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Bio-Electrical Properties of Body Tissues in Obese Women after a Program Composed by Physical Exercises and Nutrition Education

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Abstract

Aims: To identify changes in bioelectrical properties of body tissues in obese women, after a program composed by physical activity and nutrition education.

Methods: We studied 24 women, 30 to 50 years old, BMI ≥ 30 kg/m² (obese). The participants were submitted to a 16 weeks program composed by nutrition education (lectures and practical activities, 13 meetings) and physical exercise (walking and resistance exercises, 60 minutes per day, three days per week).

Variables investigated: Fat- free mass (FFM) by DEXA; bioelectrical properties of the tissues by bioimpedance vectorial analysis (BIVA); total body water (TBW) by deuterium dilution (only at the end of the program), serum albumin (by colorimetric method).

Main results: On average, FFM was reduced, however, the values presented a great individual variability; some of the participants reduced, others maintained or increased FFM; - TBW was considered within normality; - from BIVA analysis, most of the individuals who reduced the fat-free mass showed a poor hydration status. Putting the albumin, TBW and BIVA analysis together, we can indirectly suppose changes in the ratio of extracellular water/total body water in some of the participants.

Conclusions: The combination of different analysis of water balance (BIVA, TBW and serum albumin) gave us an indirect inference that our program was not able to prevent imbalances. These imbalances can give an explanation to the fat-free mass losses.

Keywords: Obesity, Physical exercise, Body composition, Nutritional status

Introduction

Attempts to control the obesity epidemic [1-3] have led to the application of different strategies, such as nutritional education, together with physical exercise [4]. Both aerobic and resistance physical exercises have shown health benefits, in order to reduce body fat and enhance muscle mass. In turn, nutritional education allows and stimulates individuals to make their own choices, from the perspective of developing autonomy [5,6]. As such, the combination of different types of exercise and nutrition education can be considered a good strategy in order to control body weight [7,8].

However, we need to be aware to the possible risks related to any kind of intervention in obesity, for instance, loss of muscle mass. It is important to highlight that the analysis of body composition, which includes muscle mass, is difficult to perform in obese individuals. Obesity is associated with specific and varied features of body composition, which makes it difficult to identify the most appropriate manner to monitor changes. For example, Dual-Energy X-ray Absorptiometry (DEXA), despite of be considered a gold-standard, is not able to monitor body water [9]. Additionally, the use of stable isotopes can identify cell mass integrity by monitoring the extracellular water/total body water (ECW/TBW) ratio. Water, labeled with deuterium, tritium or oxygen-18, has been used to measure TBW [10], and methods such as sodium bromide dilution have been employed to measure ECW [11].

Despite their unquestionable value, the application of images and/or isotope techniques is limited by requirements for expensive equipment, trained technicians, and dedicated facilities. Therefore, it is important to identify alternative forms to monitor body composition. The analysis of the bioelectrical properties of tissues has been suggested to be a reasonable tool; this kind of analysis can be performed from bioelectrical impedance (BIA), which has been reported to be a practical, non-invasive and easy-to-perform [10,12].

Among different manners of analyze BIA, the vector analysis (BIVA- bioelectrical impedance vectorial analysis) has gained attention as a valuable tool to assess hydration status and cell mass, and these assessments are associated to bioelectrical properties of tissues. In this technique, the body resistance (R) and reactance (Xc), obtained at 50 kHz, are plotted as a bivariate vector. One of the positive aspects of this kind of analysis, in addition to its low cost, is that it provides values that are independent of regression equations and body weight [13-15]. To our knowledge, there is not, currently, any published data describing the use of BIVA analysis in programs of physical activity in healthy obese subjects.

In a previous study of our group [16], we identified the combination of physical exercises and nutritional education to be able to improve cardiovascular conditioning and to reduce abdominal fat. However, the fat-free mass was not improved as expected. As such, this study has the aim to go back to the data file of the previous study, in order to investigate factors associated to changes on fat-free mass, now using the bioelectrical properties of body tissues.

Methods

The present manuscript is part of a broader study, published elsewhere [16]. We conducted a *quasi-experimental* study, comparing "before" and "after", in a non-probabilistic sample of obese women, from 30 to 50 years old, without any menopausal signs, and body mass index (BMI) above 30 kg/m². Exclusion criteria were the use of drugs that could modify metabolic rate and participation in any program aimed to body weight reduction during the last 6 months or during the study. Subjects also needed to be free from any pathologic thyroid alterations (checked by TSH analysis), free of type 1 or 2 diabetes mellitus (checked by self-referred information together with fasting blood glucose analysis) and were non-smokers, at least during the previous six months. Written agreement was obtained from all individuals, and local Ethics Committee approved the study.

The protocol of the study was published elsewhere [16]. The women participated in a four-month physical exercise program that included 30 minutes of aerobic and 30 minutes of resistance exercise, three times a week. The combination of aerobic and resistance exercise was based on the recommendation of the American College of Sports Medicine [17]. During the first two weeks of familiarization, sessions were carried out together with a baseline one maximal repetition (1MR) strength test, and the ergo-spirometric test. The exercise protocol consisted of walking on a treadmill at 65% VO_{2peak}, with heart rate monitored (Polar®). The resistance training consisted of three sets of 10 repetitions of different muscle groups, at 60-70% of the 1MR. Once a week, the women undertook a sub-maximal 8MR strength test to enable progressive adjustment of the weight lifted and a 12 minute run test, to adjust the intensity of the aerobic exercise within a given heart rate zone.

Together with the physical training, the women were submitted to a nutrition education program. The aim of the program was to promote healthy behavior in relation to food choices, without recommending severe energy restriction or food exclusion or restriction. The subjects participated in weekly sessions in which topics such as the importance of nutrients and the definition of a healthy diet were presented and discussed. These meetings included lectures, discussions and practical activities in a dietetic laboratory. The total number of meetings, including practice and theory, was 13.

Before and after the program, the women were submitted to an aerobic power test (in a treadmill), food consumption evaluation (from three food records, on non-consecutive days, recording all food and drink intake on the specified days, using domestic measurements), anthropometric measures, and DEXA analysis. In the present study, we will investigate variables associated to fat-free mass of these women, adopting the methods described below.

BIVA analysis- bioelectrical properties of body tissues

BIA measurements were carried out after an overnight fast. Participants arrived at the laboratory at between 7:00 and 9:00 am. They were previously advised not to exercise or to change their food and water ingestion during the previous 24h. BIA analysis (Biodynamics 450e®) was taken while the participants were in a supine position and on a non-conductive surface, with the electrodes placed on the recommended locations on the hands and feet. The data were analyzed for resistance (R) and reactance (Xo), plotted in an R/H Xc/H graph. Xc is the resistive effect produced by the tissue interfaces and cell membranes to the flow of an alternating electric current, whereas bioelectric R is the pure opposition of a biological conductor. The phase angle reflects the contributions between R and capacitance (arc tangent of the ratio of capacitance to R transformed to degrees). To plot the graphs, the BIVA software was utilized (Department of Medical and Surgical Sciences, University of Padova, Padova, Italy, 2002).

Dual energy X-ray absorptiometry (DEXA- Lunar®)

Body composition was determined using dual-energy X-ray absorptiometry (DEXA) with the scans analyzed for total fat- free mass, using adult software (Lunar Radiation corp, Madison, WI).

Total body water (TBW)

A subsample of the women (n=11) were analyzed only at the end of the program for total body water by deuterium analysis. On the morning of day 1, subjects arrived at the laboratory after a 10-hour fast and provided a baseline urine sample. The water was given orally at a dose of 0.12 g of 99.9 atom percent deuterium labeled-water per kilogram of estimated total body water, along with a subsequent 50 mL water rinse of the dose bottle. Urine specimens were collected at 2, 3, 4, and 5 hours after dose administration. Enrichment of deuterium in the specimens was analyzed by isotope ratio mass spectrometry (ANCA 20-20, Europa Scientific, Cheshire, UK). The laboratory where the analysis was performed, Mass Spectrometry Laboratory of the School of Medicine of Ribeirão Preto- SP-Brazil, was evaluated and approved for total body water and energy expenditure analysis by the International Atomic Energy Agency (Vienna, Austria), in 2007. Tap water was collected and analyzed, and all calculations were adjusted for the content of isotopes in the drinking water. Isotope dilution spaces were calculated, according to Schoeller [18], using the baseline specimen and the specimen collected at 5 hours after dosing. For all urine samples, some analyses were performed as quality controls. Total body water was calculated using the 4^{th} and the 5^{th} samples and the difference between them was <2.0% (21). The results were expressed as TBW (L) and % FFM.

Serum Albumin

Blood samples were collected during the period of 8:00 and 10:00 am, after an overnight fast. Serum samples were analyzed for albumin, using a colorimetric method.

Data analysis

Firstly, the normal distribution of the data was confirmed using the Komolgorov-Smirnov test. Initial and final values were compared using the paired t-test for dependent variables. Data were analyzed using the Statistica software (Copyright StatSoft, Inc, 1984-2005). An acceptable level of statistical significance was established as $p< 0.05$ for all the analyses performed.

With regard to BIVA, there are different ways to plot the BIVA graphs, and in the present study, we choose the individual point graph. This type of graph allows us to identify the individual vector migration from the start to the end of the program, using reference population ellipses. In order to explain how we interpret the location of the vector, we are showing in Figure 1 the classification proposed to BIVA analysis [13-15]. Vector analysis was performed with BIVA software (Department of Medical and Surgical Sciences, University of Padova, Padova, Italy, 2002).

Results

After checking the inclusion and exclusion criteria of 250 women who volunteered, 50 were included in the study. Of these women, 33 finished the program, but only 24 attended the minimal number of sessions required for inclusion in the analyses. The participants' mean age was 41.3 ± 7.5 years.

The main results previously published [16] will be summarized in order to contribute with the discussion of the present study. We found, after the physical exercise and nutrition education program, a reduction in trunk fat mass and an improvement in $VO_{2\ peak}$; BMI and body weight did not present any modification. With regard to diet, at the end of the study, the women presented an increase in lipids intake. In addition, the intake of proteins did not modify throughout the study, which was within the recommended level (0.8g/Kg body weight/ per day).

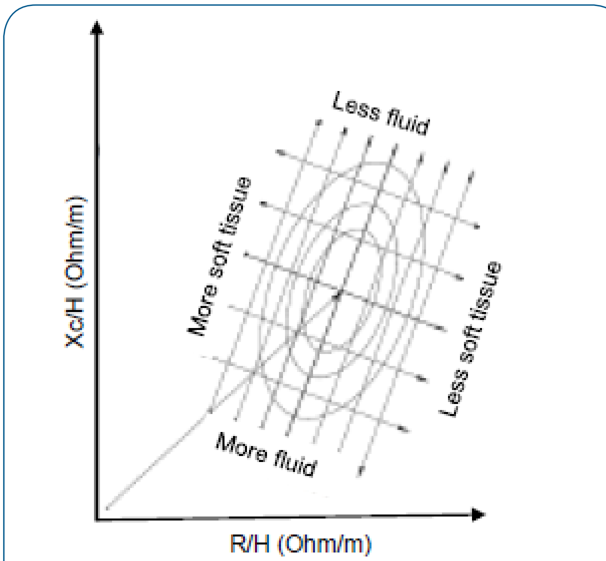

Figure 1: BIVA patterns. Different trajectories indicate combined changes in both hydration and tissue mass. The major axis indicates tissue hydration and minor axis indicates soft tissue mass. BIVA software manual (Department of Medical and Surgical Sciences, University of Padova, Padova, Italy, 2002)

In Table 1 we present the results of variables associated to fat-free mass. From DEXA analysis, the absolute values of fat-free mass were reduced, but it did not change in percentage values. The total body water at the end of the program (expressed as a percentage of fat-free mass) was within the expected range values, which are around 73% of fat-free mass [19]. Serum albumin was reduced at the end of the program; however, the values remained within the expected range. In addition, Figure 2 shows the frequency histogram of individual variation of fat-free mass, and it is evident the large variability in these values.

Figures 3 and 4 depict the individual BIVA analysis. We plotted the graphs together with the label describing the difference in fat-free mass (final values subtracted from initial values). Figure 3 shows the women who maintained or migrated to a good hydration status (therefore, they are located in lower quadrant); from the 12 women included in this figure, six of them (50%) enhanced their fat-free mass. Figure 4 shows the women who maintained or migrated to a low-hydration status (therefore, they are located in upper quadrant); from the 10 women included in this figure, only one enhanced her fat-free mass. From both figures, it can be noted that none of the women presented any sign of loss of soft tissue in comparison to the reference population (which in indicated by the localization at the left side of the graphs).

Table 1: Variables associated to fat-free mass obtained at the start and at the end of the program

Variable	Initial values	Final values
	DEXA analysis	
Fat-Free mass (kg)	49.2±6.2	48.6±6.7*
Fat-Free mass (%)	50.9±3.0	50.5±2.7
	Total body water (deuterium analysis)	
Total body water (%FFM)	-	76.6±3.1
	Serum analysis	
Serum albumin (g/dL)	4.4± 0.3*	3.7± 0.3*

FFM: Fat-Free Mass; *= p<0.05

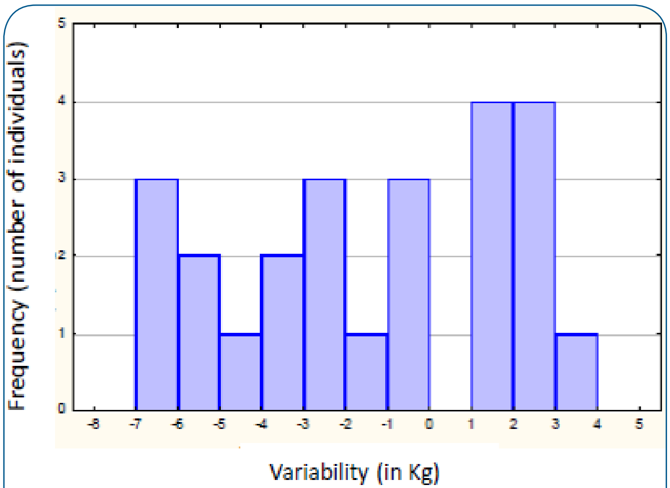

Figure 2: Variability in fat-free mass (analyzed by DEXA), from the start to the end of the program

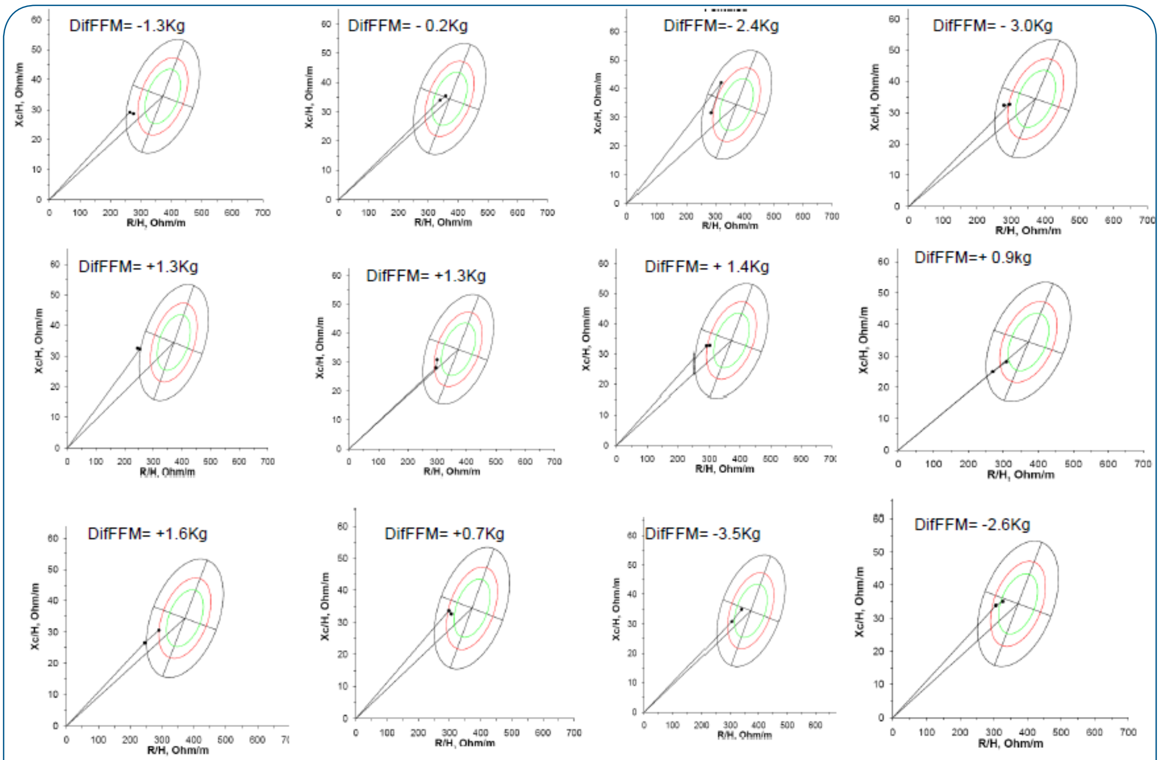

Figure 3: RXc path graph of vector migration; women who maintained or migrated to the “good hydration status” (n=12). DifFFM= free-fat mass at the end- fat-free mass at the start of the program. The vectors were plotted on the reference 50th, 75th, and 95th tolerance ellipses of a reference healthy population. The path graph starts with an arrow from the origin (start of the program) to the point vector of the sequence (end of the program). BIVA software (Department of Medical and Surgical Sciences, University of Padova, Padova, Italy, 2002)

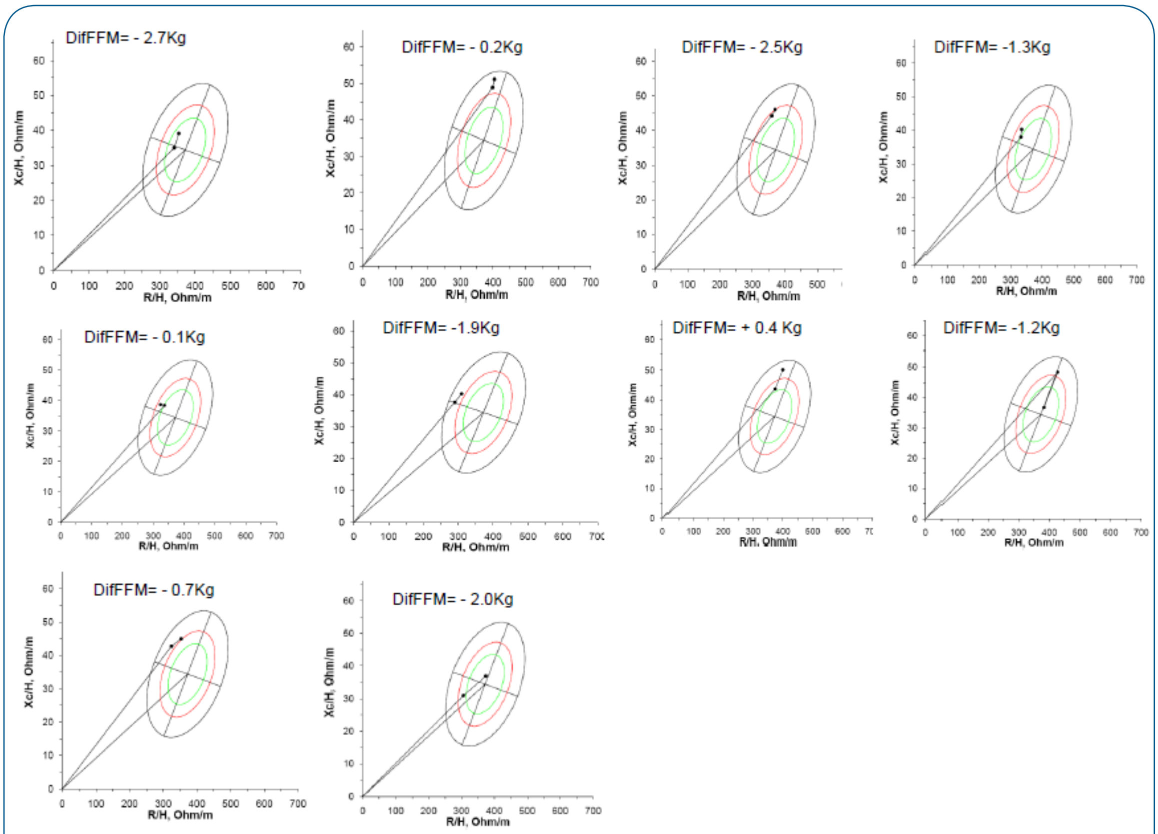

Figure 4: RXc path graph of vector migration; women who maintained or migrated to the “low hydration status” (n=10). DifFFM= fat-free mass at the end- fat-free mass at the start of the program. The vectors were plotted on the reference 50th, 75th, and 95th tolerance ellipses of a reference healthy population. The path graph starts with an arrow from the origin (start of the program) to the point vector of the sequence (end of the program). BIVA software (Department of Medical and Surgical Sciences, University of Padova, Padova, Italy, 2002)

Discussion

This study investigated variables associated to fat-free mass changes in obese women submitted to a program that combined physical exercises and nutritional education. We found a reduction in the absolute values of fat-free mass, with a great individual variability. In addition, we noticed an association between the hydration status and changes in fat-free mass, that is, the better hydration status meant the better results in fat-free mass.

Many authors have described, in similar studies, a great variability in body composition (including fat-free mass) after this kind of interventions. They have brought many hypotheses for that, without, however, a definite explanation [20-22]. In our previous study [16], we suggested that the dietary intake could contribute to this variability. Lipid ingestion was increased at the end of the study by some participants; we understood this as "compensatory behavior". King et al [21] in a study close to our in some aspects, also found, especially in the individuals who did not reduce their body weight, differences in food preferences, especially in the fat intake. The authors hypothesized as enhance in hungry/appetite mechanisms, which in turn could promoted a selective enhance in fat intake. The authors defined these individuals as "compensatory". As such, an imbalance in diet could contribute to negative outcomes in fat-free mass.

The present study tried to identify other factors associated to the variability in the body composition. We identified a water imbalance in some of the women; these imbalances can explain, at least in part, the fat-free mass losses. Savastano et al [23] studied the bioelectrical properties of morbid obese individuals and observed a similar vector position; i.e. a state of poor cell hydration. Indeed, Cigarran et al [24] suggested that low albumin concentration may be due to extracellular dilution. In the same way, in our study we identified, besides the vector position, a reduction in serum albumin. In turn, looking at the deuterium analysis, TBW showed to be within normal ranges.

Therefore, the imbalances identified by BIVA probably was not due to total body water losses, but rather to any imbalance between extra and intracellular water. Taken together the analysis of TBW, albumin and BIVA, we can reinforce this hypothesis. In a similar manner, Marken et al [25], using dilution techniques in obese individuals, reported that the ECW/ICW ratio was not normalized after a weight-loss program and found that the disequilibrium was maintained after nine months of follow-up of the same type of program. These authors suggested that the poor nutritional status of obese individuals could be responsible for this imbalance. Bringing this possibility to our results, we may suggest that the women did not adjust their own food and water intake to the exercise demands.

We can mention some statements explaining the importance of hydration in preservation of fat-free mass, based in molecular studies, different from our study. Cell dehydration could be responsible for inactivation of molecules members of insulin signaling. As consequence, processes such as glucose uptake and protein synthesis, which are dependent on insulin, are slowed or even prevented [26]. Therefore, the water imbalance could impair the appropriate protein synthesis, necessary to the maintenance of fat-free mass. Although our study did not evaluate those signaling molecules, we can find an indirect explanation for our hypothesis and results on these statements.

It is important to mention some limitations of our study. We adopted a gold standard to measure the TBW, but not to measure the ECW. Analysis of sodium bromide could give a more detailed information on ECW/TBW, although previous study showed a good agreement between BIVA analysis and isotopes [10]. In addition, we measured TBW only at the end of the program, which did not allow us to discuss the changes. However, as mentioned before, we did not find any study of this type with healthy obese women.

As main conclusions, the combination of different analysis of water balance (BIVA, TBW and serum albumin) gave us an indirect inference that our program was not able to prevent imbalances, which can be one factor associated to fat-free mass losses. As an important message, a strict control of food and water intake in this kind of program assumes fundamental position.

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