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Does performing moderate-to-vigorous physical activity in 12 to 24 hours prior Bioelectrical Impedance Analysis affects the validity of body water compartments assessment?

Dissertação elaborada com vista à obtenção do Grau de Mestre em Exercício e Saúde

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To my grandmother, the most powerful human being that I ever meet.

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List of Acronyms

ACSM – American College of Sports Medicine

ACT – Água Corporal Total

AEC – Água Extracelular

AIC – Água Intracelular

BIA – Bioelectrical impedance Analysis

BIS – Bioelectrical impedance spectroscopy

BM – Body mass

BMI – Body mass index

CV – Coefficient of variation

DXA – Dual energy X-ray absorptiometry

ECF – Extracellular fluid

ECW – Extracellular water

FM – Fat mass

FFM – Fat free mass

ICF – Intracellular Fluid

ICW – Intracellular water

MF – BIA – Multifrequency bioelectrical impedance analysis

MVPA – Moderate to vigorous physical activity

NaBr – Sodium bromide

PA – Physical activity

R – Resistance

SF- BIA – Single frequency bioelectrical impedance analysis

SMOW – Standard mean ocean water

TBW – Total body water

Xc – Capacitance

Z – Impedance

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Abstract

Purpose: One ofthe bioelectrical impedance spectroscopy (BIS) requirement is to avoid performing physical activity in the 12-24h prior measurement, which can be difficult in highly active populations. This investigation aimed: to examine if moderate to vigorous physical activity (MVPA) performed in 12-24h prior BIS testing, affects the validity of determining total body water (TBW), extracellular water (ECW) and intracellular water (ICW) using dilution techniques as the reference method.

Methods: Twenty-seven healthy highly active males, aged 20 to 39 years (72.4 ± 8.7 Kg;1.77 ± 0.07 m) were evaluated. BIS and dilution techniques were used to assess TBW, ECW, and ICW. Pearson coefficient of correlation was used to analyze if MVPA was associated with the difference between methods for TBW, ECW, and ICW.

Results: No significant differences between BIS and the dilution technique for TBW, ECW, and ICW volumes were observed ($p>0.05$). For all water compartments, no association was found between MVPA in the previous 12-24h with the differences between methods ($p>0.05$).

Conclusion: This study showed that performing MVPA 12-24h prior measuring water compartments may not compromise a valid estimation of TBW, ECW, and ICW from BIS compared to dilution techniques. Indeed, the magnitude of the differences between methods in body water assessment was not related with the amount of MVPA performed by highly active adults.

Key words: Physical activity, MVPA, body water compartments, bioelectrical impedance, dilution technique, total body water, extracellular water, intracellular water,

bioelectrical impedance spectroscopy

Resumo

Objetivo: Um dos requisitos de bioimpedância elétrica por espectroscopia prende-se com evitar a prática de atividade física nas 12-24h antes da medição, o que pode ser difícil em populações altamente ativas. Esta investigação teve como objetivo: examinar se a atividade física moderada a vigorosa (AFMV) realizada entre 12-24h antes do teste de bioimpedância elétrica por espectroscopia afeta a validade da determinação da água corporal total (ACT), água extracelular (AEC), e água intracelular (AIC) usando técnicas de diluição como o método de referência.

Métodos: Foram avaliados vinte e sete homens saudáveis, altamente ativos, com idades entre os 20 e os 39 anos (72,4 ± 8,7Kg; 1,77 ± 0.07m). A bioimpedância elétrica por espectroscopia e as técnicas de diluição foram usadas para avaliar TBW, ECW e ICW. O coeficiente de correlação de Pearson foi usado para analisar se a AFMV estava associada à diferença entre os métodos para ACT, AEC e AIC.

Resultados: Não foram observadas diferenças significativas entre a bioimpedância elétrica por espectroscopia e a técnica de diluição para os volumes ACT, AEC e AIC ($p > 0,05$). Para todos os compartimentos de água, não foi encontrada associação entre AFMV com as diferenças entre os métodos ($p > 0,05$).

Conclusões: O presente estudo mostrou que a realização de AFMV 12-24h antes da medição dos compartimentos de água poderá não comprometer uma estimativa válida de ACT, AEC e AIC do BIS em comparação com as técnicas de diluição. De fato, a magnitude das diferenças entre os métodos na avaliação da água corporal não demonstrou associação com a quantidade de AFMV realizada por adultos altamente ativos.

Palavras-chave: Atividade física, AFMV, compartimentos de água, bioimpedância elétrica, técnicas de diluição, água corporal total, água extracelular, água intracelular,

bioimpedância elétrica por espectroscopia

1. Background

Water is an essential chemical substance for all known forms of life[1]. It is widely accepted that water involves about 60% of the entire body mass[2]. It has an important role in many of the metabolic processes within the body, such as circulatory function, biochemical reactions, metabolism, subtract transport cross cellular membranes, temperature regulation and other various physiological processes[1].

Water must be consumed by humans because the amount lost in metabolism can be superior to the amount synthesized by the body. Daily water balance depends on the difference between water gains and water losses. Water gains occur from the ingestion of liquids and food, and metabolic production. Water losses can occur in many ways, such as respiratory processes, gastrointestinal and renal tract, and sweat losses[3–5].

Two compartment body composition models divide body mass (BM) in fat mass (FM) and fat free mass (FFM). This two components have different percentages of water in their constitution, being the FM less hydrated (around 10% of water) in comparison with FFM (73,2% of water), in normally hydrated adults[6–9].

The term euhydration is synonymous with the "normal body water content". Euhydration is not a specific point, but rather is best represented by a sinusoidal wave that oscillates around an average[10]. Despite no consensus exist regarding the definition of dehydration[11], it refers to the process of uncompensated water loss that decreases the volume of total body water to levels below the basal value. Dehydration can be acute, as a result of a bout of intense exercise, or chronic, resulting from less than adequate rehydration of daily water losses over a period of time[12]. The lack of consensus exists because physiologists use different techniques to evaluate dehydration (e.g. plasma osmolality, urine-specific gravity, or body weight). The term hyperhydration refers to the state that exists when ingested total body water increases above the average basal level prior to its removal by the kidneys. Hydration, therefore involves the point at which the body presently lives and can assume any of the previous explained states[11].

When body water loss occurs, various effects on neuromuscular function and short-term power have been reported[13], and they vary with the degree of water deficit[14]. For example, a fluid loss of 1% of body weight impairs thermoregulation and, thirst

occurs at this level of dehydration. Thirst increases at 2%, with dry mouth appearing at approximately 3%, at the same level occurs a reduction of 2% of muscular power [15]. At 4% dehydration, decrements of 20-30% are seen in physical work capacity. As long as dehydration increase, tingling and numbness of extremities can be seen at 6%, and at 7% dehydration level, collapse can occur. A 10% level of dehydration, is life-threatening[14].

Approximately 5% to 10% of total body water is turned over daily[4], being fluid-electrolyte turnover and whole-body water balance changing constantly because water is lost from the lungs, skin and kidneys. In sports, changes are increased and there is a high incidence of chronic and acute dehydration.

Individuals perform physical activity (PA) under certain conditions (temperature, humidity, sun, wind exposure) that may induce a significant elevation in the body temperature. Elevations on body temperature lead to increased skin blood flow and increased sweat secretion[3]. Sweat is composed of water and electrolytes, when the sweating rate it's too high, it will lead to an increase electrolyte loss. If not appropriately replaced, water and electrolytes can progress to an adverse impact on the individuals exercise performance and health.

Most evidence in the field of hydration, PA, and water compartments were performed in clinical populations or professional athletes. Love, Baker, Healey, & Black, 2018, recently have shown that professional rugby players underestimate sweat losses and fluid intake during training and just few players used recommended strategies to monitor levels of hydration. In weight restricted sports, acute dehydration, approximately 4.8% of body mass, negatively influences exercise performance at both 3 and 24h following weight loss[17].

Hydration status has been attempted to be assessed in a vary of situations for a number of years. Plasma osmolality, urine osmolality and urine specific gravity are the most widely used markers of hydration[18]. Bioelectrical impedance analysis (BIA) has been widely investigated as a tool for assessing body composition. It has the potential to assess hydration status by the determination of body water and its cellular divisions if a multifrequency device is used[19]. The National institute of Heath technology assessment statement (National Institute of Health, 1994) concluded that BIA provides a reliable estimate of total body water under most conditions. However, BIA values are affected

by numerous variables including hydration status, which means that standardization and control of these variables[19] is required.

Factors that can potentially influence BIA measurements include level of hydration of the subject, posture, measurement protocol, environmental and or/ skin temperature, age, gender, athletic status, body composition and ethnic origin. Moreover, the use of BIA to assess changes in an individual over time must control for biological and environmental variables such hydration status, timing and content of last ingested meal, skin temperature, menstrual cycle, and exercise performed several hours before measurements[20–22].

Various factors affect the validity of the BIA measurements. This dissertation will address on of the factors that affect hydration status, namely, MPVA performed in the 12-24h prior measurement.

This dissertation includes 6 sections, background, methodology, results, discussions, conclusions and references. The background (section 1), describes the overall findings of the available literature on the topic of body water compartments and water compartments assessment. The methodology (section 2) provides a description of the participants, as well the experimental design. In section 3 the results are presented being mainly organized in tables and figures. The discussion (section 4) is focused on contrasting the present findings with those of previous investigations. It also identifies the major limitations to this study. Finally, the sections 5 and 6 integrates the more important conclusions

1.1. Body water assessment

1.1.1. Water compartments

Water is the major constituent in the human body, representing between 40 to 70% of the total BM. The percentage of water in the human body depends on several factors such as sex, age, diet and body composition[6,23].

The percentage of water in FFM and in FM is not the same, with the majority of the water within the lean tissues. Water constitutes approximately 72-74% of FFM, in normal hydrated adult and approximately 10% of FM (relatively nonaqueous)[6,24].

Men have, in general, more body water than women, conversely women have higher FM[6,7] and lower sweeting rates and electrolyte losses than man, which is explained by their small sizes and lower metabolic rates

The total amount of water in the human body is distributed within the numerous organs and tissues. These innumerable fluids together can be referred as total body water (TBW). TBW is divided in two main compartments: intracellular water (ICW) and extracellular water (ECW)[26,27]. ICW corresponds to the fluid within the cells and it is ~66% (2/3 of TBW), and ECW represent all fluid outside the cells ~33% (1/3 of TBW). The separation of both compartments is made by the plasma membrane surrounding the cells[28]. The water movement across membranes is called osmosis and is achieved by facilitated diffusion by the water channel aquaporin**.** Water flows from the compartment where concentration of water is high to the compartment with "concentrated" solutions (where concentration of water is low) until the concentrations of water and solute are equal in both compartments.

When conditions external to the body change, these changes are reflected in the composition of ECW, which surrounds the individual cells of the body. Uncorrected deviations of certain factors, such oxygen, carbon dioxide, or temperature, change the composition of ECW leading to disease and even death [29,30]. So, it is crucial to have a homeostasis of the gradients and the movement of solutes and water across barriers to preserve normal body function[28]

As stated before, the water varies across the life span and within the day. In healthy persons TBW tends to be well regulated, on the other hand in some clinical conditions under the use of some drugs, the body can lose or retain significant amounts of water, presenting different proportions of TBW [29,31].

1.1.2. Dilution Techniques

The principle of the dilution techniques for water pools analysis is that the volume of a compartment can be defined as the ratio of dose of a tracer, administered orally or intravenously, to its concentration in that body compartment within a short time after the dose is administered.

This principle was first used in the study of body composition in the living human being in 1915. The investigators wanted to extrapolate the use of a red dye to measure the plasma volume and they observed that the concentration of the dye after mixing was not constant, as it disappears from blood plasma. By mathematical means a reasonable estimate was made and the volume of plasma in which the dye was initially diluted was calculated[32].

Nowadays, the most used tracers to measure TBW is deuterium (^{2}H), while bromide is the common tracer used to ECW. Usually two fluid samples (blood, saliva, or urine) are collected: one priori the administration of the tracer dose, to determine the natural background levels, and the second after waiting a sufficient amount of time for the penetration of the tracer within the compartment of interest[31,33].

When the amount of the tracer is known and the baseline and equilibration concentrations are measured, the volume into which the tracer has been diluted can be calculated[33].

Total Body Water

As previously stated, deuterium (^{2}H) is the most commonly used tracer for the measurement of TBW. The solute whose dilution is being measured distributes itself in a very uniform way in all solvent, in this case, body water[34].

Each aspect of the measurement, including preparation of the participant, dosing and sampling collection, and isotope analysis, must be in tight control. The dose of the isotope must be given to the participant while fasted and to minimize discomfort this procedure must be performed in the morning. The isotope attains the equilibration time within 3 to 4 hours after the ingestion of the dose, in that time, all body fluid compartments will have the same concentration of the isotope[35]. The most rigorous preparation is required for TBW extrapolation. Overhydration or dehydration reduces the accuracy of the extrapolation[33].

Physiological samples, usually urine must be collected before and after the isotope equilibration[33]. It has been identified that deuterium overestimates the body water pool by 4.2% in adults and children[35–37].

Extracellular Water

The ECW volume can be obtained by two different methods. First by the direct in vivo measurement of total body potassium, by whole body counting and, secondly, by dilution techniques[31].

In the 80's, bromide was identified as the most closed to ideal in ECW volumes assessment[38–41]. The measurement of ECW volume by bromide dilution, assumes the same principles as TBW measures, but saliva or plasma samples should be used as biological fluids[33].

The corrected tracer space can be calculated from the concentration after the administration of a known amount of bromide, giving an excellent approximation of ECW pool. Oral bromide is completely absorbed in the intestine, has a biological half-life of about 12 days, and is lost in urine and feces in insignificant amounts for 2 to 4 hours after an oral dose[39]. So ECW can be determined from Br when administered orally or intravenously within 2-4 hours period[40].

Intracellular Water

ICW volume can be obtained by direct in vivo measurement of total body potassium, by whole body counting, or by subtractive ECW from TBW using dilution techniques[31]. The later procedure implies the oral administration of combined dose of deuterium and bromide to assess both TBW and ECW, and then, ICW is calculated as the difference[33].

1.1.3. Bioelectrical impedance analysis

The principles of BIA have been established for more than 40 years, but methods for estimating components of body composition, specifically TBW, ECW, ICW and FFM, are comparatively recent[42].

The use of BIA to estimate body composition is based on the ability of tissues, and therefore the whole body, to conduct an electrical current. The aqueous tissues of the body, are the major conductors of electrical current, whereas non-aqueous have poor conductance properties[43,44].

Tissue conductivity is directly proportional to the amount of electrolyte-containing in the fluid. The current is conducted well by water and electrolyte-rich tissues (e.g. blood and muscle) and is poorly conducted by fat, bone and air-filled spaces[45]. Therefore, the main principle of the bioelectrical impedance method is based on an electrical current of a given frequency that flows in the human body and is resisted by body tissues and water. The result, of the current passage, gives the value of resistance (R), and reactance (Xc), that composes body's impedance (Z) value. Impedance is a function of two separated quantities, resistance and reactance, and is also frequency dependent.

Resistance, reactance and body's impedance are inversely related to biological volumes[38]. The R is described as pure opposition of the conductor the flow current being directly related with the amount of water present in the tissues. Resistance is also proportional to the length of the conductor and inversely proportional to its cross-sectional area. The Xc is the resistive effect produced by tissues interface and cell membranes, the inverse of capacitance, whereas capacitance is the storage of energy in a circuit by a capacitor[46]. In human body, capacitance shows when regions of high conductivity (i.e. ECW and ICW) are separated by regions of low conductivity (i.e. cell membranes)[46]. Therefore, ECW and ICW work as resistors in parallel while the cell membrane behaves as an imperfect capacitor, contributing to a frequency-dependent reactive component to the total impedance[20]. Depending on the frequency, the electric current crosses through ECW and ICW differently.

At 5 kHz signal pathway is conducted entirely through extracellular water because there is very little capacitive penetration of the signal into intracellular volume, assuming that measured impedance is all-out resistance[20]. At midrange frequencies (e.g50 kHz) some capacitors will be electrically charged, helping the penetration of the current in the intracellular water [47]. Therefore, at 50 kHz, the resistance is lower than at

0 kHz because there is a greater cross sectional area and a shorter path to destination electrode [46]. At high frequencies (>500 kHz), the current is able to penetrate the cell membrane of the intracellular compartment[48] and it is assumed that resistance index at this frequency is linearly correlated with TBW[49]. Resistance is very low because of the large cross-sectional area of the conductor[50].

The Cole-Cole model (0/∝ kHz), is the most accurate model used in the analysis of water compartmentalization. The success of this method can be attributed to the fact that ECW and ICW are the major electrical conductors in the body and they belong adjacent to each other, with ICW being apart from ECW by low conductivity membranes[51].

Therefore, measurements have underlying assumptions that will affect differently the path of the current passage in each participant. The major assumption that underlies the measurement is that human body has uniform cross-sectional area and homogeneous conductivity. However, these conditions are not fulfilled in humans as the human body is not a cylindrical conductor, and the tissues are not electrically isotropic[52].

Body is considered as the sum of 5 uniforms conductive cylinders (2 arms, 2 legs and a trunk) whose dimensions are proportional to the participant's stature[53]. It is predicted that an arm is about 4% of BM and a leg is about 17% of BM, but they contribute approximately 47% and 50% (respectively) of whole-body resistance when using wrist-to-ankle electrode configuration. Conversely, the trunk, that represents 50% of the BM, contributes only 5% to 12% of whole-body resistance[53,54].

Like other body composition methods, bioelectrical impedance depends on static assumptions and dynamics relationships regarding electrical properties of the body, its composition, hydration and density, age, sex, race and physical condition of the participant[55,56]. These parameters are very important and should be addressed and standardized to diminish the effects of interindividual variance in R and Z values, linked to differences in body shape and size and to increase the prediction (accuracy of body composition estimates)[57].

1.1.3.1. Factors affecting Bioelectrical Impedance Analysis

There is substantial evidence that impedance measurements are highly reliable based on the interobserver and intraobserver[55]. Body composition measurements must be accurate to a well-defined precision; they must be repeatable in several, preferably in many laboratories who speak a common language; and they must be related to a well-defined range of normal for the particular population being studied[58].

Considering the above statements, several factors affecting BIA measurements are considered during measurements. Firstly, the orthostatic fluid shifts. While a subject is standing, sitting, or ambulatory, gravitational forces tend to sequester ECW in the capacitance vessels and interstitium of the lower distal extremities. When subjects assume a supine position, as when BIA is performed, interstitial fluid is absorbed into the intravascular compartment and fluid shifts to the central pool[59]. Upon recumbence from standing, there is a ≈3% increase in low frequency impedance (decrease in ECF volume) and an additional ≈2% increase at 10 minutes[60]. For an average 70 kg man with nearly 42 liters (L) TBW, 17 L ECF and 25 L ICF, after 10 minutes, this is roughly a 0.68 L and 0.75 L error for ECF and ICF, respectively. Thus, for the prediction of absolute volume, subjects should always be measured at the same time or within 10 minutes following recumbence.

Secondly, the abduction of the limbs. In whole-body BIA, body segments are assumed to be connected in series (ie, arm to trunk to leg). Due to their geometrical shape, the extremities contribute 90% to whole-body Z. Furthermore, most of the extremity Z is accounted for by the distal segments of the upper and lower limbs as a result of their lower cross-sectional area. Published BIA prediction equations are based on this electrical series paradigm. Thus, adduction or crossing of limbs would "short circuit" the electrical path, giving rise to spurious low Z values. These errors can range from 18% for contact of crossed legs to 43% for contact of the hands to the waist, both of which are errors that occur with skin-to-skin contact conditions[60].

Third, the consumption of food or beverages meals. Consumption of food or fluid before BIA measurements are made could influence Z by changing TBW and ECW volumes. However, depending on the timing of the measurement and the amount of food or fluid ingested, one could hypothesize that a meal could have little or no effect in the prediction of TBW[60]. Recent ingestion of a meal or beverage appears to have a minimal effect on whole-body Z during the first hour. Weight, however, will be increased and thus body fat may be overestimated. Depending on the experimental

condition, Z may decrease over a 2-4-h period after a meal, generally representing a change of <3% in Z[60].

Fourth, changes in ion concentration demonstrate that a 5 mmol change in ion can affect the predicted extracellular fluid (ECF) 1-2%, and intracellular fluid (ICF) 4-5%[61]. A 2% and 5% error on ECF and ICF, represents 0.34 L and 1.2 L error, respectively, for the standard 70 Kg man with 17 L ECF, and 25 L ICF. For the prediction of absolute volume in liters, the error caused by ion is small on ECF and moderate for ICF[22].

Fifth, skin and core temperatures and the effect of cleaning the skin with alcohol. Modifications in skin temperature caused by a variety of health conditions, changes in environment, blood flow and heat dissipation, can affect the measurement adversely. In addition to these effects, changes in core temperature can also affect the prediction[60]. Respectively to cleaning skin with alcohol, a previous study shown that the use of alcohol increased the impedance between 1.3% and 1.5%[62].

Sixth, electrode position. Displacement of the source electrodes proximally by 1 cm, on either the hand or the foot, reduces the measured resistance by 2.1%. If the source and receiving electrodes are placed closer together than 4-5 cm, electrode polarization may occur that will increase resistance. Interobserver differences associated with the placement of electrodes can be reduced if the sites of electrodes placement are marked[55]. The side on which impedance is performed is also important being resistance systematically grater on the left side than the right side[63].

Seventh, dehydration. There is evidence to suggest that when a subject becomes dehydrated due to insufficient water intake, ion concentration changes 3-5 mmol/L. As stated before, a 5 mmol change in ion consumption can affect the predicted ECF 1-2%, and ICF 4- 5%. Unless the change in ion is accounted for, it is advised to fully rehydrate the subject before performing a measurement[22].

Eighth, exercise performed prior BIA measurements. Exercise can affect BIA measurements by at least three hypothesized mechanisms. 1) The hemodynamic response to exercise consists of increased cardiac output and blood flow to skeletal muscles. Increased vascular perfusion and warming of muscle tissue will reduce Z and muscle resistivity. 2) The process of heat dissipation includes increased cutaneous blood flow and vasodilation, increased skin temperature, and sweating. These changes should also reduce Z. 3) Sensible and insensible fluid losses result in dehydration, loss of

TBW, and an increase in Z. Thus, BIA measurements should vary depending on the muscle groups involved and the intensity of exercise, changes in skin blood flow and heat production, and amount of fluid loss[60]. The errors caused by exercise varies within 3% to 5%, therefore, avoiding exercise or MVPA the day before BIA measurements is usually required[22].

1.1.3.2. Single Frequency Bioelectrical Impedance Analysis

Single-frequency bioelectrical impedance analysis (SF-BIA) is the most widely available bioimpedance methodology, which involves the application of a bioelectrical current for the measurement of impedance at a single frequency, typically 50 kHz.

The classic 2-component model of body composition divides weight into FM and FFM, and because water is comprised within the FFM, TBW can be used to provide an estimate value of FFM, and consequently, FM[64]. In SF-BIA, FFM is calculated with the assumption of the constant hydration of ~0.73 of FFM[9], and FM is estimated as the difference of BM and FFM. The major limitation of using TBW to assess FFM and FM is the same as that underlying all 2-component models of body composition: the assumption of constant hydration of FFM. This fact may not hold for all populations.

SF-BIA allows estimation of TBW but cannot determine differences in the water compartments. A problem using a single-frequency measurement to predict TBW is that the sensitivity of single high-frequency measurement to changes in ECW and ICW is different due to their different resistivities, a simple change in the ratio ECW/ICW will alter TBW resistivity and cause error[65].

At 50 kHz, impedance is a mix on R an Xc, and so it has been assumed that the conductor path is a mix of both ECW and ICW pathways. This assertion has been challenged[66,67] and now appears that penetration of cells is minimal and that TBW is highly correlated with 50 khz resistance only because of its close relationship with ECW in normal hydrated states[67]. Although single frequency BIA is not valid under conditions of significantly altered hydration, this does not negate its use to predict absolute TBW in normal hydrated subjects[67–69].

1.1.3.3. Multi Frequency Bioelectrical Impedance Analysis

As previously mentioned, at 50 kHz, the electrical pathway is mainly extracellular, because there is a very little capacitive penetration of the current into the intracellular volume. In this case, the cell membrane work as an insulator, and can be assumed that the impedance is essentially a function of ECW, responsible for the measure of the R at R_0[50,65,67,68]. As a consequence, SF-BIA is limited in the ability to distinguish the distribution of body water in to its extra- and intracellular compartments[70].

Therefore, with the advent of the multi frequency method, through the application of defined frequencies (e.g. 5, 50 or 200 kHz) TBW, ECW, and ICW, are potentially more accurately assessed.

Multiple Frequency Bioelectrical Impedance

Multiple Frequency BIA uses defined frequencies to assess TBW, ECW, ICW and FFM[68,71]. MF-BIA was first introduced by Thomasset el al. and used impedance data from very low frequencies (5kHz) through high frequencies (50, 100, 200 to 500kHz)[44].

Low frequencies, like 5kHz, are commonly used to predict the resistance of extracellular fluid (Re), because its believed that there is an insignificant penetration of ICW at this frequency. High frequencies, such 100 or 500kHz, are used to predict resistance of whole fluid (Rt), as it enables current penetration through the cell membrane. The resistance of the intracellular fluid (Ri) is a function of both low and high frequencies[50,72]. In most situations of altered body water compartmentalization, this approach would not accurately predict TBW, because it assumes equal resistance per unit of ICW or ECW[50,67].

The use of more than one frequency gives an uncertainty of results and no conclusions can be made concerning the validity of one frequency over another, in the perdition of body fluid compartments[50].

Bioelectrical Impedance Spectroscopy

Bioelectrical impedance spectroscopy (BIS), it is a multifrequency method that measures the impedance across a spectrum of frequencies. BIS uses physical and mathematical modeling and mixture substitute of regression equations[68]. Therefore, BIS supply a more direct, individualized measurement of ECW and ICW than other impedance approaches[45].

The model that best explains the application of this model is the Cole-Cole model. In the Cole-Cole model, human body is viewed as an electrical circuit with intracellular and extracellular pathways in parallel and with cell membranes working as capacitors for the intracellular pathway[51].

Cole model is computed by using nonlinear curve fitting to extrapolate data to the low and high frequency limits. This process generates Cole model terms, including R_e (resistance associated with ECW), R_i (resistance associated with ICW), C_m (cell membrane capacitance) and exponent α. The terms of Cole model are then applied to equations derived from Hanai mixture theory, which is based on the notion that the body is conducting medium of water, electrolyte-rich (e.g. blood and muscle) tissues in addition to nonconducting material within it (e.g. bone fat and air-filled spaces)[72].

The Hanai theory was used to improve the Cole-Cole linear model as i) it accounts for the effects of non-conductive substances in the body water, ii) removes the apparent population-specificity found with Cole-Cole linear equations, iii) improves sensitivity to body water changes[73].

1.2. Relevance of the study

BIS is an easy applied, non-expensive, and non-invasive method that evaluates total body composition and individual body segments[74]. However, the validity and accuracy of the method is influenced by several factors such as instrument type, electrode placement, hydration level, feeding, PA levels, menstrual cycle, ambient

temperature, and prediction equation[68]. In order for the measures to be valid and accurate, there is a need to control these factors.

It is difficult to some populations, namely highly active individuals, to meet the requirements for BIS, such as to avoid exercising in the previous 12-24 hours (since in the high competition the athletes have to perform exercise in a daily basis).

To date there is no study that verified the impact of moderate to vigorous physical activity (MVPA) performed on the 12-24h prior BIS evaluation. Therefore, it is unknown whether the magnitude of MVPA performed the day before BIS measurements affect the validity of TBW ECW an ICW estimations from BIS compared to reference methods.

1.3. Purpose

The aim of this study is to examine if the validity of TBW, ECW, and ICW estimated from BIS against dilution technique is affected by MVPA performed 12-24h prior the test.

2. Methodology

In this section we will present the methods used in this thesis.

2.1 Study Design and Sample

Twenty-seven healthy non-smoking males were recruited through advertisements around the University of Lisbon who volunteered to participate in this study. The participants were non-athletes but were engaged in general fitness activities for up to 2h/day. This study was part of a larger intervention where the aim was to determine the effect of caffeine ingestion on TBW and is compartments (ClinicalTrials.gov; nº NTC01477294).

Inclusion criteria were age between 20 and 39 years-old; body mass index (BMI) ranging from 18,5–29,9 kg/m^2; not taking any medication or dietary supplement;

being physically active (defined as ≥30min per day of MVPA, according to the recommendations of the ACSM); all participants were informed about the potential risks of this investigation before they gave written informed consent to participate in the study. All procedures were approved by the Ethics Committee of the Faculty of Human Kinetics, University of Lisbon.

2.2. Body composition measures

Participants were weighted to the nearest 0.01kg on an electronic scale connected to a plethysmograph computer (BOD POD®, Life measurement, Inc., Concord, Ca, USA). Height was measured to the nearest 0.1cm with a stadiometer (Seca, Hamburg Germany) respecting standardized procedures. BMI was calculated as body mass (kg) divided by the square of the stature (m).

2.2.1 Dual Energy X-Ray Absorptiometry (DXA)

To estimate total fat mass and fat free mass, Dual Energy X-ray absorptiometry (Hologic Explorer-W, fan beam densitometer, software QDR for windows version 12.4, Waltham, USA) was used.

A whole-body scan was performed and the attenuation of X-rays pulsed between 70 and 140 kV synchronously with the line frequency of each pixel of the scan was measured. According to the protocol for DXA described by the manufacture, a phantom with six folds of acrylic and aluminium of varying thickness and known absorptive properties was scanned alongside each subject to serve as an external standard for the analysis of different tissue components.

The same technician positioned the participants, performed the scans and executed the analysis protocol. Based on test-retest using ten subjects, the coefficient of variation (CV) in the laboratory for the fat mass and fat free mass, are 1.7% and 0.8%, respectively[75].

2.2.2. Bioelectrical Impedance Spectroscopy (BIS)

The present research used Bioelectrical Impedance Spectroscopy analyzer model 4200B Xitron Technologies (San Diego, Ca, USA). The multifrequency bioelectrical impedance analysis allows the generation of frequencies from 1 kHz to 1.35 MHz.

A priori to the examination, anthropometric variables and gender were inserted into the interface of BIS software. A few requirements were also taken into consideration to guarantee the validity and reproducibility and precision of the measurement. They were the position of the body, length of time in supine position before examination, correct stature measurement, skin preparation with alcohol before electrode placement, electrode position, hydration status, consumption of beverages, and diuretic drinks before test, recent MVPA, ambient air and skin temperature, the use of metals and conductance of the examination[66,68,69].

The protocol of examination was followed, being the participants in the supine position with legs and arms abducted at a 45º angle. Before the placement of the electrodes, the skin was cleaned with alcohol, to ensure a better adhesion and to remove anything that could diminish the efficacy of the electrode. Four electrodes, two electrodes for injecting current (I) and two for sensing voltage(V) were placed on the dorsal surfaces of the right foot and hand.

The injecting current (I) electrodes were place on the foot, in the middle of dorsal surface proximal to the metatarsal-phalangeal joint, and in the middle of the dorsal surface proximal metacarpal-phalangeal joint. The sensing voltage (V) electrodes were placed in the ankle joint at the line between the malleoli and on the wrist at the midline between the distal prominences of the radius and ulna. The injecting and sensing electrodes should be with a 5cm distance between each other.

After placement of the electrodes, the red cable was connected to the proximal surface and the black cable was connected to the distal surface. The alternating current is passed through the outer electrodes, while the voltage drop across the body is measured using the inner electrodes.

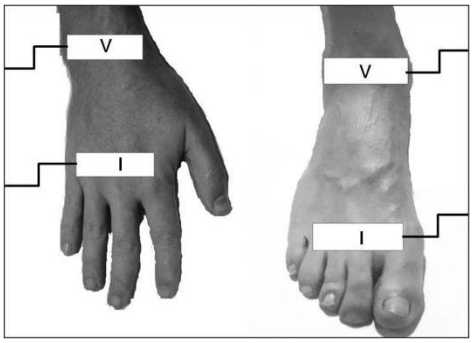


Figure 1. Electrodes placement according Xitron Hydra 4200 user's manual.

Measurements were performed after 10 minutes resting in the lying position without move. A total of 50 frequencies between 5 and 500 kHz were selected. The impedance spectrum was modelled according to the Cole-Cole (1941) cell suspension model to derive a theoretical impedance at zero and infinity frequency based on a nonlinear curve fitting from the measured resistance and reactance.

ECW and ICW were predicted from the Hanai mixture theory. The total body water was estimated from the sum of intra and extracellular water.

Based on test-retest reliability of BIS in 10 participants for TBW, ECW, ICW in our laboratory is 0.3%, 0.7%, and 0.3%, respectively[76].

2.2.3. Deuterium Dilution

TBW was assessed by deuterium oxide (^2H_2O) dilution, using a stable Hydra gas isotope ratio mass spectrometer (PDZ, Europa Scientific, Crewe, UK). After a 12-h fast, a baseline urine sample was collected prior to the administration of an oral dose of 0.1g/Kg/body mass of ^2H_2O, diluted in 30 ml of water. A second urine sample was obtained after 4-h equilibration period and then was analyzed the abundance of ^2H_2O.

Urine and diluted dose samples were prepared for $^1H/^2H$ analysis using the equilibration technique. After the tubes were filled, they were equilibrated at 20 ±1ºC for 3 days. The tubes were then introduced sequentially into a helium flow that was dried by magnesium perchlorate and were then analyzed by a Hydra gas isotope ratio mass spectrometer to detect $^1H/^2H$. The enrichments of equilibrated local water standards were calibrated against standard mean ocean water (SMOW). According to delta SOWM, TBW was estimated by including a 4% correction to account for equilibration with nonaqueous compartments. The TBW was then converted in kg by

multiplying the TBW values in liters by 0.9937kg*L^{-1} assuming an average body temperature of 36ºC. Based on test re-test in 10 participants, the CV for TBW with the stable isotope ratio mass spectrometry in our laboratory was 0.3%[77].

2.2.4. Bromide Dilution

ECW was assessed by the sodium bromide (NaBr) dilution. The participants were asked to drink an oral dose of 0.03g*kg*bodymass^{-1} of NaBr. The NaBr concentration in saliva was measured by high-performance liquid chromatography (Dionex Corporation, Sunnyvalr, Calif., USA) before and 3h after tracer administration. The volume of ECW was calculated by the following equation:

$$ECW\ (L) = [dose/(post[Br]_{saliva)} - (pre[Br]_{saliva})] \times 0.90 \times 0.95$$

Where $pre[Br]_{saliva}$ and $post[Br]_{saliva}$ is the saliva bromide concertation before and after tracer administration, respectively; 0.996 is the correction factor for intracellular bromide found in saliva. The ECW was also converted in kilograms by multiplying the ECW values in liters by 0.9937 kg*L^{-1} assuming the average body temperature of 36ºC. Based on test re-test in 7 participants, the CV for ECW with ionic chromatography in our laboratory was 0.4%[76].

2.2.5 Intracellular water

ICW was calculated as the difference between TBW and ECW as determined by the aforementioned dilution techniques.

2.3. Moderate-to-Vigorous Physical Activity Assessment

All participants were asked to wear an accelerometer (Actigraph, GT1M model, Fort Walton Beach, Fla., USA) on the right hip, near the iliac crest, for 11 consecutive

days. They were given detailed instructions by the study investigators for the correct use of the accelerometers. The devices were activated on the first day at 07h00 and data were recorded in 10s-epoch.

The device activation and data download were performed using ActiLife Lifestyle Software (V 3.2. ActiLife Pensacola, Fla., USA). For the analysis, a valid day was defined as having 600 or more minutes of monitor wear, corresponding to the minimum daily use of the accelerometer. In addition to when it was removed for sleeping or water activities, periods of at least 60 consecutive minutes of zeros were considered as non-wear time.

The total amount of activity assessed by accelerometery was expressed as counts*min^{-1}*day^{-1}. In addition, the minutes per day spent sedentary, in light, moderate, and vigorous intensities were estimated. The cut-off values used to define PA intensities and therefore to quantify the mean time in each PA intensity were sedentary, <100 counts*min^{-1}; light 100-2019 counts*min^{-1}; moderate, 2020-5998 counts*min^{-1}; and vigorous ≥ 5999 counts*min^{-1} [78].The total amount of MVPA was determined by summing the time spent in MVPA.

For the present study, only was considerate the physical activity performed in the day before the evaluation.

For the present study only was considered the MVPA performed in the day before the evaluation. Thus, the day before the examination the accelerometer has to record at least 600 min of PA, to be valid.

2.4. Statistical Analysis

Statistical analysis was performed using IBM SPSS Statistics for Mac OS 24 (SPSS Inc., an IBM Company, Chicago IL, USA). Descriptive analysis included means ± SD for all measured variables. Before performing a paired sample t-test, data were tested for normality with the Shapiro-wilk test. All variables presented a normal distribution ($p>0.05$).

To compare TBW, ECW, and ICW from BIS with dilution technique, a comparison of means was performed using a paired sample t-test to determine the

differences in body water compartments between both methods. Also, the degree of association between BIS and dilution techniques in detecting TBW, ECW, and ICW, was tested using the Pearson coefficient of correlations.

	Mean ± SD	Range

To explore if PA performed in the 24h prior test affected the validity of the BIS in determining TBW, ECW, and ICW from the reference method, the Pearson coefficient correlation was performed to test if a correlation between the methods with MVPA existed. Statistical significance was set at $p<0.05$.

3. Results

Table 1 shows the baseline characteristics of the sample.

Table 1. Sample Characteristics: Body Composition and Physical Activity Outcome

Age (yr)	24.7 ± 5.0	20 – 39
Height (m)	1.77 ± 0.07	1.64 – 1.87
Body Mass (kg)	72.7 ± 8.7	53.0 – 89.5
Body Mass Index (kg/m^2)	23.3± 2.4	19.3 – 29.7
Fat Mass (kg)	12.2 ± 4.4	4.9 – 23.2
Fat mass (%)	16.7 ± 4.6	9.3 – 26.2
Fat Free Mass (kg)	59.2 ± 6.1	46.7 – 70.3
TBWREF (kg)*	45.9 ± 4.9	37.2 – 56.0
ECWREF (kg)*	17.7± 1.4	15.4 – 20.2
ICWREF (kg)*	28.2 ± 5.3	17.8 – 36.7
TBWBIS (kg)*	45.9 ± 4.8	37.5 – 56.5
ECWBIS (kg)*	17.6 ± 1.6	15.2 – 20.5
ICWBIS (kg)*	28.3 ± 4.0	21.8 – 37.2
MVPA 12-24h	53.2 ± 29.0	10.7 ± 131.9

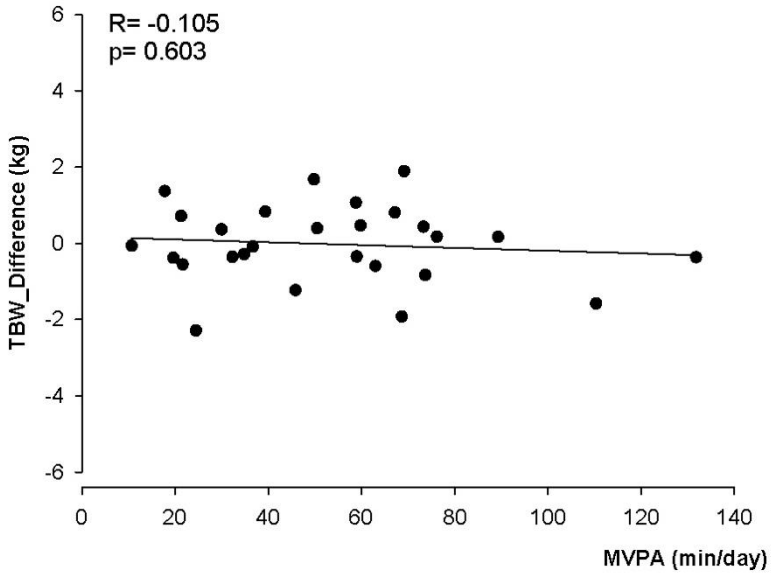
Abbreviations: ECWBIS, Extracellular water from BIS, ECWREF, Extracellular from bromide dilution, TBWBIS, Total body water from BIS, TBWREF, total body water from deuterium dilution, MVPA, Moderate to vigorous physical activity.

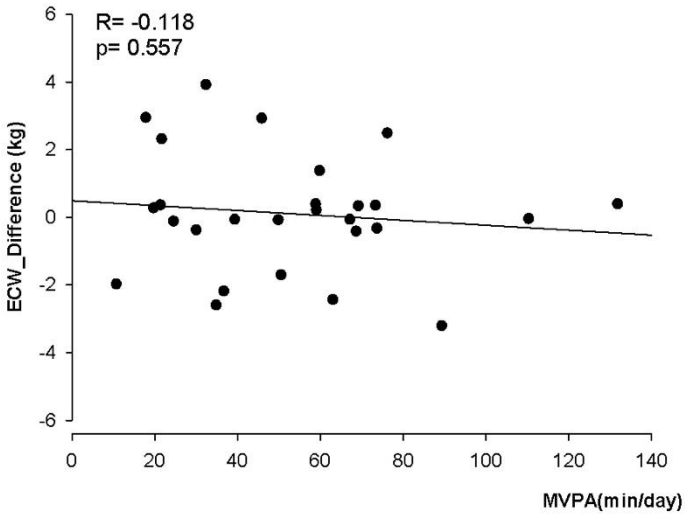
The sample consisted of 27 men aged between 20 and 39 years with a mean age of 25 years. The sample was non-obese with a body mass index ranging between 19.3 and 29.7 kg/m^2, with an average of 23.3 kg/m^2. Percentage of fat mass by DXA varied between 9.3 and 26.2% with a mean relative fatness of 17%.

The percentage of fat mass by DXA ranged between 4.9 and 23.2 kg, with a mean relative fatness of 12.2 kg. The percentage of fat free mass by DXA ranged between 46.7 and 70.3kg, with a mean relative fatness of 59.2kg.

The majority of the participants were physically active before the BIS measurement as the mean value was above 30 min/day of MVPA (53.2±29.0 min/day). Only seven participants had values below 30 min/day. No significant differences were observed between both methods when measuring TBW, ECW, and ICW ($p= 0.934$; $p= 0.762$; $p=0.756$ respectively for TBW, ECW and ICW) and the methods were highly associated for TBW ($r=0.967$; $p= 0.001$) and ICW ($r=0.915$; $p=0.001$), but moderately associated for ECW ($r=0.385$; $p= 0.035$),

Figure 2 presents the degree of association between MVPA 12-24h prior measurements with the differences between the methods in water compartments assessment. For all water compartments, no association was found between MVPA and the differences between methods ($p>0.05$), that is the magnitude of MVPA performed 12-24h prior measurements was not related with the differences between BIS and reference methods for TBW, EWC, and ICW ($p>0.05$).





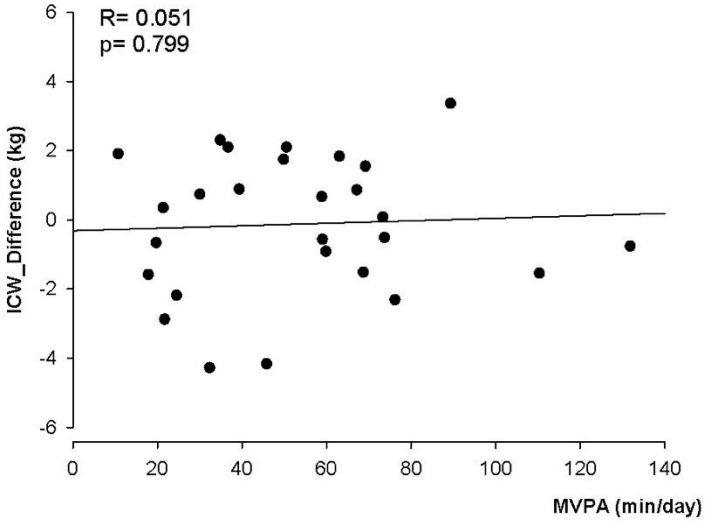


Figure 2. Correlation between MVPA 12-24h priori BIS examination with the difference of methods in body water compartments assessment.

4. Discussion

The BIS allows the monitorization of the hydration status in a non-invasive, fast and non-expensive way. Compared with other methods, BIS provides a good estimation of the volume of water present during changes in the hydration status, being highly sensitive to changes in the electrical properties of muscle tissue and, consequently, loss of body fluid during the practice of PA[79,80].

However, for monitoring to be more precise, it is necessary to control the conditions under which the measurements are made, in order to avoid the influence of external factors, such as the placement of electrodes, the posture of the individual and / or body movements[81].

Regarding our investigation, the factor that was aimed was the MVPA performed the day before the evaluation. Individuals who perform MVPA regularly may present differences compared to sedentary individuals, which may affect the measurements performed by BIA[82].

Associated with MVPA, it is necessary to take into account the ambient temperature in which it is performed, since as the ambient temperature increases, there is a decrease in the impedance of the body[83].

MVPA combined with heat also alters the distribution of fluids between the vascular and interstitial compartments[21]. MVPA leads to increased vascular perfusion, cutaneous blood flow and vasodilatation, and increased vascular perfusion and heating of muscle tissue will reduce impedance and muscle resistivity[82]. The production of sweat results in a better conductivity of the skin and, therefore, in a reduction of the impedance thereof. O'Brien et al. (2002) report that all these factors influence body impedance and may complicate the detection of loss of body fluids[21].

Jürimäe et al. (2000) conducted a study where they observed changes in body fluids during rowing endurance training. The changes were significant in TBW content during the first 30 minutes of recovery. The amount of ECW decreased over the same period of time[84].

Perrella et al. (2005) concluded that two hours of rugby play had little effect on BIA measures. On the other hand, Neves et al. (2005) used several methods to evaluate changes in body composition of participants after four hours of intense PA and observed

a decrease in the percentage of fat mass evaluated by BIS, thus indicating a strong relation of PA with the results obtained[85].

Koulmann et al. (2000) concluded that after an exercise-induced dehydration state, the BIS predicted only half of the total body water lost, and that the main factors that usually affect the accuracy of the BIS measurements appeared not to be involved in the result obtained[86].

In another exercise-induced dehydration study Beckmann et al. (2009), used the BIS to see the variations occurring in the water compartments. Regarding total body water, study participants dehydrated, having lost between 0.8 and 1-6 kg of body weight. Regarding extracellular water, all participants showed losses in the order of 0.4l-0.65L. With regard to intracellular water, the variations were not uniform[87].

Thomas et al. (1999) concluded that BIA is only able to accurately detect changes in water volume when the electrolyte concentration is maintained constant[88].

In all of the aforementioned studies, water compartment measurements were performed in a short period of time after PA practice, in order to verify acute changes in the state of hydration. The present study differs from previous ones in the context in which hydration status evaluations were performed the day after PA practice.

Possible explanations for the results obtained in the present study differ from the previous ones are time elapsed between the practice of MVPA and the measurements, i.e., it is expected that the participants of this study had the possibility of restoring all the water lost during the activity practiced the day before. This is possible if liquids and electrolytes are ingested in sufficient quantities and have been undergoing an extended period of recovery (eg 8-12 hours) since the last physical activity session until the time of the evaluation. Water and sodium retention are associated with the replacement of liquid electrolytes, which are crucial to the active lifestyle, reducing the likelihood of dehydration[89].

Since the interval between MVPA and BIS measurement is quite prolonged (12-24 h), it is expected that the physiological mechanisms associated with the homeostasis of water compartments will be triggered. Since the hypothalamus is responsible for the regulation of water levels, it will stimulate the release of antidiuretic hormone in order to retain as much water as possible and thus reestablish normal levels of hydration[28].

Another major factor is skin temperature. Cornish et. al. (1998) investigated the influence of temperature on skin impedance and, demonstrated that skin resistance and

reactance decreased by 35% and 18% within a temperature range of 20 ° C to 40 ° C 90. During sports, skin temperature rises due to increased muscle activity and skin perfusion, and after 15 minutes of rest, the temperature remains high[87].

As previously mentioned, the results of this study show that the performance of MVPA on the day before the measurement of the water compartments did not affect the validity with which the BIS estimated the TBW, ECW, ICW, against the reference method. As this is the first study to clarify the effect of MVPA in the 12-24 hours before the exam, and depending on the results, the relevance of the requirement of not exercising 12-24h before the BIS examination.

Limitations

Despite the encouraging results of this study, some limitations should be addressed. First, the size of our sample (n = 27) is reduced, which impossibilities to generalize the results to other populations.

Second, in the present study, the consumption of liquids and solids on the day before the assessment was not considered. A diet with a low intake of micronutrients leads to lower concentrations of electrolytes present in plasma which may affect the conductivity of the electrical current in the body.

Third, a distinction between time spent in moderate and vigorous intensity was not made and may affect the results of this study, especially if vigorous intensities are achieved. In the scope of the activities performed, if any of the participants had practiced muscular strength training, that would lead to a measurement error of the levels of physical activity intensity performed, since the accelerometer does not capture MVPA with additional loads

Fourth, the time of day (morning, lunch or afternoon) when MVPA was performed was not determined. If MVPA was performed in the morning participants would still have almost 24 hours of recovery before the assessment of body water compartments, however, if it was performed in the afternoon, the recovery time would be approximately 12 hours, and at night would be of 8 -10h.

Therefore, more studies are needed to clarify the impact that MVPA levels performed on the day before the assessment can have on the evaluation of water compartments.

5. Conclusions

This research demonstrated that preforming MVPA in the day before the BIS measurement does not affect the validity of TBW, ECW, and ICW obtained by BIS against dilution technique.

Therefore, these results may, question the relevance of avoiding exercise the day before performing the BIS test

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