suplementação nutricional na BIVA, em pacientes em HD. **Método:** O protocolo do estudo foi previamente inscrito no Registro Brasileiro de Ensaios Clínicos. Os pacientes foram randomizados em dois grupos: suplementação, que recebeu 20 g de proteínas do soro do leite, 250 mg de vitamina C e 600 UI de vitamina E e placebo que recebeu 20 g de farinha de arroz e cápsulas de celulose microcristalina. Os dois grupos receberam as intervenções imediatamente após a sessão de HD, 3 vezes por semana, durante 8 semanas. **Resultados:** Dentre os 29 pacientes incluídos houve seis perdas e finalizaram o estudo 15 mulheres e 8 homens, com idade média de 54,3±13,1 anos. Após a intervenção, o vetor médio da BIVA do grupo suplementado foi deslocado para região central dentro da elipse de 50% e o vetor médio do grupo placebo foi deslocado da elipse de 95% para a elipse de 75%. Houve aumento significativo ao longo do tempo nos desfechos massa de gordura (kg) (p=0,020), % de massa de gordura (p=0,034), reatância (Xc) (p=0,008), Xc/altura (p=0,014), R (p=0,040) e Z (p=0,037), independente do tratamento (grupo suplementado ou placebo). Não foi observado efeito significativo do tratamento e da interação entre o tratamento e tempo. Houve uma diferença significativa nos desfechos Xc (p=0,039) e Xc/altura (p= 0,046) entre os grupos suplementado e placebo, independente do tempo. **Conclusão:** Houve deslocamento dos vetores da BIVA nos dois grupos de intervenção, sugerindo a detecção de alterações positivas na massa celular corporal e na hidratação.

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INTRODUCTION

Nutritional status assessments and monitoring of patients with chronic kidney disease (CKD) receiving haemodialysis (HD) are fundamental to the prevention, diagnosis, and treatment of malnutrition[1]. However, in this population, the assessment of nutritional status can be compromised by changes in hydration[2,3]. For a more complete approach, the Kidney Disease Outcomes Quality Initiative (KDOQI) guideline suggests the use of more than one method to assess nutritional status. Among the methods available, bioelectrical impedance analysis (BIA) has been suggested to estimate the body composition of HD patients[4].

The human body can be divided into different compartments (Figure 1A). BIA is based on the principle that body tissues offer different opposition to the passage of an electrical current (Figure 1B). Impedance (Z) refers to this opposition and is composed of 2 vectors: resistance (R) and reactance (Xc). The R component of BIA represents the opposition to the flow of an alternating current through ionic solutions and is inversely related to intracellular and extracellular water volume. Due to its large water and electrolyte content, muscle tissue is a good conductor of electrical current, presenting low resistance to the passage of electrical current. In turn, tissues which contain less fluid, such as adipose tissue, skin, and bones, are worse conductors, and therefore present higher resistance (R). The number of structures containing soft tissues is directly related to Xc, a component of Z. Xc is related to the capacitance properties of the cell membrane, that is, the capacity to store electrons produced by the interfaces of tissues and cell membranes, so that variations can occur depending on the integrity, function, and composition of the cell membrane (Figure 1C)[5-8].

The estimation of body composition by BIA is subject to various technical and physiological sources of variation, which can reduce its accuracy. These include the need to use specific equations validated for sex, the population of interest, and each BIA device/brand. Therefore, especially in populations with known fluid disturbances, such as dialysis patients, using generic equations or algorithms provided by each manufacturer is not ideal. In this context, analysis with BIVA, similarly to the phase angle, minimizes these sources of error, as it exclusively uses raw parameters (R/H and Xc/H), without relying on predictive equations or algorithms[9].

Bioelectrical impedance vector analysis (BIVA) is based on the evaluation of the values of R and Xc, normalized to the patient's height (H), and provides information about hydration and nutritional status in the resistance-reactance (RXc) graph[10]. The differential of BIVA is that it is an autonomous procedure, independent of equations or predictive models, and allows assessment of the patient through direct measurements of impedance vectors[3]. Therefore, BIVA can be interpreted accurately even if patients are at extremes of weight or volume distribution[11].

Patients receiving HD generally consume less protein and energy than nutritional recommendations and this can lead to loss of lean mass and malnutrition[4,12,13]. Oral nutritional supplements might be a good strategy to improve nutritional status[14]. In addition, due to the complexity of malnutrition in these patients, joint interventions including proteins and anti-inflammatory nutrients have been suggested[12,15,16], highlighting the anti-inflammatory and antioxidant effect of vitamins C and E[17,18].

Thus, the purpose of the current study was to verify the effects of protein supplementation combined with 2 antioxidant micronutrients on BIVA in patients receiving HD. Our hypothesis was that supplementation using whey protein combined with vitamins C and E would promote displacement of BIVA vectors.

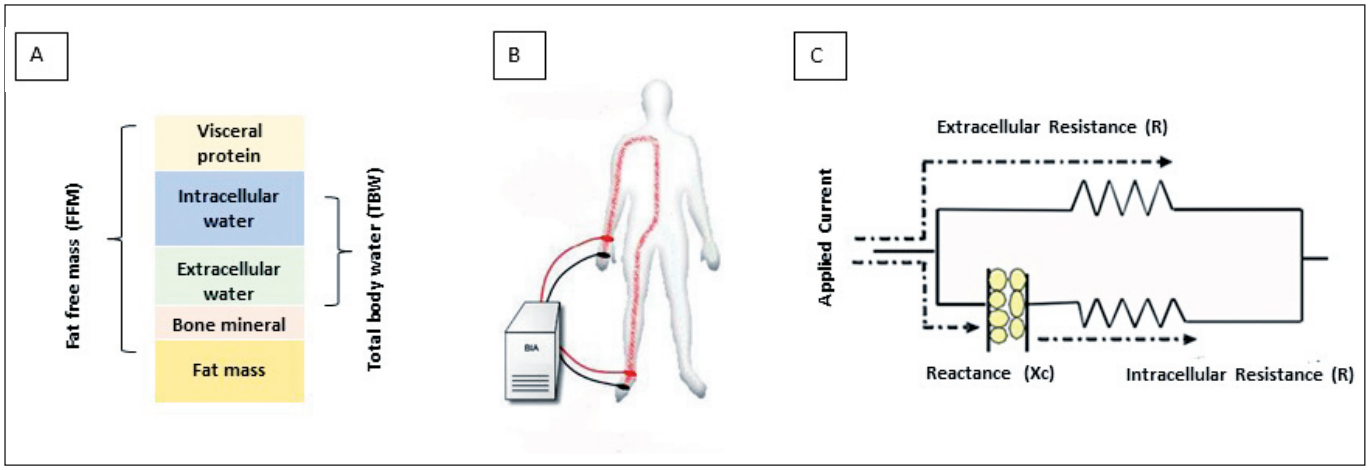

Figure 1 - *A = different compartments of the human body; B = a typical four-electrode configuration for BIA measurements; C = electrical circuit used to interpret data measured in BIA.*

METHODS

Ethics

The present study received approval from the local Research Ethics Committee and was officially registered at Brazilian Registry of Clinical Trials (https://ensaiosclinicos.gov.br, code: RBR-978GY8). All participants signed a statement of informed consent.

Study design and subjects

This is a randomised, double-blind, placebo-controlled clinical trial, conducted from January to March 2020 at a Hospital Dialysis Unit. The research protocol followed the CONSORT guidelines.

The sample consisted of men and women who underwent HD in a dialysis centre during the period of data collection, and who agreed to participate and were eligible for the study. The inclusion criteria were: ≥19 years old; having a diagnosis of CKD; and performing HD two or three times a week for a period ≥ three months. The exclusion criteria were: recent intake of antioxidants (i.e., vitamin C or E), amino acids, protein, N-acetylcysteine, keto acid, or immunosuppressives; allergy to milk protein; having a potentially catabolic diagnosis, such as infection by human immunodeficiency virus; a diagnosis of cancer; advanced heart failure; chronic lung disease or liver disease; hospitalization; pregnancy or breastfeeding; physical and/or psychological incapacity to participate; and not being able to comprehend or communicate.

Sample size and Randomisation

Sample size was determined according to resistance (as one of the primary outcomes assessed by BIA) in a previous study with HD patients who received nutritional supplementation[19]. Patients were randomised into two groups at a ratio of 1:1 and stratified by HD frequency (2 or 3 times per week), with homogeneity in the number of patients in each group. Eligible patients were subsequently allocated sequential codes generated in Research Randomizer® and assigned to each group randomly. Other investigators labelled the sachets and capsules for supplementation according to the previously generated 5 codes for each patient and managed the nutritional intervention at the HD clinic.

Blinding

The visual similarity between the supplements and the identical sachet designs and capsules for the two groups ensured blinding of the participants, the researchers who managed the supplementation at the HD clinics, and the investigator in charge of the statistical analyses.

Nutritional Intervention

The intervention was administered 3 times a week immediately after the HD session. Patients in the supplementation group received whey protein, 1 capsule containing vitamin C, and 2 capsules containing vitamin E. The placebo group received rice flour, 1 vitamin C placebo capsule, and 2 vitamin E placebo capsules. The placebo capsules were composed of microcrystalline cellulose and were identical in appearance to the capsules received by the supplementation group. At the clinic, the researchers diluted the sachets with whey protein or rice flour in a black cup containing 50 mL of filtered water and administered the capsules with the liquid. The protein module and white rice flour were both soluble in water, equivalent in colour, and packaged in identical sachets. The sachets were metallized on the outside and opaque on the inside, to minimize the influence of light and maintain the stability of the interventions.

During recruitment, participants were instructed to reach the recommended protein intake proposed by the National Kidney Foundation of 1.0 to 1.2 g/kg/day through diet[4]. In addition to the initial guidance, participants were encouraged weekly to maintain the previously recommended dietary intake and an adequate caloric consumption.

Whey protein

The protein offered to each group was previously separated, weighed on a precision scale, and placed in identical aluminium foil sachets. The protein module used was whey protein isolate from the brand Fresenius Kabi: Fresubin Protein Powder, and each intervention dose contained 20.0 g of the module, equivalent to 19.8 g of amino acids (Table S1). As a placebo, 20.0 g of white rice flour were administered. The placebo did not contain Na, P, K, or vitamins C and E, and its energy value per dose was equivalent to that of the supplemented whey protein (Table S2).

Vitamin C

Vitamin C was administered in the form of 1 capsule containing 250 mg of the vitamin in the supplemented group.

The 250 mg dose was chosen based on previous studies, without prejudice to HD patients. In addition, this dose was considered safe to prevent oxalosis (<500 mg/day)[20].

The vitamin C and the vitamin C placebo (microcrystalline cellulose) used in the research were compounded and donated by Farmácia Biodora (Pharma & Phormula Compounding Pharmacy Eireli).

Supplementary table 1 – Amino acid composition of whey protein module.

Essential amino acids (g)	1-day dose (20 g)	1-week dose (60 g)
Lysine	1.80	5.40
Threonine	1.40	4.20
Methionine	0.40	1.20
Phenylalanine	0.56	1.68
Tryptophan	0.36	1.08
Valine	1.16	3.48
Leucine	2.00	6.00
Isoleucine	1.32	3.96
Conditionally indispensable amino acids (g)		
Tyrosine	0.56	1.68
Cysteine	0.48	1.44
Histidine	0.36	1.08
Arginine	0.36	1.08
Glutamine	1.08	3.24
Non-essential amino acids (g)		
Glycine	0.32	0.96
Alanine	0.96	2.88
Proline	1.28	3.84
Serine	1.00	3.00
Glutamic acid	2.32	6.96
Aspartic acid/Asparagine	2.08	6.24

Source: Fresubin® technical file.

Supplementary table 2 – Nutritional content in 1 dose of whey protein and its placebo (white rice flour).

Nutrients	Whey protein module (20.0 g)	White rice flour (20.0 g)
Energy (kcal)	72.0	73.2
Protein (g)	17.6	1.36
Lipids (g)	0.2	0.28
Carbohydrate (g)	0.2	16.4
Calcium (mg)	12.0	0.2
Phosphorus (mg)	48.0	7.2
Sodium (mg)	110.0	-
Potassium (mg)	240.0	2.6
Vitamin C (mg)	-	-
Vitamin E (mg)	-	-
Vitamin E (mg)	-	-

Source: Fresubin® and RisoVita® technical files.

Vitamin E

The 600 IU of vitamin E were administered in 2 capsules containing 300 IU each, at the same time as the vitamin C and whey protein, immediately after the HD session.

Vitamin E (alpha-tocopherol acetate) and the placebo capsules (microcrystalline cellulose) used were compounded and donated by Biodora Pharmacy (Pharma & Phormula Compounding Pharmacy Eireli).

Adherence

To ensure compliance, priority was given to managing the interventions soon after the HD session at the dialysis clinic. Patients who received HD twice a week were instructed weekly to prepare and ingest the content of the third supplementation sachet at home, and in the following HD session, the patients were asked about whether they had taken the supplement at home.

Body mass index

Weight and height were obtained to determine the body mass index (BMI)[21]. Height was measured using a portable stadiometer from Alturaexata®. The patient stood upright, barefoot, with feet together and weight evenly distributed on the platform surface, arms hanging by the sides, and the posterior surface of the body (head, back, buttocks, and heels) touching the measuring scale of the instrument. To ensure an accurate measurement, the head was positioned so that the individual's gaze was perpendicular to the body. At the time of measurement, the participant was instructed to inhale and hold the position until the movable ruler was brought to the highest point of the head.

For patients unable to stand, height was estimated using the knee height[22]. To measure the knee height, the patient remained seated with the right leg positioned at a 90° angle between the knee and ankle. A non-elastic tape measure with 0.1 cm increments was used.

Bioelectrical impedance analysis (BIA)

The BIA evaluation was carried out at the beginning of the study and after 8 weeks. Single frequency (SF) BIA was performed to determine R and Xc, using a portable tetrapolar device (Biodynamics - model 310e, Biodynamics Corporation, Seattle, WA) with a current intensity of 800 μA and a single frequency of 50 kHz. The patients were positioned in a supine position on a stretcher made of non-conductive material, relaxed and comfortable, with arms and legs apart and hands open. All metallic adornments, such as piercings, rings, earrings, chains, and wristwatches, were removed. The electrodes were placed on the midline between the prominent ends of the radius and ulna of the wrist and midline between the medial and lateral malleoli of the ankle. The patients

were evaluated approximately 20 minutes after the HD on the side of the body without vascular access, as recommended by the U.S. National Institutes of Health[23]. The resistance (R) and reactance (Xc) measurements were recorded in ohms (Ω) for subsequent calculations.

BIA parameters

For BIA parameter analysis, R (ohm) and Xc (ohm) were used to obtain the Z (ohm)[24]: $Z = \sqrt{(R^2 + Xc^2)}$.

R, Xc, and Z were standardized by height (H) in meters (R/H, Xc/H, Z/H) (ohm/m).

Phase angle (PA)

The R and Xc measures were used for the calculation of the PA (°) = arctangent [(Xc(Ω)/R(Ω)) x (180/π)] (Figure 2A)[6].

Bioelectrical impedance vector analysis (BIVA)

BIVA 2002 software[7] was used to determine the BIVA. The normalisation of R and Xc by H was expressed as z-scores (z-R/H and z-Xc/H) with the reference population described by Piccoli et al[25]. Patients with vectors outside the 75% ellipsis in the upper quadrant (underweight) and lower quadrant (cachexia) on the right side of the graph were classified as malnourished. Patients with vectors outside the 75% ellipsis in the major axis in the direction of hyperhydration (lower quadrant) were classified as hyperhydrated and outside the 75% ellipse in the upper quadrant as dehydrated. Variations in bioelectric vectors along the major axis of the tolerance ellipse indicate progressive changes in tissue hydration. Variations along the minor axis indicate changes in body cell mass (BCM), with vectors located to the left or right of the axis respectively, indicating higher or lower BCM[5,7,10,26] (Figure 2B). Group vectors were plotted as the mean and 95% confidence intervals of the z-score (Figure 2C).

Statistical analysis

The data were analysed using Data Analysis and Statistical Software (STATA, version 13 for Windows; Stata Corporation, College Station, USA). The sample description was performed as absolute and relative frequencies, means and standard deviations for parametric variables, and medians and interquartile ranges for non-parametric variables. Between these variables, according to the intervention group (supplementation vs placebo), the chi-square test was used for categorical variables and the Student's t-test or Mann-Whitney test for numerical variables.

Any differences promoted by the two interventions (supplementation or placebo) as well as at the different times analysed were detected using two-way repeated measures analysis of variance (two-way RM-ANOVA). The paired T-test was also performed to verify the differences between the times of each variable in each intervention.

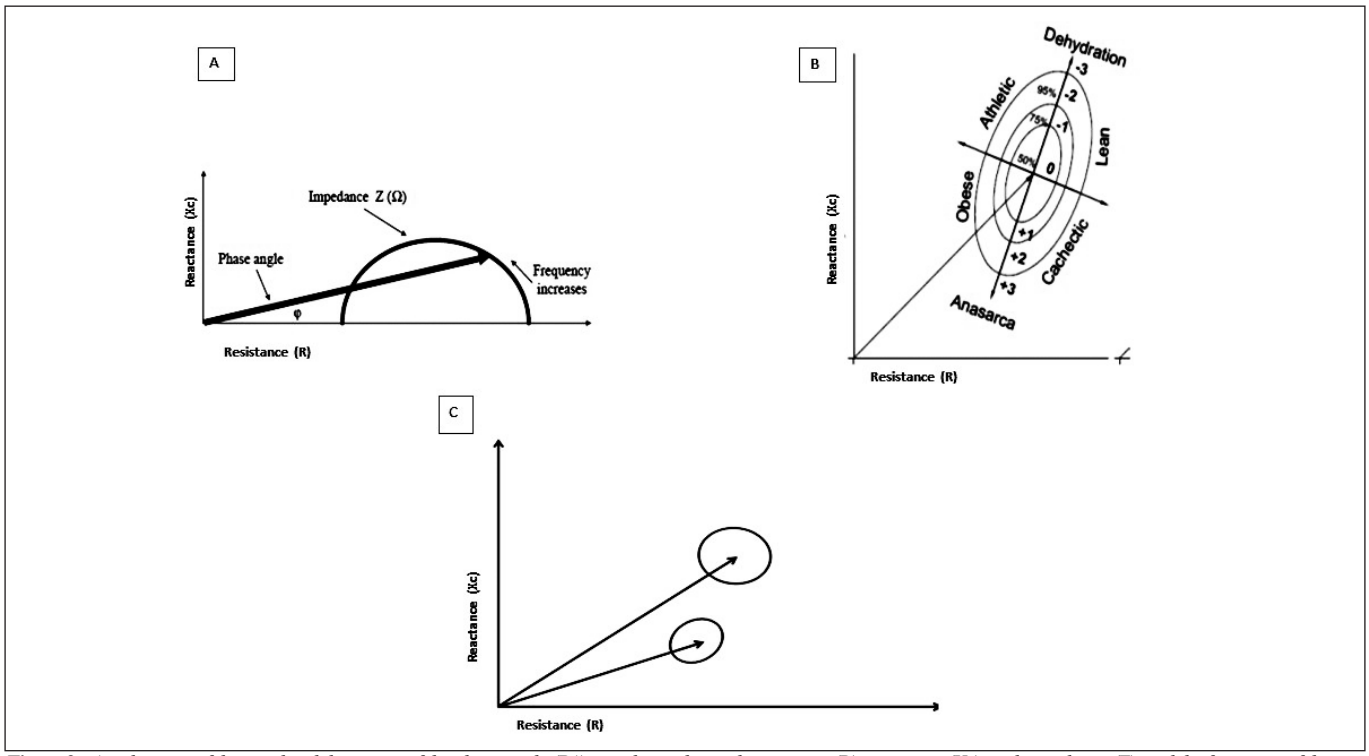

Figure 2 - *A = diagram of the graphical derivation of the phase angle (PA), its relationship with resistance (R), reactance (Xc), and impedance (Z), and the frequency of the applied current; B = RXc graph with 95%, 75%, and 50% tolerance ellipses; C = RXc graph with confidence ellipses for group comparisons.*

The vector means of the resistance-reactance graph were analysed using the Hotelling's T^2 test and univariate analysis (F test). A $p<0.05$ was considered statistically significant.

RESULTS

Out of the 50 patients who underwent HD, 29 met the inclusion criteria for the study and were randomly assigned to either the supplementation or placebo group (Figure 3). Six patients were lost during follow-up, resulting in a final sample of 15 women and 8 men, with a mean age of 54.3±13.1 years.

Regarding the parameters of bioelectrical impedance (BIA), Table 1 shows that the supplementation group demonstrated significantly higher Xc values than the placebo group, indicating higher boy cell mass (BCM). When analysing the raw data, it was observed that one patient in the supplementation group was an outlier in relation to Xc. After exclusion of the data for this patient (data not shown), the analysis showed no significant differences between the groups for Xc (p=0.128).

The BIVA results are shown as explained according to the schedule in Figure 2B. Figure 4A shows the vector of each individual in the supplementation and placebo groups at baseline after the transformations of the impedance measures into z-scores. Figure 4B presents the vectors of each individual in the supplementation and placebo groups at the end of the study. Based on the resistance-reactance graph, the malnutrition rate was 62.5% (n=5) in the supplementation group at baseline and 37.5% (n=3) at the end of the study. One patient left the cachectic quadrant and

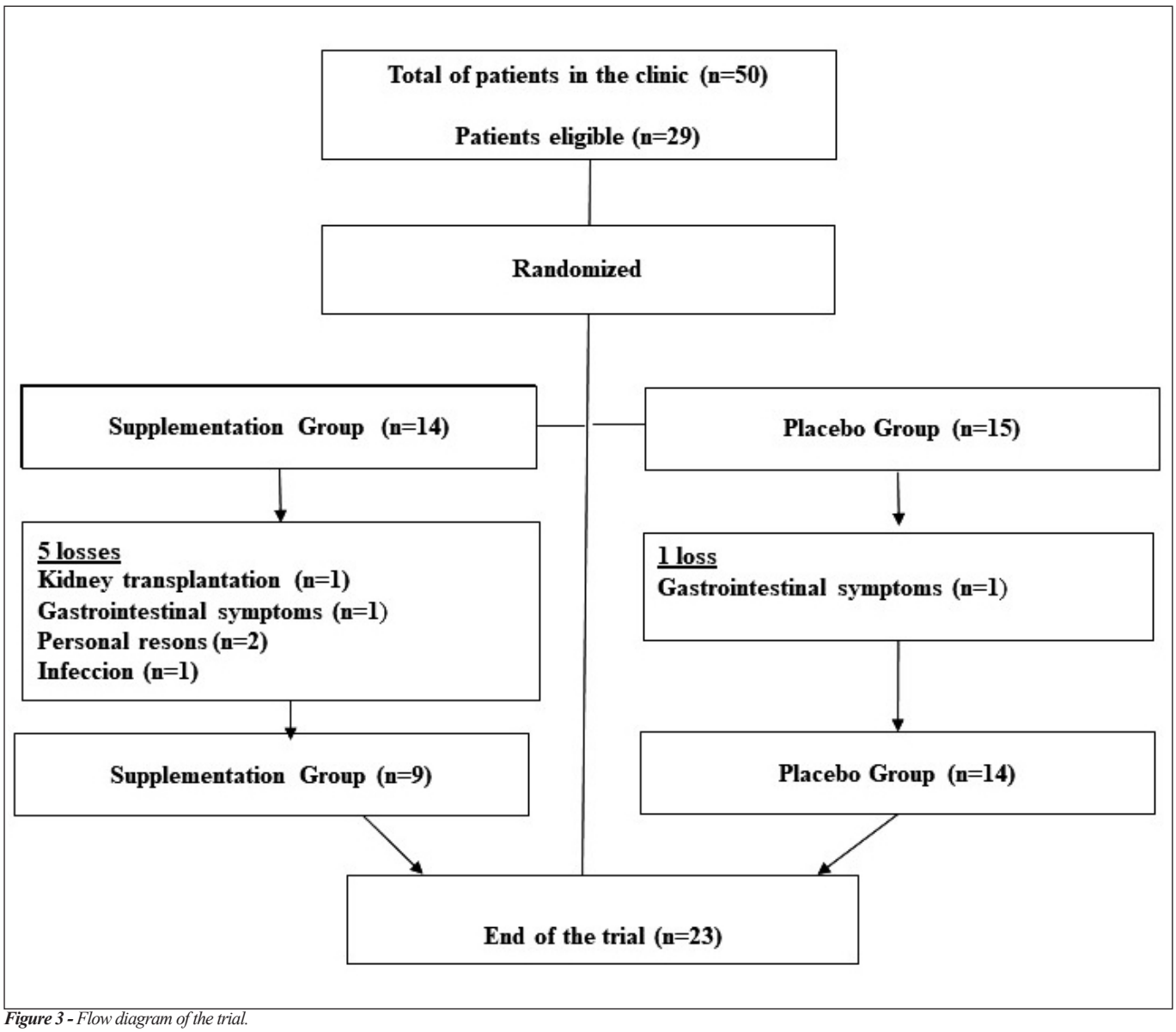

Figure 3 - *Flow diagram of the trial.*

Table 1 – Baseline clinical, bioelectrical impedance, and nutritional parameters of patients receiving haemodialysis, stratified by intervention.

	Supplementation (n=9)	**Placebo (n=14)**	**p-value**
Age (years)	54.9 (±12.7)	54.0 (±13.8)	0.878[a]
Sex (n, % of women)**	6 (40%)	9 (60%)	0.907[b]
BMI (kg/m²)	26.8 (±4.95)	29.9 (±5.63)	0.187[a]
Smoking			
Smoker	1 (11.1 %)	0 (-)	0.315[b]
Ex-smoker	2 (22.2%)	6 (42.9%)	
Never smoked	6 (66.7%)	8 (57.2%)	
Comorbidity - n (%)**			
Systemic arterial hypertension	8 (88.8%)	13 (92.9%)	0.742[b]
Diabetes mellitus	5 (55.5%)	9 (64.3%)	0.675[b]
Other	1 (11.1%)	3 (21.4%)	0.524[b]
Haemodialysis duration (months)**	27.0(±11.2)	27.8(±22.7)	0.926[a]
HD Frequency			0.809[b]
3 times a week	6 (66.6%)	10 (71.4%)	
2 times a week	3 (33.3%)	4 (28.6%)	
Dialysis dose (Kt/V)	1.26 (±0.25)	1.33(±0.28)	0.540[a]
BIA parameters			
Resistance (ohm)	569 (±125.0)	518.2 (±70.1)	0.244[a]
R/H (ohm/m)	353.1 (±83.1)	328.7 (±60.5)	0.445[a]
Reactance (ohm)	64.4 (±15.4)	53.3 (± 8.2)	0.045*[a]
Xc/H (ohm/m)	39.8 (±9.70)	33.5 (±4.49)	0.054[a]
Impedance (ohm)	572.7 (±125.5)	521(± 69.9)	0.237[a]
Z/H (ohm/m)	355.4 (±83.4)	330.4(± 60.4)	0.436[a]
Phase angle (°)	6.51 (±1.17)	5.94 (±1.11)	0.284[a]
Fat mass (%)	31.1 (±9.43)	32.7 (±10.1)	0.725[a]
Fat mass (kg)	21.9 (±8.87)	22.3 (±10.9)	0.939[a]

Data presented as mean and standard deviation. ** = absolute and relative frequency. BIA = bioelectrical impedance analysis; BMI = body mass index; R/H = resistance by height; Xc/H = reactance by height; Z/H = impedance by height; BMI = body mass index; [a] = Student's t-test; [b] = chi-squared test; * = p<0.05.

moved to the lean quadrant. In the placebo group, the malnutrition rate was 69.5% (n=9) at baseline and 61.5% (n=8) at the end of the study. Regarding hydration, at baseline, the supplemented group contained 37.5% hyperhydrated patients (n=3) and 12.5% dehydrated (1) and at the end of the study 12.5% hyperhydrated patients (1) and the same dehydrated patient (12.5%). In relation to the placebo group, 46.2% (n=6) were hyperhydrated at the beginning of the study and 38.5% (n=5) at the end of the study. Figure 4C shows the confidence interval between the R/H and Xc/H vectors of the supplementation group and placebo group at the beginning of the study. The Hotelling's test (T^2=4.5) and F test (F=2.1) showed no significant differences (p=0.1445) between the groups. Figure 4D presents the confidence intervals between the R/H and Xc/H vectors of the supplementation and placebo groups at the end of the study, the Hotelling's test (T^2=4.1) and the F test (F=1.9) also showed no significant differences (p=0.1712) between groups at the end of the study.

Table 2 presents the analysis of variance for repeated two-way measurements (two-way Repeated Measures -ANOVA). The results showed a significant increase over time in the outcomes fat mass (kg) (p=0.020), % fat mass (p=0.034), Xc (p=0.008), Xc/H (p=0.014), R (p=0.040), and Z (p=0.037), regardless of the intervention. The analysis also demonstrated a significant difference in the outcomes reactance (p=0.039) and Xc/H (p=0.046) between the supplemented group and placebo group, regardless of time (baseline or after 8 weeks). There was no significant effect for the interaction between the intervention and time.

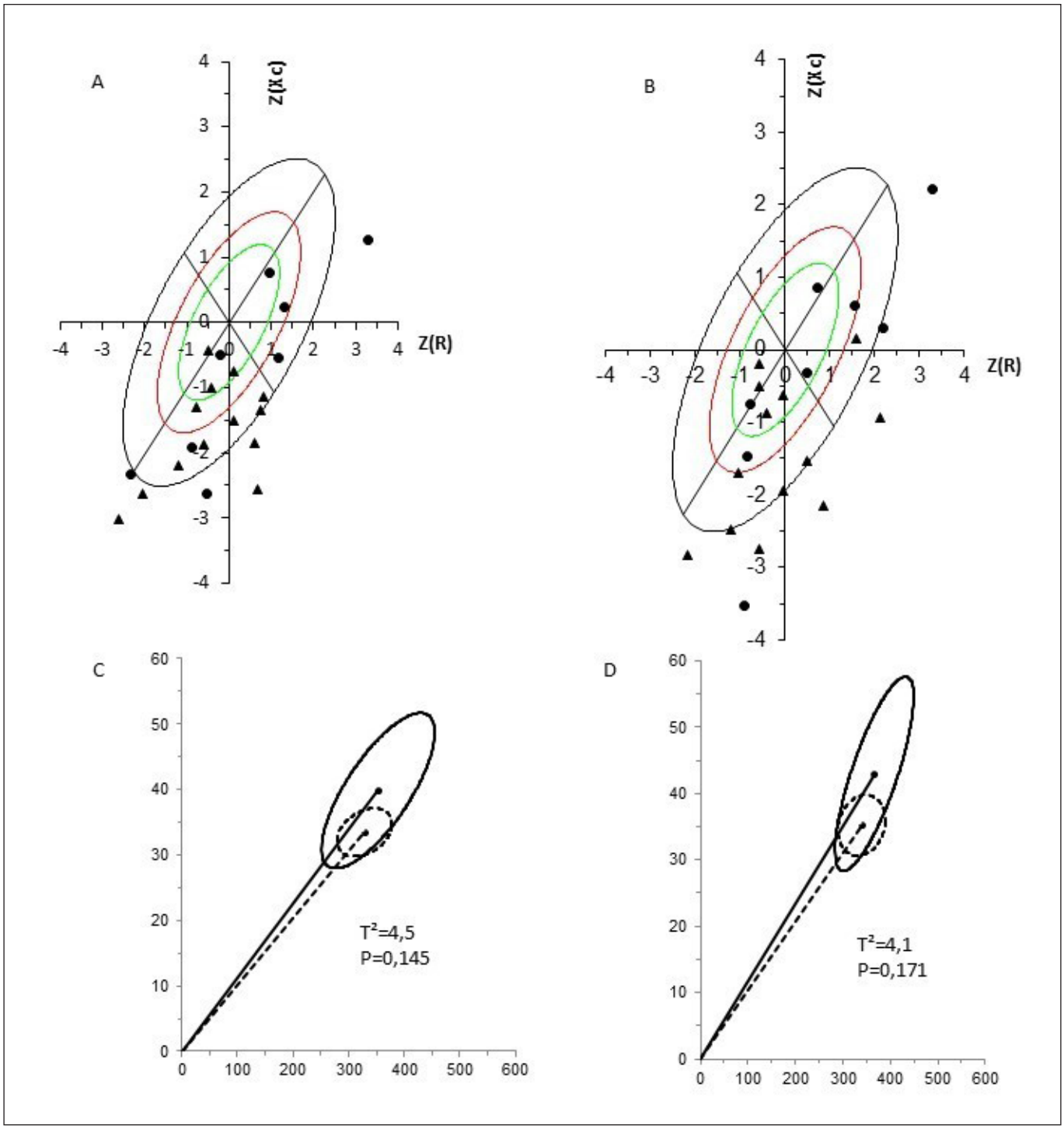

Figure 4 - *Graph with regions of elliptic probability on the RXc plane normalized by height (R/H and Xc/H, in Ω/m); Position of individual vectors for supplementation group (●) and placebo group (▲). A = distribution of individual vectors on resistance-reactance graph at baseline; B = distribution of individual vectors on resistance-reactance graph at the end of the study; C = confidence ellipses for supplementation group (black line) and placebo group (dashed line) at baseline; D = confidence ellipses for supplementation group (black line) and placebo group (dashed line) at the end of the study.*

DISCUSSION

It has been postulated that BIA can be used to detect alterations in BCM and hydration earlier than changes in nutritional parameters[27]. At the beginning of the current study, the mean of the supplementation group vector was in the 50% tolerance ellipse, in the lower right quadrant. The mean vector of the placebo group was located outside the 75% ellipse in the lower right quadrant. Figure 5 represents the displacement of the mean vector of the patients in the supplementation group and the placebo group at the beginning and at the end of the study, indicating improvement in both groups in the BIVA RXc plane. The mean

Table 2 – Effects of the nutritional intervention on bioelectrical impedance in patients receiving haemodialysis.

Parâmetro	Supplementation (n=9)			Placebo (n=14)			p-value		
Time	**T0**	**T8**	**p-value[a]**	**T0**	**T8**	**p[a]**	**p[b] (G1 vs G2)**	**p[c] (T0 vs T8)**	**p[d] (G vs T)**
Resistance (ohm)	#569±125.0	#601.9±110.0	0.068	#518.2±70.1	#535±67.8	0.282	0.147	0.040*	0.486
R/H (ohm/m)	#353.1±83.1	#366.5±67.6	0.260	#328.7±60.5	#339.4±60.8	0.285	0.386	0.123	0.861
Reactance (ohm)	#64.4±15.4	#70.8±20.0	0.068	#53.3±8.42	#56.2±11.0	0.053	0.039*	0.008*	0.286
Xc/H (ohm/m)	#39.8±9.70	#43.0±12.0	0.260	#33.5±4.49	#35.2±5.61	0.053	0.046*	0.014*	0.454
Impedance (ohm)	#572.7±125.5	#606.1±111.1	0.102	#521.0±69.9	#538.1±67.6	0.274	0.141	0.037*	0.479
Z/H (ohm/m)	#355.4±83.4	#369.1±68.2	0.144	#330.4±60.4	#341.3±60.6	0.278	0.375	0.117	0.854
Phase angle(°)	#6.51±1.17	#6.65±1.25	0.066	#5.95±1.11	#6.07±1.32	0.510	0.291	0.391	0.943
Fat mass (%)	#31.1±9.43	#33.3±9.97	0.253	#32.7±10.1	#34.0±8.25	0.214	0.787	0.034*	0.569
Fat mass (kg)	#21.9±8.87	#23.5±9.54	0.589	#24.2±9.26	#25.2±8.87	0.153	0.620	0.020*	0.591

G1 = supplementation group; G2 = placebo group; T0 = baseline; T8 = after 8 weeks; BIA = bioelectrical impedance analysis; R/H = resistance by height; Xc/H = reactance by height; Z/H = impedance by height; # = data on 1 patient lost; [a] = paired t test; [b] = two-way RM-ANOVA (analysis by group); [c] = two-way RM-ANOVA (analysis by time); [d] = two-way RM-ANOVA (analysis by group and time interaction); * = p<0.05.

vector of patients in the supplementation group shifted to the most central region within the 50% ellipse and the mean vector of the placebo group shifted from the 95% ellipse to the 75% ellipse, indicating improvement in nutritional status in both groups. Regarding the displacement in the mean vector of the placebo group, it cannot be ruled out that interference from the frequent incentives given to both groups with respect to adequate food intake, contributed to the improvement, including in the placebo group, since the groups were blinded.

BIVA has been shown to be useful for assessing the effectiveness of nutritional interventions. The path of the vector in the graph indicates the response to the intervention, providing treatment feedback, which is useful in clinical practice[28,29]. BIVA was used to assess the effectiveness of nutritional supplementation with zinc in pre-pubertal young people and children not deficient in zinc[28,30,31], to evaluate the effect of symbiotic supplementation in older adults[31], and to assess the effect of a nutritional intervention in breast cancer patients[29]. Recently, BIVA was used with serum brain natriuretic peptide to assess adequate dry weight and nutritional status in 50 patients receiving HD[33].

To our knowledge, only two studies have utilized BIVA to measure the impact of nutritional intervention on HD patients. In one study, the author evaluated the impact of a nutritional intervention in 36 patients on HD, regardless of if they underwent a physical exercise program, over a period of 12 weeks. The authors reported only a migration of vectors towards dry weight and an improvement in lean body mass at the end of the study, suggesting that the interventions were effective and safe strategies to prevent or treat malnutrition in HD patients[19]. In another study, thirty-two chronic HD patients were included in a 6-month randomized pilot study[34]. Patients in the supplementation group received a simultaneous intervention consisting of a personalized diet and nutritional supplementation and dry weight adjustment. Patients in the control group received only a personalized diet and dry weight adjustment. During the intervention, the vector of the control group moved on the major axis, to the top of the ellipses, indicating fluid removal and improved hydration status. However, the nutritional status of these patients when measured by other parameters did not change. In the supplementation group, the vector mean migrated, on the major axis, to the top of the ellipses, and to the left on the minor axis, being located at the end of the intervention inside the ellipse of 50%, indicating significant improvement in nutritional status and the normalization of hydration status[34]. The present study is similar to the aforementioned studies which used BIVA

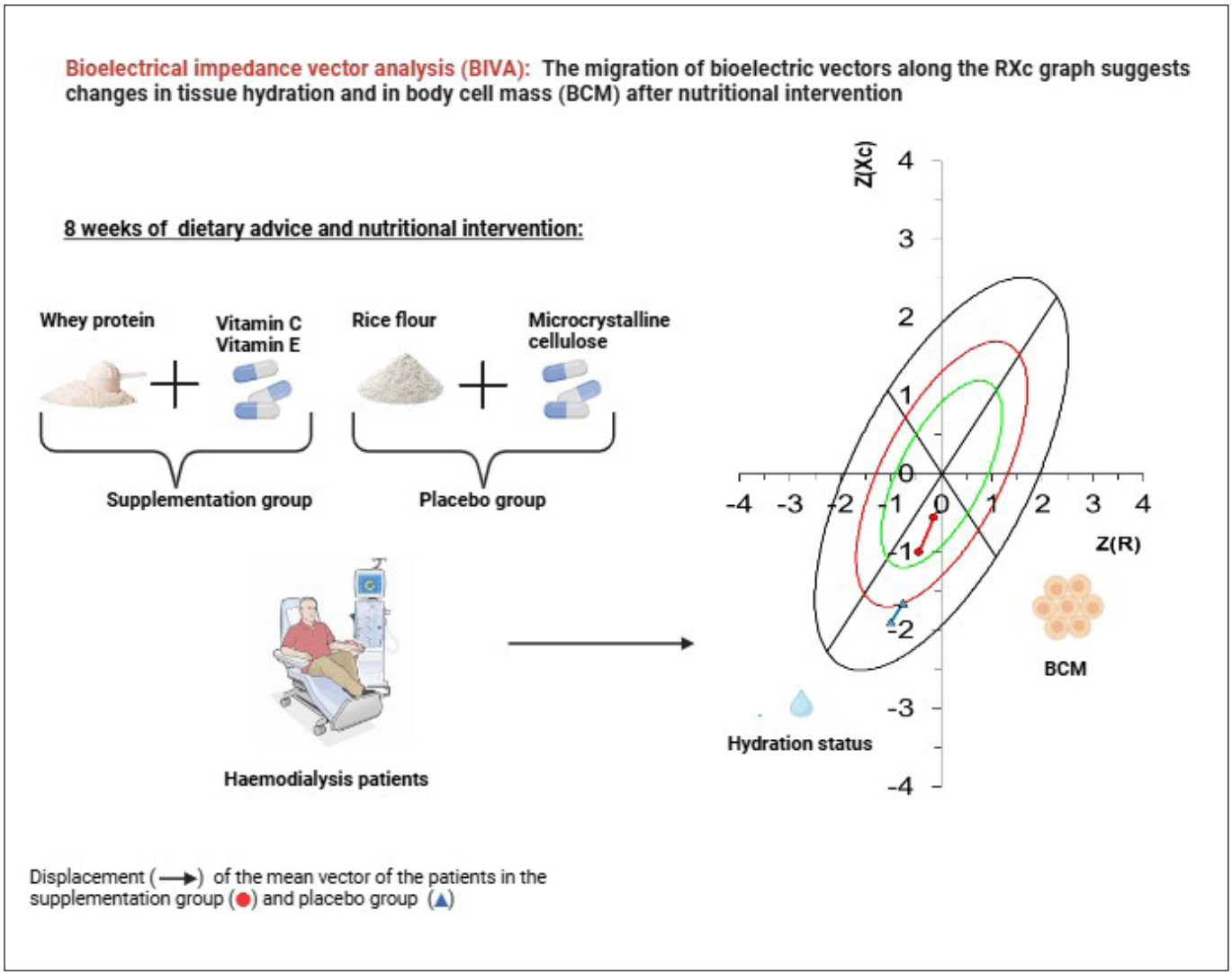

Figure 5 - *Displacement of mean vectors for supplementation and placebo groups after the transformation of impedance measures into z-scores from the baseline to the end of the study. The mean BIVA vector of the patients in the supplementation group was located within the 50% tolerance ellipse at the beginning of the study and shifted to the most central region within the same ellipse at the end of the study, while the mean vector of the placebo group shifted from the 95% ellipse to the 75% ellipse.*

to measure the effect of nutritional intervention on HD patients. Our supplementation in the current study contained less kcal than the others mentioned, while the quantity of protein was quite similar[19,34]. In addition, we included vitamins C and E.

Another difference from the previously mentioned two studies that included supplementation and BIVA in HD patients is that the current work is the first randomized controlled double-blinded study with patients on HD which used BIVA to verify the effects of a nutritional supplementation protocol compared with a placebo group.

One of the limitations of the current study was that the height of one patient was estimated based on knee height, due to the inability to stand for measurement. It should be considered that, even when using a validated formula, the estimation error of this equation is high and can directly impact BIVA, since this evaluation method employs electrical parameters normalized by each patient's height.

Another limitation of BIVA assessments using the RXc graph in our population is that there are no available BIA parameters in the Brazilian population to use as a reference for creating tolerance ellipses. Therefore, the relationship between R and Xc and height in meters was expressed as a z-score (z-R/H and z-Xc/H) and the tolerance ellipses were created according to the population of Piccoli et al.[25], which refers to the American population. This was done because, among the populations available, this one most closely matched the body composition of our sample. However, it should be noted that there is a difference between the body composition of different populations, due to genetic variations and differences in dietary habits.

The small sample size was a limiting factor. Despite this,

we used a BIA parameter that allowed observation of the displacement of the vectors on the BIVA graph, suggesting early detection of changes, before they could be perceived by other nutritional parameters. The loss of patients during the study may have contributed to the power of the study. Regarding the subtle improvement in the placebo group, these results should be interpreted with caution, since studies with supplementation, BIVA, and HD are scarce. In addition, another limitation of the study was that we did not control the patients' food intake or physical activity patterns, which may have influenced the results.

To minimize methodological biases, randomisation, stratification, and double-blinding were applied, and as there was no control group, one group received the placebo intervention. It is noteworthy that supplementation was standardized for all patients, so the results may not demonstrate the dose-dependent effect of supplementation. Furthermore, although the patients were randomized and stratified by HD frequency, with a homogeneous number of patients in each group, randomisation did not result in a homogeneous distribution between men and women, so the results may be influenced by differences in body composition between the sexes.

Considering that changes in nutritional status can be masked when using other parameters, the results of the current study could motivate new research with supplementation and BIVA in HD, since several factors interfere in the assessment of nutritional status and studies with BIA could serve as a prognosis[35-37].

CONCLUSION

Eight weeks of nutritional intervention promoted displacement of the BIVA vectors in both the supplementation group and the placebo group, suggesting early detection of changes in body cell mass and hydration status. BIVA, in addition to being used for scientific research, is a tool for monitoring nutrition and hydration in clinical practice. We recommend individual nutritional monitoring of HD patients, aiming to reach nutritional recommendations through diet and nutritional supplementation.

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