AMERICAN UNIVERSITY OF BEIRUT

BIOELECTRICAL IMPEDANCE VECTOR ANALYSIS (BIVA) FOR ASSESSMENT OF HYDRATION STATUS: A COMPARISON BETWEEN ENDURANCE AND STRENGTH UNIVERSITY ATHLETES

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A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science to the Department of Nutrition and Food Sciences of the Faculty of Agricultural and Food Sciences at the American University of Beirut

> Beirut, Lebanon April 2024

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Date of thesis defense: April 8, 2024

ACKNOWLEDGEMENTS

First and foremost, I would like to express my sincere gratitude and appreciation to my advisor Dr. Elie-Jacques Fares for his continuous guidance and cooperation. His expertise profoundly contributed to the success of this thesis.

Special thanks to my committee members Dr. Lara Nasreddine, Dr. Afif Mufarrij, and Dr. Rédina Berkachy for their insightful comments, invaluable advice, and efforts.

I am deeply thankful for my beloved family members who continuously support me and believe in me. They made this remarkable journey possible.

I am also grateful to all the students who participated in my study for their time, effort, and contribution.

And to my friends, thank you for being there through the ups and downs. I would not have done it without you.

Finally, I would like to express my sincere gratitude to God for his blessings and unwavering strength, which have empowered me and guided me during the whole process, enabling me to persist with determination, consistency and hard work.

ABSTRACT

OF THE THESIS OF

Maria Roger Abdel Nour

for <u>Master of Science</u> <u>Major</u>: Nutrition

Title: <u>Bioelectrical Impedance Vector Analysis (BIVA) for Assessment of Hydration</u> <u>Status: A Comparison Between Endurance and Strength University Athletes</u>

Background: Adequate hydration is essential for athletes specifically before and after training, during competitions and in extreme conditions. Hydration assessment is performed using a variety of tools, and the combination of several techniques is recommended to accurately measure water losses and gains that greatly impact athletes' performance.

Aim: The aim of this study is to assess the validity of bioelectrical impedance vector analysis (BIVA) as a tool for measuring hydration status in endurance and strength athletes pre- and post- training by plotting vectors variation by gender and sport and comparing it to reference methods.

Methods: 148 athletes (n=64) endurance and (n=84) strength divided between (n=90) males: (n=54) strength, (n=36) endurance and (n=58) females: (n=29) strength, (n=29) endurance, were evaluated on one experimental day, pre and post training. Urine samples were collected in the morning, prior to and after training for analysis of color and specific gravity (USG). Body weight changes were measured, sweat rate was calculated, and bioelectrical impedance analysis was performed prior to and post training to track changes in bioelectrical variables (R = resistance; Xc = reactance; Z = impedance vector; and PA = phase angle). Reference ellipses were plotted using data of 200 healthy non-athletic individuals equally divided between males and females.

Results: A strong agreement was noted between raw bioelectrical values standardized for height: Xc/h, R/h and Z, PA with each of USG and sweat rate. (p>0.05) The sensitivity of classic BIVA in detecting minor changes in hydration status is confirmed both graphically and statistically. No significant difference was observed in urine specific gravity values at different timepoints. The distribution pattern of raw bioelectrical values before and after training did not significantly change in the studied groups of athletes. R/h and Z statistically significantly decreased post-training in both genders and sports types. Male athletes exhibited a specific BIA vector distribution in comparison with the reference population and were slightly more hydrated than female athletes.

Conclusion: Bioelectrical variables (R, Xc, Z, and PA) assessment is gaining significant attention for the purpose of monitoring hydration levels in both healthy and unwell individuals. The impact of hydration on sports performance is undeniable, necessitating the development of easy and practical monitoring methods to ensure athletes can consistently perform at their peak.

Keywords: bioelectrical impedance vector analysis, resistance, reactance, phase angle, urine specific gravity, hydration, athletes

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ABBREVIATIONS

UC: Urine Color BIVA: Bioelectrical Impedance Vector Analysis USG: Urine Specific Gravity PA: Phase Angle TBW: Total Body Water ECW: Extracellular Water ICW: Intracellular Water D2O: Deuterium Oxide **BIA: Bioelectrical Impedance Analysis** FFM: Fat Free Mass **R**: Resistance Xc: Reactance Z: Impedance PRE: Before **POST:** After CHSC: Charles Hostler Student Center CHDC: Charles Hosler Diet Clinic DXA: Dual-energy X-ray Absorptiometry ACSM: American College of Sports Medicine AUB: American University of Beirut **IRB:** Institutional Review Board EMPr: Endurance Males Pre **EMPo: Endurance Males Post** SMPr: Strength Males Pre SMPo: Strength Males Post EFPr: Endurance Females Pre EFPo: Endurance Females Post SFPr: Strength Females Pre SFPo: Strength Females Post

CHAPTER I INTRODUCTION AND LITERATURE REVIEW

A. Fluid balance in athletes

Water is the most essential nutrient for athletes. (Fink HH, Mikesky AE, 2013) The American College of Sports Medicine (ACSM) recommends the maintenance of adequate body hydration status before, during and after exercise to improve training capacity and performance, decrease the risk of heat illness and injury and to achieve euhydration. Fluid balance maintenance is crucial for body temperature regulation, cellular metabolism, cardiac function and exercise performance. Moreover, overhydration and excessive dehydration can result in fluid-electrolyte balance disturbance. Excessive dehydration leads to at least two percent body weight loss, negatively affecting performance. In terms of fluid imbalance, hypohydration is more common than hyperhydration. It may impair physical performance, specifically in endurance sport players, by increasing fatigue and perception of effort. It also increases the risk of injuries. Additionally, factors that affect hydration status include habitual fluid intake, exercise type, intensity and duration, sweat loss, sweat rate during exercise and environmental conditions. (Sports Nutrition Care Manual, 2023, Meyer, F., Kimberly, Volterman, K.A., Timmons, B.W., Wilk, B., 2012)

B. Total body water assessment

An adult's body mass is made up of 63% water distributed as follows: 30-35% in intracellular fluid, 20-25% in interstitial fluid and 5% in plasma. Total body water (TBW) can be measured using deuterium oxide (D2O) dilution, a safe, precise and one of the most accurate methods to monitor hydration status, however, it is costly and

requires tedious specimen preparation. (Barley OR, et al., 2020) Moreover, dual-energy X-ray absorptiometry (DXA) is a body composition method that gives information about TBW by measuring changes in lean body mass. (Achamrah, N., Colange, G., Delay, J., et al., 2018) However, its measurements in athletes are affected by exercise and food ingestion. Furthermore, a combination of alternative methods is recommended for assessment of hydration status to obtain accurate and valid results. Multiple methods (gross and body fluids) allow better understanding of the location of water retention in the body in the absence of direct assessment of intra- and extra- cellular hydration. (Barley OR, et al., 2020) Other commonly used methods include urine color, urine-specific gravity, osmolality, body weight changes, sweat rate and bioelectrical impedance (BIA). (Campa F, Toselli S, Mazzilli M, Gobbo LA, Coratella G., 2021)

C. Urinary indicators of hydration

During exercise, or shifts in body position, changes in total body water (TBW) may vary in different body fluid compartments, hence the importance of following standardized protocols to control for possible confounders when assessing hydration status and not solely relying on TBW. Urine color and specific gravity are two inexpensive, convenient, easy, fast collection and analysis methods and are minimally invasive compared to blood variables for hydration assessment.

1. Urine color

Urine color consists of subjectively evaluating urochrome in the urine and using a Likert scale. Pale color indicates higher excretion of water whereas dark color indicates less water excretion. On the other hand, urine color chart 1- to 8-level classifies

individuals as well (1 to 3) hydrated and having minimal (3-4), significant (5-6), and serious (>6) dehydration. (Casa, D. J., 2000)

2. Urine specific gravity

Urine specific gravity (USG) consists of placing a small volume of urine onto a refractometer, followed by comparison of the urine density to double distilled water (density = 1.000). As the volume of water decreases, the concentration of substances present in the urine increases. Several factors such as fluid intake, diet, alcohol, caffeine and/or illness may influence urine output. Urine collection several times per day rather than once in the morning may better reflect overall hydration status. Urine specific gravity can be performed using a handheld pocket refractometer. Values >1.020 are indicative of hypohydration and >1.030 of significant hypohydration. (Meyer, F., Kimberly, et al., 2012, Barley OR, Chapman DW, Abbiss CR., 2020) There is no conclusive evidence regarding the accuracy of urinary assessment tools use on their own. It is recommended to combine urinary measures with other methods due to the following factors: concentration-based assessments may be affected by the urinary excretion rate and urinary measures do not represent hydration status at the cellular level but are rather indicative of the renal response to fluid homeostasis.

Moreover, comparisons to baseline changes within the individual are preferred over single cut-off limits.

3. Urine osmolality

Urine osmolality of \leq 700 mOsmol kg-1 is indicative of euhydration, according to The American College of Sports Medicine. Plasma osmotic pressure increases in proportion to the decrease in total body water. Moreover, dehydration is indicated by a urine

osmolality over 700 mmol/kg. (Cheuvront, S.N., Sawka, M.N., 2006) Urine osmolality and urine specific gravity are considered more accurate than urine color. (Barley O.R, et al., 2020)

D. Blood indicators of hydration

Measurements of hemoglobin and hematocrit can be used to calculate changes in blood volume, cell volume, and plasma volume under controlled exercise conditions (temperature, posture). However, the transfer of fluid from ICW to the ECW space and renal retention mediate the recovery of serum osmolality, which lowers its sensitivity as a hypohydration indicator. Hence, USG is a better indicator of fluid deficit. The aforementioned hydration assessment methods are considered adequate for sports science but are not practical for hydration status monitoring during training because they are expensive, necessitate methodological control and analytical expertise. (Zubac, D., Marusic, U. & Karninčič, H., 2016)

E. Sweat rate

Changes in body mass before and after training are used to estimate the volume of sweat loss during training while correcting for fluid intake. Fluid intake is assessed by calculating the change in water bottle mass before and after training. External water sources, for example water fountains, may bias results.

One liter of sweat loss is estimated to be equivalent to one kilogram of body mass loss. However, limitations in sweat rate include the possible additional water losses from respiration or water produced from glycogen and fat substrate oxidation and the affected substrate oxidation from carbohydrate consumption during exercise.

However, the aforementioned potential errors are ignored in sweat rate calculation because of the minimal water losses they induce.

Factors that affect sweat rate include but are not limited to:

At the individual level (genetics, level of acclimatization, level of fitness, age); at the activity/sport level (intensity, intermittent vs continuous, land vs water, modality); at the environmental/external conditions level (humidity, temperature, clothing, solar radiation).

Exercise type plays an essential role: swimmers dissipate greater body heat through convection and conduction in water compared to runners. Additionally, heat exposure (going from cold to warm conditions) increases sweat rate. These factors are to be accounted for when adjusting volumes of fluid intake. (Meyer, F., et al., 2012)

F. Gross measures incorporation for hydration assessment

It is recommended to combine urinary measures with gross measures such as body mass, bioelectrical impedance analysis (BIA) and/or dual-energy X-ray absorptiometry. One advantage of their use is the alleviation of the issue of using fluid from one body compartment to predict whole body hydration status.

1. Body mass changes

Body mass changes are used to estimate whole body fluid loss in terms of volume, during exercise. It requires careful consideration of fluid, food intake and excretion. For accurate measurements, shorter time durations between measurements are essential. One gram of body mass is equal to 1 mL of water. (Barley O.R, et al., 2020)

2. Bioelectrical impedance analysis (BIA)

BIA is a doubly indirect practical quantitative body composition assessment method for estimation of TBW, intracellular and extracellular water content, in relation to body mass or fat-free mass. It allows quick and non-invasive assessment of TBW with an error of 1.5-2.5 kg. (Achamrah, N., Colange, G., Delay, J., et al., 2018) It is based on impedance and necessitates calibration against another method, deuterium oxide (D2O). BIA is commonly used at 50 kHz single frequency in humans and measurements are derived from regression equations, deemed to be inaccurate in the athletic population at the individual level. (Campa F, et al., 2021) On the other hand, BIA presents accurate measurements for body composition and fluid volume change tracking on a group-level in athletes. However, the model and make of BIA affect TBW measurements and hydration in fat free mass (FFM) is not well studied. (Cataldi D, Bennett JP, Quon BK, Liu YE, Heymsfield SB, Kelly T, Shepherd JA., 2022, Sports Nutrition Care Manual, 2023). BIA measurements may be affected by posture, electrolyte balance, skin temperature, food and alcohol ingestion, physical activity intensity and malnutrition and its accuracy is affected by body fluid volume changes and tonicity. (Barley, O.R., 2020)

G. Qualitative hydration assessment by phase angle (PA)

Alternatively, body composition assessment can be performed qualitatively by evaluating the bioelectrical phase angle (PA) calculated as the arctangent of Xc/R × $180^{\circ}/\pi$, where Xc is the reactance representing the delay in the flow of current measured as a phase shift and indicating dielectric properties, and R is the resistance representing the decrease in voltage indicating conductivity through ionic solutions. PA is expressed in degrees and is represented as the angle between impedance and the x-axis,

graphically. The phase angle is an indicator of the intra/extracellular water ratio and allows for the detection of acute dehydration when high values are recorded and acute hyperhydration or chronic dehydration when lower phase angle values are noted. In the athletic population, PA is likely higher in males than females. PA largely varies between individuals for the same sport type, however, it is unclear to what extent it differs between different types of sports. Interestingly, differences may be noted for the same sport when physical characteristics of the individual athlete differ. (Di Vincenzo O, Marra M, Scalfi L., 2019) Also, further research is needed to understand the relation of PA to performance levels and training. (Di Vincenzo O, Marra M, Scalfi L., 2019) PA ranges between 5 and 7 degrees in healthy individuals whereas in athletes it is estimated to be 9.5 degrees where it is used as an index of muscularity. (Campa, F., et al., 2021)

H. BIVA technique for hydration assessment in athletes

Another method that is being used for hydration assessment in athletes is the bioelectrical impedance vector analysis (BIVA), first utilized in 2007 and consisting of simultaneous evaluation of the raw parameters (R and Xc) by studying the spatial relationship between the two, known as impedance Z, displaying differences in hydration and body cell mass (BCM) and graphically plotted as a vector for soft tissue hydration. It is a qualitative measure of soft tissue that does not depend on body size. BIVA requires the performance of multiple measurements in a short period of time for accuracy and it allows for the easy classification and ranking of athletes' hydration, independent of body mass and equations. (Nwosu, AC et al. 2019, Tinsley GM, et al., 2020) BIVA is classified into two types: classic and specific. Classic BIVA consists of

the standardization of bioelectrical values by height H to account for inter-individual variations in conductor length (R/H, Xc/H), whereas specific BIVA consists of the standardization of values for height and cross-sectional areas of body segments (arms, waist, calf). (Stagi S, Silva AM, Jesus F, Campa F, Cabras S, Earthman CP, Marini E., 2022) Specific BIVA, first proposed in 2013, has been recently validated for assessment of body fat mass percentage changes in athletes. (Castizo-Olier J, Irurtia A, Jemni M, Carrasco-Marginet M, Fernández-García R, Rodríguez FA., 2018, Sandra de la, C. M., et al., 2021)

Several studies have confirmed the accuracy and replication of the BIVA technique in measuring body composition by comparing its results to the gold standard methods such as DXA and dilution technique for body hydration assessment during the competitive season in athletes. (Sandra de la, C. M., M^a Paz Redondo del Río, & Beatriz de, M. S., 2021)

Therefore, classic BIVA may detect TBW (variations mainly associated with vector length, Z), whereas specific BIVA correlates with fat mass or percentage fat mass (vector length, Zsp). Together, classic and specific BIVA take into account the PA, which makes it clear that they are both sensitive to intracellular water/extracellular water (ICW/ECW) ratio. Measurements of R and Xc provide a vector of length and direction (at 50 kHz). The length of the Z vector is inversely proportional to TBW, and the PA is a measure of tissue hydration status (Nwosu, AC et al. 2019, Stagi S., et al., 2022) However, a recent systematic review indicates that « classic BIVA » may not be reliable for detection of dehydration in athletes on an individual level. (Castizo-Olier J, et al., 2018)

Furthermore, the vector position within tolerance ellipses is evaluated using BIVA, specifically drawn for each population and reflecting the percentile in body composition parameters. Reference target zones can be obtained from tolerance ellipses, useful for identification of the individual athlete's profile by sport type and by competitive level. To date, a limited number of papers examined raw BIA variables in athletes. Hence, there are no definite conclusions on the variation of PA between sports types (endurance vs resistance training, recreational vs competitive sports) (Di Vincenzo O, Marra M, Scalfi L., 2019) making it necessary to further validate BIVA for different sports types and to design reference tolerance ellipses for athletes based on sport type, age, gender and ethnicity. (Castizo-Olier J, et al., 2018)

I. Sweat loss variation by gender and sport type

Endurance sports players have been studied more than high-intensity exercise players due to greater sweat loss during exercise. (Maughan, R.J. and Shirreffs, S.M., 2010) Moreover, male adult athletes have greater sweat losses compared to females, despite similar fluid consumption relative to body mass in both genders. (Suppiah, H. T., Ng, E. L., Wee, J., Taim, B. C., Huynh, M., Gastin, P. B., et al., 2021). Each method of hydration status assessment has its flaws, and the accuracy and validity of measures vary by situation. There is no single universally accepted method for hydration assessment in athletes. Sensitive and accurate hydration biomarkers must detect body water fluctuations of ~3% TBW. (Zubac, D., et al., 2016) Multiple measurements are necessary to increase validity and accuracy, reduce measurement error that results in incorrect categorization of hydration. (Barley, O.R., 2020)

Noting the importance of hydration in sports, in the absence of sufficient data and with the need to further validate hydration assessment techniques in athletes, this study will be the first of its kind to extensively explore, evaluate and plot phase angle variations by gender and sport type among athletes pre- and post- training and design reference tolerance ellipses for each. This study will be the first in the Middle East and North Africa Region to use classic BIVA techniques to assess hydration status pre and post training in athletes and compare it to reference methods.



Figure 1 Graphical representation and interpretation of the impedance vector Z by means of the plot R/H-Xc/H

R: resistance; Xc: reactance; H: height; BCM: body cell mass

Taken from Sandra de la, C. M., Mª Paz Redondo del Río, & Beatriz de, M. S. (2021)

J. Aim and specific objectives

The overall aim of this study is to assess the agreement between BIVA as a hydration monitoring tool and reference methods (urine specific gravity, urine color, sweat rate) in Lebanese strength and endurance university athletes pre and post training.

The specific objectives of this study are:

- (a) To assess and compare the hydration status of endurance and strength athletes preand post- training using different methods
- (b) To assess the sensitivity of classic BIVA technique in detecting minor changes in hydration by interpreting bioelectric variables Z, R and Xc distribution pattern in athletes pre and post training by gender and sport type
- (c) To design specific confidence and tolerance ellipses for the Lebanese athletic and non-athletic population

CHAPTER II METHODOLOGY

A. Study Design and Sampling

A pre-post quasi-experimental study was carried out between September and November 2023, among 148 AUB endurance and strength athletes. The sample size was calculated based on a 95% confidence level, 5% margin of error, 0.8 statistical power and 50% response rate. Athletes were divided into (n=64) endurance and (n=84) strength selected by convenience from the American University of Beirut's varsity teams and Charles Hostler Student Center (CHSC) gym respectively. The study also included 200 non-athletic individuals equally divided between males (n=100) and females (n=100) constituting the reference population, selected by convenience from CHDC database, to plot reference ellipses for the athletic population. The study included 348 individuals in total. Male athletes (n=90) were divided into (n=54) strength and (n=36) endurance and female athletes (n=58) were divided into (n=29) strength and (n=29) endurance, respectively. (Martins, P. C., Gobbo, L. A., & Silva, D. A. S., 2021, Sanz de la Garza, M., Adami, P.E., 2020)

1. Athletic population

Participants inclusion criteria: healthy adult between the ages of 18 and 35 years, part of AUB's varsity team athletes or weightlifter members at CHSC, not injured at the time of the study, training at least 3 times per week, females in a post-menstruation state with the ovarian cycle between days 5 to 11, not on contraceptives or drugs for menstrual cycle treatment, not pregnant or planning to become pregnant. Exclusion criteria: not

part of AUB's varsity teams, not weightlifters at CHSC, not being able to visit the clinic facility, injured/having health problems/previous surgeries that alter body composition (bariatric surgery), pregnant or on contraceptives.

2. Reference population

The reference population inclusion criteria: healthy adults aged 18-35 years and part of the AUB community, females in a post-menstruation state with the ovarian cycle between days 5 to 11, not on contraceptives or drugs for menstrual cycle treatment, not pregnant or planning to become pregnant. Exclusion criteria: not part of AUB's community, not being able to visit the clinic facility/having health problems or surgeries that alter body composition (bariatric surgery), pregnant or on contraceptives.

B. Recruitment

Participants were recruited by the investigating graduate student by direct approach to take part in this study. Individuals were approached while exercising at the gym and during training hours for varsity teams, in the afternoon depending on each team's training schedule for Fall 2023-2024. Physiotherapists personnel at CHSC and varsity teams' managers helped identifying potential participants suitable for the study. The student investigator approached team members before their training session and informed them about the study. Also, advertisements were used to recruit athletes and they were contacted through WhatsApp and email. Flyers were posted at CHSC gym, in the training rooms of varsity teams and in different faculties on campus. It included the email address and phone number of the student investigator to be contacted if they were interested to participate in the study. Eligibility for participation in the study was

determined by assessing if the individual meets the inclusion criteria through answering a series of questions posed by the student investigator in CHDC after obtaining consent, using the "eligibility questionnaire" (Refer to Appendix C). The asked questions were: Are you a healthy adult between the ages of 18 and 35 years? Do you have any medical problems/illnesses/allergies? Are you part of AUB's varsity teams or weightlifter members at CHSC? Are you injured? How often do you train? For females only: when was the last time you got your period? When do you expect to get it again? What is your normal menstruating cycle? Are you on contraceptives or drugs for menstrual cycle treatment? Are you pregnant or planning to become pregnant?

Personnel at CHSC helped determine if the individuals provided correct information regarding their age, the varsity teams they are part of, whether they are members of the gym and their injury state.

Participants who voluntarily consented to participate in the study were informed on the day of signing the consent form that they will be asked to present one week before the experiment and one day before the experiment, and they were allocated the exact time and day on which they were required to present to CHDC through outlook invitations scheduling to discuss the necessary steps prior to the experiment. If they chose to be reminded through WhatsApp, they had to consent to provide their phone number next to their signature in the consent form. Otherwise, they were reminded through e-mail. All arrangements and experimental procedures were performed by the student investigator.

C. Data Collection

The study took place during the Fall semester at CHDC located on the first floor in CHSC at the American University of Beirut. Measurements were taken on one experimental day, during two experimental sessions, one prior to training and the second after training, to assess hydration status of athletes.

1. Pre-hydration assessment questionnaire

One week prior to the experiment, a short questionnaire was administered to participants (Refer to appendix E) to gather information regarding their lifestyle and to provide them with the necessary recommendations that will help them maximize their readiness for the study. It included questions about demographics, medical history, physical activity level, type of sports and frequency, diet and anthropometry. The questionnaire provided the researcher with additional insight about the individual's medical, nutritional and health status, making it easier to select individuals based on their inclusion criteria. Furthermore, the following instructions were discussed to reach euhydration prior to the experiment: avoidance of caffeine and alcohol consumption 4 hours prior to the experiment, not exercising intensely 12 hours prior to the experiment and fasting 4 hours prior to the experiment. (Martins, P.C., Alves Junior, C. A., Silva, A.M., & Silva, D. A. S., 2023, Boykin, J. R., Tinsley, G. M., Harrison, C. M., Prather, J., Zaragoza, J., Tinnin, M., Smith, S., Wilson, C., & Taylor, L. W., 2021).

This questionnaire helped in recruiting participants based on the inclusion/exclusion criteria and it will provide the researcher with information about the medical, nutritional and health status of participants prior to the experiment to familiarize them with testing protocols and instructions.

On the day of the experiment, a data collection sheet was used (Refer to appendix F) to record the athlete's first name, middle name, last name, date, time, email address, participant #, phone number (optional) and the total training duration. Additional information that was recorded included the athlete's gender (male/female), date of birth, sport type, whether the eligibility questionnaire and pre-hydration assessment questionnaires were filled, the empty urine cups weight, the time of urine collections, height, body weight measurements, water bottle weight A (before) and after (B) training and whether all 4 BIA tests were performed (2 prior to training and 2 post-training).

2. Anthropometric measurements and body composition

Height was measured using a stadiometer (portable, SECA 213, Hamburg, Germany).Measurements were taken in an orthostatic position and barefoot on a platform.(Martins, P.C., Gobbo, L.A., Silva, D.A.S.A, 2021)

Body composition test was performed to obtain bioelectrical data: Resistance (R), R/h (where h is the height of the individual), reactance (Xc), Xc/h, phase angle (PA), impedance (Z), and extracellular water:total body water ratio (ECW:TBW) using the InBody 770 machine (Biospace, Los Angeles, CA, USA), at a 50-kHz single frequency (Carrasco-Marginet, M., Castizo-Olier, J., Rodríguez-Zamora, L., Iglesias, X., Rodríguez, F. A., Chaverri, D., Brotons, D., & Irurtia, A., 2017) BIA analysis was performed four times for athletes: twice prior to training and twice post training for accurate results. For the reference population, bioelectrical data was obtained once for each individual. Moreover, participants were also asked to remove any metals, earrings or rings during measurements. The following steps were followed for the BIA test: First, participants were asked to remove shoes, socks, heavy clothes, and items in pockets. Then, they were asked to wipe their hands and feet with an electrolyte tissue provided by InBody.

Second, they were asked to stand on the device barefoot and align heel with the round silver electrodes and the rest of the feet with the foot electrode while staying still and waiting for weight to be measured.

Third, after weight was measured, the student investigator entered the age, height, gender and a unique ID for participants to track the difference in measurements before and after training.

Fourth, participants were then asked to grab the hand electrodes by placing their thumbs on the thumb electrodes and wrapping their fingers around the bottom electrodes, keeping their arms relaxed and extended slightly away from the torso so that their armpits are not touching one another (around 15 degrees).

The InBody Test took 60 seconds and results were automatically shown on the connected PC screen. Results were saved in a folder which allowed for comparison of measurements before and after training. Confidence and tolerance ellipses were then created based on BIVA values. (Martins, et al., 2021)

3. Urine collection

Urine cups labeled 1, were provided to athletes at CHDC one day prior to the experiment. Athletes were instructed on proper midstream voiding and the student investigator filled information regarding the date and time on the data collection sheet.

(Davy, K., Davy, B., Neilson, A.P., 2017). They were instructed to void in the morning in cup 1 and to handle it back as soon as they arrive to the university. Urine cups were weighed empty prior to handling to athletes and the empty weight was saved as a reference. Cups were labeled using numbers and letters, for example: the first morning cup labeled 1 as B001_ELIFAR (first 3 letters from the first and last name of the individual) for the only reason of not mixing the individuals' cups prior to analysis. Once the urine inside each cup was analyzed, the label was discarded and cups were emptied and were only accessed by the student investigator.

Moreover, on the day of the experiment, each athlete was given another urine cup labeled 2 after handling the cup labeled 1, and they were asked to void in the cup labeled 2 right before training. Right after the training session, they were asked to void in a cup labeled 3 and give it back immediately.

After collection, samples were analyzed in Agr 517 Lab, FAFS, AUB, for color scoring and urine specific gravity measurements.

a. Urine color scoring (UC)

Urine color chart 1- to 7-level was used to classify individuals as hydrated (1), well hydrated (2), very well hydrated (3), extremely hydrated (4), mildly dehydrated (5), dehydrated (6), severely dehydrated (7) (Armstrong, 2000) (Refer to appendix A).

b. Urine specific gravity (USG)

Urine specific gravity refractometer (Pen–Urine S.G., Atago, Tokyo, Japan) was used to measure urine specific gravity. Urine specific gravity values were categorized as follows based on the National Athletic Trainers' Association position statement: Fluid replacement for athletes": <1.010 well hydrated, 1.010-1.020 minimal dehydration, 1.021-1.030 significant dehydration, >1.030 serious dehydration. (D.J. Casa et al., 2000)

4. Sweat rate

Sweat rate was assessed by calculating body mass changes. Pre-exercise body mass were measured after voiding, and fluid intake was tracked after this measurement. Postexercise body mass was taken before voiding and the following equation was used:

Whole body sweating rate (WBSR) = [Body massPre-Ex - (Body massPost-<math>Ex - fluid intakeEX) / exercise duration (Baker, 2017)

Body weight was measured individually before and after training using the BIA machine right after handling urine cup 2 prior to training. They were asked to remove their shoes and heavy clothes, stand with their feet on the center of the scale and remove accessories. For the measurement of post-training weight, the same conditions were applied, after voiding in the third urine cup and without sweaty clothes.

Moreover, sweat rate analysis necessitates the collection of volume of fluid consumed (in liters) during training, therefore, each athlete's water bottle was weighed before and after training and the weight difference was recorded using a calibrated scale. Furthermore, the total training duration in hours was obtained from the athlete and training schedules.



Figure 2 Summary of the experimental procedure

D. Ethical considerations

Participation in this study was voluntary, and the individual had the right to terminate his participation at any point during the experiment.

Individuals who agreed to participate signed the informed consent provided by the student investigator at CHDC and approved by the American University of Beirut's Institutional Review Board (IRB), BIO-2023-0121. The consent form includes a brief description of the study with a highlight on what will be required from the participants and the time frame of the study. All rights and privacy are preserved by signing the consent form.

Also, participants' privacy is protected by keeping the responses in this study confidential, storing the data in a password-protected computer, presenting research report findings on a group basis without any personally identifying information. Participants were informed about the protection of their privacy in the consent form.

The data was stored as coded, on a password-protected computer for 3 years that only researchers will have access to and destroyed 3 years later.

The privacy of participants was maintained by only having the participant and student investigator present in the room at the time of asking questions and noting their answers.

E. Data Analysis

To assess hydration status, classic BIVA was used and compared to the following methods: urine color, urine specific gravity, sweat rate and phase angle R statistical software (version 4.2.3, R Foundation for Statistical Computing) was used to generate Bland- Altman plots, boxplots and to perform linear regression. Statistical Package for the Social Sciences (SPSS, version 25) was used for Spearman correlation test for urine color and raw bioelectrical parameters. Descriptive statistics were also generated for urine specific gravity. Multivariate repeated measures ANOVA were performed for USG morning, USG PRE, USG POST between the 4 sports groups. A one-sample paired Hotelling's T-test was used to analyze pre- to post- training changes in the vector through the 95% confidence ellipses. Two-sample Hotelling's T-Test was used to determine the BIA vector differences between sports groups and the reference population by studying multivariate raw BIA data. Welch two sample T-Test was used to compare univariate BIA data between sports groups and reference groups. P < 0.05

was considered significant. Point graphs and R-Xc mean graphs were performed using the BIVA 2002® software (Microsoft, Padova, Italy). All bioelectrical impedance raw values were standardized by height (meters).

CHAPTER III

RESULTS

Bland-Altman was used for assessment of agreement between BIVA and reference hydration assessment methods (urine specific gravity and sweat rate). The difference between measurements of two standardized datasets is calculated, followed by a onesample independent T-test. Pre- and post-training values of USG and sweat rate were compared with raw bioelectrical impedance raw values Z, Xc and R standardized for height, respectively. All T tests have a p-value of 1 strongly indicating no statistical difference between the two compared measurements. Moreover, the majority of data points fall between the 95% upper and lower limits of agreement indicating a high level of agreement between techniques. The mean difference of 0 is shown in the plots below suggesting no systematic bias. No proportional bias is noted since all plots have approximately equal numbers of data points above and below the mean difference line compared to below the line. Results are further explained in the figures 3-10 below.



Figure 3 Comparison of urine specific gravity before training (USG PRE) and raw bioelectrical value impedance Z using Bland-Altman Plot

The Bland-Altman plot is shown in Figure 3. A linear regression is performed. The model coefficients are carefully studied. Given that the statistical null hypothesis is that there is no proportional bias, the model coefficients should be close to 0, particularly the slope of the line to not reject the null hypothesis and indicate that there is no trend between the differences and the means. In the case of USG PRE vs. Z PRE, the slope of the regression line is 7.326e-17 with a standard error of 0.191. In addition, the t-score of the model is not significant at the 5% level of significance with a p-value of 1, confirming the conclusion of no particular trend or no significant proportional bias between the differences and the means. The independent t-test between the distributions: t = 7.0808e-14, df = 140, p-value = 1. The null hypothesis of this test is that the mean of the difference distribution is equal to zero. Since the p-value is equal to 1, this means that this null hypothesis strongly cannot be rejected, e.g. that the mean of the distribution of differences is not significantly different than zero.


Figure 4 Comparison of urine specific gravity before training (USG PRE) and raw bioelectrical value resistance (R) using Bland-Altman Plot

The Bland-Altman plot is shown in Figure 4. The linear regression performed for USG PRE and R PRE has a slope of 8.662e-15 with a standard error of 5.492e-16. In addition, the t-score of the model is not significant at the 5% level of significance with a p-value of 1, confirming the conclusion of no particular trend or no significant proportional bias between the differences and the means. The independent t-test between the distributions: t = 6.9219e-14, df = 140, p-value = 1. The null hypothesis of this test is that the mean of the difference distribution is equal to zero. Since the p-value is equal to 1, this means that this null hypothesis strongly cannot be rejected, e.g. that the mean of the differences is not significantly different than zero.



Figure 5 Comparison of urine specific gravity before training (USG PRE) and raw bioelectrical value reactance Xc using Bland-Altman Plot

The Bland-Altman plot is shown in Figure 5. The linear regression performed for USG PRE and Xc PRE has a slope of -2.306e-16 with a standard error of 0.196. In addition, the t-score of the model is not significant at the 5% level of significance with a p-value of 1, confirming the conclusion of no particular trend between the differences and the means. The independent t-test between the distributions: t = 6.3817e-14, df = 140, p-value = 1. The null hypothesis of this test is that the mean of the difference distribution is equal to zero. Since the p-value is equal to 1, this means that this null hypothesis strongly cannot be rejected, e.g. that the mean of the distribution of differences is not significantly different than zero.



Figure 6 Comparison of urine specific gravity before training (USG PRE) and phase angle before training (PA PRE) using Bland-Altman Plot

The Bland-Altman plot is shown in Figure 6. The linear regression performed for USG PRE and PA PRE has a slope of 8.638e-15 with a standard error of 0.161. In addition, the t-score of the model is not significant at the 5% level of significance with a p-value of 1, confirming the conclusion of no particular trend between the differences and the means. The independent t-test between the distributions: t = 7.4856e-14, df = 140, p-value = 1. The null hypothesis of this test is that the mean of the difference distribution is equal to zero. Since the p-value is equal to 1, this means that this null hypothesis strongly cannot be rejected, e.g. that the mean of the distribution of differences is not significantly different than zero.



Figure 7 Comparison of sweat rate and phase angle before training (PA PRE) using Bland-Altman Plot

The Bland-Altman plot is shown in Figure 7. The linear regression performed for Sweatrate and PA PRE has a slope of -1.060e-16 with a standard error of 0.152. In addition, the t-score of the model is not significant at the 5% level of significance with a p-value of 1, confirming the conclusion of no particular trend between the differences and the means. The independent t-test of the distribution of differences is t=3.9545e-15, df=146, p-value=1. The null hypothesis of this test is that the mean of the difference distribution is equal to zero. Since the p-value is equal to 1, this means that this null hypothesis strongly cannot be rejected, e.g. that the mean of the distribution of differences is not significantly different than zero.



Figure 8 Comparison of sweat rate and raw bioelectrical value resistance (R) using Bland-Altman Plot

The Bland-Altman plot is shown in Figure 8. The linear regression performed for Sweatrate and R PRE has a slope of 4.969e-16 with a standard error of 0.189. In addition, the t-score of the model is not significant at the 5% level of significance with a p-value of 1, confirming the conclusion of no particular trend between the differences and the means. The independent t-test of the distribution of differences is t=-2.1434e-15, df=146, p-value=1. The null hypothesis of this test is that the mean of the difference distribution is equal to zero. Since the p-value is equal to 1, this means that this null hypothesis strongly cannot be rejected, e.g. that the mean of the distribution of differences is not significantly different than zero.



Figure 9 Comparison of sweat rate and raw bioelectrical value impedance (Z) using Bland-Altman Plot

The Bland-Altman plot is shown in Figure 9. The linear regression performed for Sweatrate and Z PRE has a slope of -1.966e-16 with a standard error of 0.189. In addition, the t-score of the model is not significant at the 5% level of significance with a p-value of 1, confirming the conclusion of no particular trend between the differences and the means. The independent t-test of the distribution of differences is t=3.8972e-16, df=146, p-value=1. The null hypothesis of this test is that the mean of the difference distribution is equal to zero. Since the p-value is equal to 1, this means that this null hypothesis strongly cannot be rejected, e.g. that the mean of the distribution of differences is not significantly different than zero.



Figure 10 Comparison of sweat rate and raw bioelectrical value reactance (Xc) using Bland-Altman Plot

The Bland-Altman plot is shown in Figure 10. The linear regression performed for Sweatrate and Xc PRE has a slope of -2.063e-16 with a standard error of 0.182. In addition, the t-score of the model is not significant at the 5% level of significance with a p-value of 1, confirming the conclusion of no particular trend between the differences and the means. The independent t-test of the distribution of differences is t=-2.5022e-15, df=146, p-value=1. The null hypothesis of this test is that the mean of the difference distribution is equal to zero. Since the p-value is equal to 1, this means that this null hypothesis strongly cannot be rejected, e.g. that the mean of the distribution of differences is not significantly different than zero.







The x-axis of the boxplot represents the variable urine color (UC) and the y-axis represents the phase angle (PA). Urine color is divided into 7 categories: hydrated, well hydrated, very well hydrated, extremely hydrated, mildly dehydrated, dehydrated, severely dehydrated. The hydrated group has a median PA of 6.3 with two outliers: one exceeding the upper limit of PA value 7.3 and the other below the lower limit of 5.2. Negative skewness in noted in the hydrated group indicating that most values were below the median of 6.3.

The well hydrated group has a median PA of 6.6 and it is negatively skewed with an upper band of 8.3 and a lower band below 5. The very well hydrated group has a median PA of 6.8 and it is negatively skewed with an upper band of 7.8 and a lower band of 4.8. The extremely hydrated group has a median PA of 6.1 and it is negatively skewed with an upper band of 7.4 and a lower band below 5. The mildly dehydrated group has a median of 5.3 and it is positively skewed with an upper band of 6.5 and a lower band of 5. The dehydrated group has a median of 6.5 and it has no skewness with

an upper band of 7.8 and a lower band of 5.4. The severely dehydrated group has a median of 5.2 and it has no skewness. There is great variability in the median PA between different urine color groups, with the greatest PA observed in the well hydrated followed by the very well hydrated and extremely hydrated groups, respectively. However, the well hydrated and dehydrated groups have very close median PA values of 6.6 and 6.5.



Figure 12 Box and whisker plots for bioelectrical raw value impedance Z and urine color (UC) before training in the athletic population

The x-axis of the boxplot represents the variable urine color (UC) and the y-axis represents the impedance (Z). The hydrated group has a median Z of 315 (Ω /m). It has an upper limit of 510 (Ω /m) and a lower limit of 225 (Ω /m) and it is positively skewed. The well hydrated group has a median Z of 325 (Ω /m). It has an upper limit of 505 (Ω /m) and a lower limit of 240 (Ω /m) and it is positively skewed. The very well hydrated group has a median Z of 300 (Ω /m). It has an upper limit exceeding 500 (Ω /m) and a lower limit of 210 (Ω /m) and it is positively skewed. The extremely hydrated group has a median Z of 340 (Ω /m). It has an upper limit of 500 (Ω /m) and a lower limit of 250 (Ω /m) and it is slightly positively skewed. The mildly dehydrated group has a median Z of 320 (Ω /m). It has an upper limit of 450 (Ω /m) and a lower limit of 310 (Ω /m) and has no skewness. The dehydrated group has a median Z of 290 (Ω /m). It has an upper limit of 405 (Ω /m) and a lower limit of 240 (Ω /m) and it is positively skewed. The severely dehydrated group has a median Z of 450 (Ω /m). None of the groups have illustrated outliers. However, the severely dehydrated group has missing bands which might be explained by outliers. There is great variability in the median Z between different urine color groups, with the greatest impedance observed in the very well hydrated followed by the hydrated and extremely hydrated groups respectively. However, the hydrated, very well hydrated and dehydrated groups have very close median Z values.



Figure 13 Box and whisker plots for bioelectrical raw value resistance (R) and urine color (UC) before training in the athletic population

The x-axis of the boxplot represents the variable urine color (UC) and the y-axis represents the resistance (R). The hydrated group has a median R of 315 (Ω). It has an upper limit of 510 (Ω) and a lower limit of 225 (Ω) and it is positively skewed. The well hydrated group has a median R of 320 (Ω). It has an upper limit of 500 (Ω) and a lower limit of 240 (Ω) and it is positively skewed. The very well hydrated group has a median R of 305 (Ω). It has an upper limit exceeding 500 (Ω) and a lower limit of 210 (Ω) and it is positively skewed. The extremely hydrated group has a median R of 350 (Ω). It has an upper limit of 500 (Ω) and a lower limit of 250 (Ω) and it is slightly positively skewed. The mildly dehydrated group has a median R of 350 (Ω). It has an upper limit of 450 (Ω) and a lower limit of 310 (Ω) and has no skewness. The dehydrated group has a median R of 300 (Ω). It has an upper limit of 410 (Ω) and a lower limit of 240 (Ω) and is positively skewed. The severely dehydrated group has a median Z of 440 (Ω). Among all groups, only the dehydrated group has one outlier exceeding the upper limit. There is great variability in the median R between different urine color groups, with the greatest resistance observed in the very well hydrated followed by the hydrated, equally in the extremely hydrated and well hydrated groups, respectively. However, the hydrated and very well hydrated groups have very close median Z values.

Boxplot of the Xc PRE and UC PRE



Figure 14 Box and whisker plots for bioelectrical raw value reactance (Xc) and urine color (UC) before training in the athletic population

The x-axis of the boxplot represents the variable urine color (UC) and the y-axis represents the reactance (Xc). The hydrated group has a median Xc of 34 (Ω). It has an upper limit of 47 (Ω) and a lower limit below 30 (Ω) and it is positively skewed. The well hydrated group has a median Xc of 39 (Ω). It has an upper limit of 46 (Ω) and a lower limit below 30 (Ω) and it is negatively skewed. The very well hydrated group has a median Xc of 37 (Ω). It has an upper limit of 46 (Ω) and a lower limit of 29 (Ω) and it is positively skewed. It has one outlier below the lower limit. The extremely hydrated group has a median Xc of 37 (Ω). It has an upper limit of 46 (Ω) and a lower limit of 29 (Ω) and it is slightly positively skewed. It has two outliers: one above the upper limit and one below the lower limit. The mildly dehydrated group has a median Xc of 35 (Ω). It has an upper limit of 46 (Ω) and a lower limit of 46 (Ω) and is slightly negatively skewed. The dehydrated group has a median Xc of 36 (Ω). It has an upper limit of 43 (Ω) and a lower limit below 30 (Ω) and is negatively skewed. It has one outlier above the upper limit. The severely dehydrated group has a median Z of 40 (Ω) and is evenly distributed with no upper and lower bands. There is great variability in the median Xc between different urine color groups, with the greatest reactance observed in the hydrated followed by the well hydrated and extremely hydrated groups, respectively. However, the very well hydrated, extremely hydrated and dehydrated groups have similar Xc values.

Table 1 Spearman's correlation for urine color (UC PRE) and raw bioelectrical values Xc, R and Z each noted as average PRE in the athletic population.

Correlations								
			UC_PRE	average PRE	average PRE	average PRE		
Spearman's rho	UC_PRE	Correlation Coefficient	1.000	.012	.058	.057		
		Sig. (2-tailed)		.888	.494	.498		
		Ν	142	142	142	142		
Х	C average PRE	Correlation Coefficient	.012	1.000	.746**	.749**		
		Sig. (2-tailed)	.888		.000	.000		
		Ν	142	148	148	148		
	R average PRE	Correlation Coefficient	.058	.746**	1.000	1.000**		
		Sig. (2-tailed)	.494	.000		.000		
		Ν	142	148	148	148		
	Z average PRE	Correlation Coefficient	.057	.749**	1.000**	1.000		
		Sig. (2-tailed)	.498	.000	.000			
		Ν	142	148	148	148		

**. Correlation is significant at the 0.01 level (2-tailed).

The strength of the relationship between urine color and each of the Xc, R and Z raw values is examined using Spearman's rho correlation. None of the raw values were significantly correlated with urine color because these correlations are low (<0.75)

Table 2 Mean, median and standard deviation of USG in the morning (USG_M), before training (USG_PRE) and after training (USG_POST) for strength males (sport 1), endurance males (Sport 2), strength females (sport 1), and endurance females (sport 2)

Clanotico							
Gender	Sport			USG_M	USG_PRE	USG_POST	
male	1	Ν	Valid	54	53	53	
			Missing	0	1	1	
		Mean		1.020865	1.017159	1.016575	
		Median		1.021100	1.016800	1.016200	
		Std. De	viation	.0078826	.0082862	.0091917	
	2	Ν	Valid	36	34	36	
			Missing	0	2	0	
		Mean		1.019469	1.016596	1.017628	
	Median			1.019900	1.015500	1.016300	
		Std. De	viation	.0076917	.0092746	.0081198	
female 1		Ν	Valid	29	27	29	
			Missing	1	3	1	
		Mean		1.017466	1.014887	1.014226	
		Median		1.017400	1.015000	1.011600	
		Std. De	viation	.0088995	.0085637	.0097921	
	2	Ν	Valid	28	28	28	
			Missing	0	0	0	
		Mean		1.019300	1.016948	1.016348	
		Median		1.018350	1.018800	1.018350	
		Std. De	viation	.0068451	.0089406	.0091557	

Statistics

Table 3 Multivariate repeated measures ANOVA performed for USG morning, USG PRE, USG POST between the 4 sports groups: strength males, endurance males, strength females and endurance females

							Partial Eta
Effect		Value	F	Hypothesis df	Error df	Sig.	Squared
usg	Pillai's Trace	.129	2.371 ^b	2.000	32.000	.110	.129
	Wilks' Lambda	.871	2.371 ^b	2.000	32.000	.110	.129
	Hotelling's Trace	.148	2.371 ^b	2.000	32.000	.110	.129
	Roy's Largest Root	.148	2.371 ^b	2.000	32.000	.110	.129

Multivariate Tests^a

a. Design: Intercept

Within Subjects Design: usg

b. Exact statistic

The null hypothesis of this test is that there is no statistically significant differences between the means of USG morning, USG PRE and USG post between the 4 sports groups.

A p-value of 0.110 indicates that there is not sufficient evidence to reject the null hypothesis at a significance level (e.g., 0.05). There is no statistically significant difference in urine specific gravity values among the conditions (pre-training, post-training, morning).

The partial eta squared value of 0.129 indicates a moderate effect size.

	PRE	POST
	Mean ± 1	Error term
Gender = 1 (Male)		
R/h	296.49 ± 6.84	291.65 ± 6.51
Xc/h	34.93 ± 0.80	34.44 ± 0.80
Z	298.58 ± 6.86	293.73 ± 6.53
РА	6.74 ± 0.11	6.76 ± 0.12
Gender = 2 (Female)		
R/h	426.07 ± 12.70	422.09 ± 12.86
Xc/h	40.62 ± 1.12	40.44 ± 1.21
Z	428.06 ± 12.71	424.08 ± 12.87
РА	5.48 ± 0.15	5.50 ± 0.16

Table 4 Comparison of raw bioelectrical values (R, Xc, Z, PA) pre and post training in athletes by gender

Sport = 1 (Weightlifting)		POST
R/h	344.58	± 17.71
Xc/h	36.91 ± 1.10	
Z	346.62 ± 17.71	339.94 ± 17.76
PA	6.28 ± 0.19	36.30 ± 1.13
Sport = 2 (Endurance)		341.95 ± 17.76
R/h	351.68 ± 16.34	6.26 ± 0.19
Xc/h	37.51 ± 1.15	
Z	353.73 ± 16.34	347.35 ± 16.30
РА	6.20 ± 0.19	37.48 ± 1.20
		349.44 ± 16.30
		6.27 ± 0.20

Table 5 Comparison of raw bioelectrical values (R, Xc, Z, PA) pre and post training in athletes by sports type

	Delta-value	Delta-value /h	Paired-t-test	P-value	Cohen's d
	Mean ± E	rror term			
Gender = 1 (Male)					
R/h	-4.50 ± 1.20	-1.32 ± 0.34	7.38	1.1117e-12***	0.799
Xc/h	$\textbf{-0.37} \pm 0.20$	-1.00 ± 0.55	3.53	0.000549***	0.46
Z	-4.51 ± 1.21	-1.32 ± 0.34	7.33	1.4247e-11***	0.7957
PA	0.02 ± 0.02	0.32 ± 0.35	-1.82	0.07*	0.116
Gender = 2 (Female)					
R/h	-4.84 ± 1.27	-1.57 ± 0.42	7.54	3.995e-11***	0.437
Xc/h	-0.49 ± 0.22	-1.36 ± 0.62	4.34	3.794e-05***	0.118
Z	-4.85 ± 1.28	-1.57 ± 0.42	7.51	4.665e-11***	0.433
PA	$0.01\pm\ 0.03$	0.23 ± 0.42	-1.04	0.27	0.202
Sport = 1 (Weightlifting)					
R/h	$\textbf{-3.99}\pm2.40$	$\textbf{-0.94} \pm 0.57$	3.33	0.001527***	0.791
Xc/h	$\textbf{-0.17} \pm 0.39$	-0.45 ± 1.01	0.9	0.37	0.616
Z	-3.98 ± 2.42	$\textbf{-0.94} \pm 0.57$	3.3	0.001677***	0.792
PA	0.03 ± 0.03	0.46 ± 0.63	-1.54	0.13	0.179
Sport = 2 (Endurance)					
R/h	-4.64 ± 1.27	$\textbf{-1.40}\pm0.38$	7.25	1.956e-10***	0.475
Xc/h	-0.62 ± 0.22	-1.70 ± 0.60	5.65	2.202e-07***	0.0212

Table 6 Comparison of raw bioelectrical values (R, Xc, Z, PA) pre and post training in athletes by gender and sport type

Z	-4.67 ± 1.28	$\textbf{-1.39}\pm0.39$	7.26	1.896e-10***	0.468
PA	-0.02 ± 0.02	-0.32 ± 0.40	1.64	0.11	0.541

In tables 4 and 5, the first column represents the studied raw bioelectrical variables and the different categorizations by gender and sports type. The second and third columns represent mean values for each of the studied variables with the standard error and confidence interval, pre and post-training, respectively. In all the athletic population, in males and females, and in the endurance sports group, R/H, Xc/H, Z decreased post-training whereas PA slightly increased. In the strength group all raw bioelectrical values, including PA decreased post-training. In table 6, the delta-value for the difference between post-pre for all variables indicates minimal changes if not nonnegligible. The delta-value/h standardized by height has similar to the delta-value. Moreover, the paired-t-test performed between pre and post variables has Corresponding p-values indicating statistically significant difference observed for all raw bioelectrical variables R/H, Xc/H, Z and PA in the athletic population as a whole. In the male group, a statistically significant difference was noted for all except for PA with a p-value 0.2771 exceeding 0.05. In the female group, R/H and Z were statistically significantly different post-training whereas Xc/H and PA were not, with pvalues of 0.3713 and 0.1293 respectively.

In strength athletes, all were statistically significantly different except for PA with a p-value of 0.1054. In endurance athletes, all were statistically significant, except for Xc/H with a p-value of 0.8669. PA distribution pre and post training is statistically strongly significant, with a value of 6.272e-05, noting that the complete dataset differences are significant at the 10% level, and PA is not statistically significantly different post-training for males, females and for strength athletes.

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Cohen's d values, which measure the effect size, are shown in the seventh column indicate that the difference between pre and post is negligible for PA in the athletic population as a whole, in the male group for PA, in the female group for Xc/h and PA, in the strength group for PA and in the endurance group for Xc/H since all these groups have a Cohen's value of 0.2 or less, with a small effect size. Hence, the statistically non-significant difference in these groups is confirmed.



Figure 15 Point graph for individual and mean impedance vectors of endurance male athletes before training plotted on the 50%, 75% and 95% tolerance ellipses of the healthy Lebanese male reference population



Figure 16 Point graph of individual and mean impedance vectors of endurance male athletes after training plotted on the 50%, 75%, and 95% tolerance ellipses of the healthy Lebanese male reference population



Figure 17 Point graph of individual and mean impedance vectors of strength male athletes before training plotted on the 50%, 75% and 95% tolerance ellipses of the healthy Lebanese male reference population



Figure 18 Point graph of individual and mean impedance vectors of strength male athletes after training plotted on the 50%, 75% and 95% tolerance ellipses of the healthy Lebanese male reference population



Figure 19 Point graph of individual and mean impedance vectors of endurance female athletes before training plotted on the 50%, 75% and 95% tolerance ellipses of the healthy Lebanese female reference population



Figure 20 Point graph of individual and mean impedance vectors of endurance female athletes after training plotted on the 50%, 75% and 95% tolerance ellipses of the healthy Lebanese female reference population



Figure 21 Point graph of individual and mean impedance vectors of strength female athletes before training plotted on the 50%, 75% and 95% tolerance ellipses of the healthy Lebanese female reference population



Figure 22 Point graph of individual and mean impedance vectors of strength female athletes after training plotted on the 50%, 75% and 95% tolerance ellipses of the healthy Lebanese female reference population



Figure 23 R-Xc mean graph showing the 95% confidence ellipses for the mean impedance vectors of endurance female athletes before training, strength female athletes before training and the healthy female reference population



Figure 24 R-Xc mean graph showing the 95% confidence ellipses for the mean impedance vectors of endurance female athletes after training, strength female athletes after training and the healthy female reference popoulation



Figure 25 Mean vector displacements of endurance and strength female athletes from preto post- training

Paired one sample Hotelling's T-Test was performed for each of the endurance and strength female athletic groups to evaluate mean BIA vector displacement from pre- to post-training. Endurance females are represented by the green ellipse and strength females are represented by the red dot located at the center of the graph. The vector of the mean difference for endurance females is located near the origin of the RXc graph and is slightly shifted to the right. There is no statistically significant difference between the means of BIA vectors for endurance (T = 17.3) (p=0.77) and strength (T = 11.8) (p=0.64) female athletes from pre- to post-training. (Both p-values are greater than 0.05)



Figure 26 R-Xc mean graph showing the 95% confidence ellipses for the mean impedance vectors of endurance male athletes before training, strength male athletes before training and the healthy male reference population



Figure 27 R-Xc mean graph showing the 95%. confidence ellipses for the mean impedance vectors of endurance male athletes after training, strength male athletes after training and the healthy male reference population



Figure 28 Mean vector displacements of endurance and strength male athletes from pre- to post- training

Paired one sample Hotelling's T-Test was performed for each of the endurance and strength male athletic groups to evaluate mean BIA vector displacement from pre- to post- training. Endurance males are represented by the purple ellipse and strength males are represented by the orange ellipse. The vector of the mean difference is located near the origin of the RXc graph for both endurance and strength male athletic groups and are slightly shifted to the right. There is no statistically significant difference between the means of BIA vectors for endurance (T = 22.7) (p=0.79) and strength (T = 43.7) (p=0.9) male athletes from pre- to post- training. (Both p-values are greater than 0.05)

Xc/H + R/H+ Z/H + PA (PRE)	Two sample Hotelling's T^2 test		Paired one sample Hotelling's T-test
All athletes and	T=18.2	P-value=8.79e-05***	
reference population			
Male athletes and	T=18.2	P-	
male reference		value=0.001775***	
population			
Female athletes and	T=4.8	P-value=0.3208	
female reference			
population			
Strength athletes	T=24.1	P-	
and general athletic		value=0.000179***	
population			
Endurance athletes	T=11.5	P-value=0.02721***	
and general athletic			
population			
Female endurance			Endurance females:
athletes and female			T=17.3; p=0.77
strength athletes			Strength females: T=11.8;
pre-post training			p=0.64
Male endurance			Endurance males:
athletes and male			T=22.7; p=0.79
strength athletes			Strength males: T=43.7;
pre-post training			p=0.9

Table 7 Comparison of mean BIA vector between different groups

Table 7 shows the obtained test statistic and p-value from Hotelling's T-test to compare hydration status differences using BIVA in a multivariate space. The assumptions of this test are tested and considered as met. The considered model is Xc/H pre + R/H pre + Z/H pre + PA pre ~ Group (X or Y). The tested null hypothesis is that there is no statistically significant difference between the means of the athletic and reference population. The tested alternative hypothesis is that there is a statistically significant difference between the means of the athletic and reference population. A statistically significant difference in the mean BIA vector of the athletic population and male athletic population in comparison to the reference population is noted. (p-value<0.05 noted by ***). The female athletic population's mean BIA vector distribution is not statistically significantly different than that of the reference population. (p-value= $0.3208 \Leftrightarrow 0.05$) Both strength and endurance athletes have a different mean vector distribution than the general population, stronger for the strength population (p=0.000179) Additionally, no statistically significant difference is noted in the vector shift from pre- to post- training in the same group comparison for endurance females, strength females, endurance males, strength males. (p-value>0.05)

	Welch's Two Sample T-Test					
Groups	Z/H	R/H	Xc/H	PA		
Endurance	T=94.707	T=1.1654	T=-0.6267	T=-1.8205		
females vs	df=100.05	df=72.47	df=65.93	df=42.21		
reference	p<2.2e-16***	p=0.2477	p=0.533	p=0.0758		
females	05% CI:	05% CI:	05% CI:	05% CI:		
	[417.46, 435.32]	[-7.38,28.18]	[-2.40,1.25]	[-0.45,0.02]		
	x: 430.55	x: 424.95	x: 39.34	x: 5.31		
	ÿ: 4.16	ÿ: 414.55	ÿ: 39.91	ÿ: 5.52		
Strength	T=94.642	T=-1.0636	T=-1.9459	T=-1.0687		
females vs	df=100.11	df=46.66	df=55.08	df=43.61		
reference	p<2.2e-16***	p=0.293	p=0.0568	p=0.2911		
females	95% CI:	95% CI:	95% CI:	95% CI:		
	[417.22,435.09]	[-36.58,11.28]	[-4.02,0.06]	[-0.35,0.11]		
	x: 430.55	x: 424.94	x: 39.34	x: 5.31		
	ÿ: 4.39	ÿ: 437.60	ÿ: 41.32	ÿ: 5.43		
Endurance	T=-14.444	T=7.3219	T=1.4205	T=-8.3279		
males vs	df=76.35	df=131.25	df=73.37	df=108.39		
	p<2.2e-16***		p=0.1597			

Table 8 Mean BIA vector of groups stratified by gender and sport type studied in comparison to the corresponding reference population
reference		p=2.198e-		p=2.757e-
males		11***		13***
	95% CI :	95% CI :	95% CI :	95% CI :
	[-194.14, -	[49.41, 85.98]	[-0.57, 3.40]	[-1.28, -0.79]
	147.10]			
	x: 376.25	x: 371.39	x: 36.43	x: 5.65
	ÿ: 546.87	ÿ: 303.69	ÿ: 35.01	ÿ: 6.69
Strength	T=52.851	T=8.9766	T=2.4131	T=-9.3513
males vs	df=98.008	df=148.03	df=149.38	df=148.25
reference	p<2.2e-16***	p=1.168e-	p=0.01703***	p<2.2e-16***
males		15***		
	95% CI:	95% CI:	95% CI:	95%CI:
	[359.29,387.32]	[61.40,96.07]	[0.33,3.35]	[-1.35,-0.88]
	x: 376.25	x: 371.39	x: 36.43	x: 5.65
	ÿ: 2.95	ÿ: 292.65	ÿ: 34.58	ÿ: 6.77

Table 8 shows the obtained test statistic (T), degree of freedom (df), p-value (p), 95% confidence interval (CI) and means \bar{x} and \bar{y} of the sports and reference groups, respectively. Welch two sample T-test was used to compare hydration status differences using BIVA in a multivariate space between two different populations. The assumptions of this test are tested and considered as met. Each of the raw BIA variables Xc/H pre, R/H pre, Z/H pre, PA pre- were tested individually between groups.

The tested null hypothesis is that the true difference in means is equal to 0 between the sports group and the reference group of each column. The tested alternative hypothesis is that the true difference in means is not equal to 0 between the sports group and the reference group of each column.

Z/H is statistically significantly different between all studied groups. R/H and PA are only statistically significantly different between male endurance athletes and male reference athletes, and between male strength athletes and male reference athletes, respectively, with

p-values <0.05***. Xc/H is only statistically significantly different between strength males and references males (p=0.01703).

Table 9 Anthropometric and bioelectrical	parameters for	r male athlete	s and reference
population			

	Reference males (n=100)	Endurance males (n=36)			
Parameter		PRE	POST	Δ- Pre- Post(%)	∆-value Ref-group
Anthropometric					¥
BM (kg)		76.7 ± 12.9	76.1 ± 13.0	-0.9 ± 0.8	_
Bioelectrical					
R (Ω)	631.6 ± 91.1	543.1 ± 56.7	535.5 ± 55.9	-1.4 ± 2.3	88.6
Χc (Ω)	62.2 ± 6.5	62.6 ± 8.3	63.4 ± 7.0	2.8 ± 18.7	-0.4
R/h (Ω/m)	304.5 ± 37.2	303.7 ± 32.0	299.4 ± 31.9	-1.4 ± 2.3	0.8
Xc/h (Ω/m)	45.0 ± 4.1	35.0 ± 4.9	35.5 ± 4.2	2.8 ± 18.7	10
$PA(\Omega)$	5.7 ± 0.8	6.7 ± 0.5	6.8 ± 0.5	1.3 ± 1.9	-1
Ζ (Ω/m)	634.8 ± 91.0	546.9 ± 56.8	539.3 ± 56.1	-1.3 ± 2.3	87.9
r (R/h, Xc/h)	0.67	0.47	0.75		
	Reference males (n=100)		Strength males	(n=54)	
Parameter		PRE	POST	Δ- Pre- Post(%)	∆-value Ref-group
Anthropometric					
BM (kg)		77.8 ± 11.3	77.86 ± 11.3	0.1 ± 0.7	
Bioelectrical					
R (Ω)	631.6 ± 91.1	515.8 ± 53.9	507 ± 50.0	-1.6 ± 1.9	124.6
$\operatorname{Xc}(\Omega)$	62.2 ± 6.5	60.9 ± 5.7	59.6 ± 5.4	2.18±5.5	1.3
$R/h(\Omega/m)$	304.5 ± 37.2	292.6 ± 32.8	287.7 ± 30.6	-1.6 ± 1.8	16.8
Xc/h (Ω/m)	45.0 ± 4.1	34.6 ± 3.6	33.8 ± 3.4	-2.0 ± 2.8	11.2
$PA(\Omega)$	5.7 ± 0.8	6.6 ± 0.4	6.7 ± 0.6	-0.3 ± 1.8	-1
Ζ (Ω/m)	634.8 ± 91.0	519.5 ± 53.9	510.6 ± 50.1	-1.6 ± 1.8	124.2

r (R/h, Xc/h)	0.67	0.67	0.62		

Table 10 Anthropometric and bioelectrical parameters for female athletes and reference population

	Reference females (n=100)	Endurance females (n=29)			
Parameter		PRE	POST	Δ- Pre-	Δ-value
				Post(%)	Ref-group
Anthropometric					
BM (kg)		62.2 ± 7.2	62.0 ± 7.3	0.3 ± 1.37	-
Bioelectrical					
R (Ω)	697.8 ± 69.0	680.4 ± 60.1	674.1 ± 59.3	0.93 ± 1.35	17.4
Xc (Ω)	64.6 ± 8.7	65.4 ± 5.6	65.5 ± 6.2	-0.15 ± 9.7	-0.8
R/h (Ω/m)	428.7 ± 45.2	414.6 ± 36.6	410.69 ± 35.7	0.95 ± 0.02	18
$Xc/h(\Omega/m)$	39.7 ± 4.6	39.9 ± 3.9	39.96 ± 4.2	-0.15 ± 7.14	-0.2
ΡΑ (Ω)	5.3 ± 0.5	6.7 ± 0.5	6.8 ± 0.5	-1.47 ± 0	-1.4
Z (Ω/m)	700.9 ± 69.1	683.6 ± 59.9	677.4 ± 59.2	0.91 ± 0.02	17.3
r (R/h, Xc/h)	0.60	0.39	0.27		
	Reference females (n=100)		Strength females	(n=29)	
Parameter		PRE	POST	Δ- Pre-	Δ -value
				Post(%)	Ref-group
Anthropometric		1		1	
BM (kg)	_	57.9 ± 8.1	57.9 ± 8.1	0	
Bioelectrical				1	
R (Ω)	697.8 ± 69.0	705.0 ± 85.5	698.3 ± 87.9	0.95 ± 2.73	-7.2
Xc (Ω)	64.6 ± 8.7	66.5 ± 6.1	65.8 ± 6.9	1.06 ± 11.6	-1.9
R/h (Ω/m)	428.7 ± 45.2	437.6 ± 56.0	433.5 ± 57.7	0.95 ± 2.95	-8.9
$Xc/h(\Omega/m)$	39.7 ± 4.6	41.3 ± 4.6	40.9 ± 5.1	0.98 ± 0.09	-1.6
$PA(\Omega)$	5.3 ± 0.5	5.4 ± 0.5	5.4 ± 0.6	0	-0.1
Ζ (Ω/m)	700.9 ± 69.1	708.2 ± 85.4	701.5 ± 87.9	0.95 ± 2.91	-7.3

r (R/h, Xc/h) 0.60 0.6	3 0.69		
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Values are the mean \pm standard deviation; BM, body mass; R, resistance; Xc, reactance; h, height; PA, phase angle; Z, impedance vector module; r, Pearson correlation coefficient between R/h and Xc/h; Δ ,

CHAPTER IV

DISCUSSION

This study is the first to explore raw bioelectrical values between strength and endurance university athletes from both genders and plot reference and tolerance ellipses for the general Lebanese population. It is also the first of its kind to compare raw bioelectrical impedance vector variables to USG, sweat rate and urine color. This study showed that bioelectrical impedance vector analysis (BIVA) can be used as a hydration assessment technique in athletes. The strong agreement between raw bioelectrical values standardized for height: Xc/h, R/h and Z, PA with each of USG and sweat rate makes all three techniques interchangeable. Nevertheless, no significant relationships were found with urine color in the present study. According to Barley, O.R, et al. (2020), urinary measures do not represent real-time hydration status at the cellular level, but rather the renal response to fluid homeostasis. Urinary markers, including color and osmolality did not correlate well with hydration status post-training (Maughan, R.J. and Shirreffs, S.M., 2010) despite participants arriving in a euhydration state. Urine color on its own has been deemed to be a poor hydration assessment tool, and in the present study spearman correlation proved the same (Table 1), which could explain the absent correlation with raw BIVA values. Therefore, no further testing for agreement was done. The very similar median values for raw bioelectrical variables between dehydrated and hydrated groups according to urine color could further explain the inaccuracy of urine color. Also, these results can be altered by the diet and/or vitamin intake.

Moreover, the distribution pattern of raw bioelectrical values before and after training did not significantly change in any of the four studied groups of athletes due to the short training duration not exceeding 1.41 hours on average, including rest (Tables 4-5-6). On the other hand, the lack of detection of hydration changes post-training using other variables could be affected by the well hydrated status of individuals who were aware of the study design and were biased to drink enough water between trainings. However, the amount of water intake at the end of the training should not have affected the variables since research indicates that recent ingestion of a beverage, less than one hour from the ingestion to the next BIA measurement, seems to be "electrically silent" and to have a negligible effect on whole-body impedance Z. (Evans W, McClagish H, Trudgett C., 1998) No significant differences in USG were noted when comparing values of morning, before and after training (Table 3) Thus, the agreement between USG and raw BIA variables in terms of no sensitive detection of hydration changes following training is confirmed. Hence, not only Bland Altman plots confirm the agreement between the two methods, but also the pre-post analysis using multivariate analysis. Fernandez-Elias et al. (2017) highlights urine specific gravity (USG) as a valid and practical tool for assessing hydration status in athletes, offering valuable insights into fluid balance and hydration levels crucial for optimizing athletic performance.

Only R/h and Z statistically significantly decreased post-training in both genders and sports types, to a small extent, indicating their significant role as detectors of changes in hydration in this study. Similar results were observed in a cross-sectional study conducted by Micheli et al. (2014), whereby a decrease in the R/h was noted with no change in Xc/h in elite

soccer players. Resistance may be affected by temperature control, whereby every 1.0°C increase in the skin can result in around 11% decrease in R. (Caton, J. R., Molé, P. A., Adams, W. C., & Heustis, D. S., 1988) According to Carrasco-Marginet, M., et al. (2017), vector migration along the major axis due to increased R/h and Xc/h indicates fluid loss in swimmers. Hence, in the present study, the significantly decreased resistance experienced by the athletes could be reflecting the increase in body fluids. The statistically significant decrease in Z post exercise could indicate a decrease in TBW. (Silleras BdM, Ares GC, Marcos SdlC, Enciso LC, Fernández EQ, Río PRd. et al., 2023)

Similar to the study conducted in swimmers, a displacement to the left was also observed, due to a decrease in the R/h component. Other studies have also reported a shift in vectors of athletes to the left when compared to the reference population (Koury, J., Trugo, N. and Torres, A., (2014), Gatterer, H., Schenk, K., Laninschegg, L., Schlemmer, P., Lukaski, H., & Burtscher, M.,2014, Micheli, et al. 2014), which might be reflecting the specific adaptations of body composition in different sports. Andreoli, A., Monteleone, M., Van Loan, M., Promenzio, L., Tarantino, U., & De Lorenzo, A., 2001).

It is essential to explore whether the shift in vectors is due to performance level (Koury, J., et al., 2014) or is affected by the level of exercise. (Micheli, et al., 2014)

Other studies that detected and interpreted changes in reactance after training, such as increased Xc, explained it by a possible fluid shift between intra- and extracellular compartments. (Gatterer et al., 2014)

Additionally, PA was the weakest parameter in terms of significant changes post-training.

PA has not been proven to be a reliable parameter for hydration comparison between different sports types in athletes, making it only useful for within-athlete monitoring of changes in body composition until more research is conducted. (Tinsley GM, Stratton MT, Harty PS, Williams AD, White SJ, Rodriguez C, Dellinger JR, et al., 2022) A study by Smith et al. (2020) found that phase angle, while commonly used as a marker of cellular health and hydration status, demonstrated minimal change from pre to post training within the same athlete. Several factors can affect PA measurements including exercise level and age, necessitating further exploration. (Di Vincenzo, O., Marra, M., & Scalfi, L., 2019). Furthermore, male athletes were more hydrated in comparison to the reference healthy nonathletic population and compared to females, with a decrease in R/H and they were located outside the tolerance ellipses with a shift downwards to the right on the graph. Endurance females were more deviated from the female reference population, in contrary to male athletes, with most of them locating in the 75% tolerance ellipses. The vector position in females is slightly shifted to the left, on the minor axis (Xc) of tolerance ellipses, indicating higher BCM. Similar to males, post-training minor changes were noted in graphs, with less endurance female athletes located in the 50% and some shifted to the 95%. This could be an important indicator of the sensitivity of BIVA technique in detecting minor changes in hydration status visually shown on graphs, giving it the advantage over other techniques including USG and sweat rate in being more precise as to the subject displacement on the graph.

As for strength females, they were mostly located in the 95% tolerance ellipse prior to training and remained in the same position post-training. This goes in accordance with no change graph visualization for strength males from pre- to post-training. Hence, graphs give

us insight on the minor changes in hydration status in endurance females and endurance male athletes following training. Endurance sports athletes are more prone to dehydration post-training compared to strength athletes, regardless of gender, due to higher sweat rates and fluid losses incurred during prolonged aerobic exercise. (Cheuvront et al., 2018) Moreover, graphs indicate that all sports groups are adequately hydrated, however, slightly better hydration status is noted in male athletes compared to female athletes. Similar findings were noted in a study conducted by Sagayama, H., et. al. (2020) evaluating fat-free mass hydration whereby male athletes had a mean TBW of 43.3 Kg and female athletes a mean TBW of 31.9 Kg. This could be due to males drinking greater amounts of water between the first and second BIA measurements. However, more outliers were noted in females compared to males which could have affected the results.

Findings from R-Xc mean graphs confirm those of USG indicating no major changes in hydration status post-training, giving one more reason for validating BIVA as sensitive in detecting minor changes.

The overlapping 95% confidence ellipses for the mean impedance vectors of female athletes, divided into endurance and strength sports in both cases before and after training indicates that no group is significantly more hydrated than the other. In other terms, sports type was not a factor that affected hydration status in females. Both endurance and strength females are very close to the reference population as shown on the graph and this goes in hand with the performed Hotelling's T-test between the athletic and reference female groups (p-value=0.3208) indicating similar hydration characteristics for both. This may imply similarities in body composition between the two female endurance and strength groups. Hence, again, graphical and statistical results go in hand, indicating the accuracy of

results in the present study. In the same context, fat free mass (FFM), skeletal muscle mass (SMM), TBW, and ECW composition were found to be lower in endurance females compared to team sports and can be influenced by the design of training program and requirements of each to maximize performance. (Azmy, U., Rahmaniah, N., Renzytha, A. R., Atmaka, D. R., Pratiwi, R., Rizal, M., Adiningsih, S., & Herawati, L., 2023)

Male athletes exhibit a specific BIA vector distribution in comparison with the healthy reference population, nonathletic, of similar age, in contrary to females. Also, no mean vector displacement from pre to post training was observed and it is confirmed by the Hotelling-T tests.

Shorter vector length was observed for males on the graph compared to females. The length of the vector is inversely related to TBW. (Carrasco-Marginet M., et al., 2017). This can be explained by the consumption of water due to heat and sweat loss, impacting the BIA results. Moreover, vector length could be a key indicator of hydration status worth exploring. (Heavens et al., 2016) In the same context, according to Welch T-Test results, all four raw BIA variables (Z/h, R/h, PA, Xc/h) being statistically significantly different between strength males and reference males confirms the different distribution between mean BIA vector of strength males and reference males. In general, factors such as training, large muscle mass and greater glycogen reserves in athletes affect total body fluid, hence have been shown to result in an increase in soft tissue mass in comparison to the reference population in other studies (Andreoli, A., et al., 2001), indicating more water transport to the muscle. (Carrasco-Marginet M., et al., 2017) Hence, more water is transported to the muscle (Sawka M. N., 1992). One possible confounder could be that the reference

population subjects have a great muscle mass that they had built through regular training during their life in the past years even if in the present they do not practice sports, and possibly due to greater genetic inheritability of the body composition in men compared to women. (Brener, A., Waksman, Y., Rosenfeld, T., Levy, S., Peleg, I., Raviv, A., Interator, H., & Lebenthal, Y., 2021).

The vector of the mean difference is located near the origin of the RXc graph for both endurance and strength male athletic groups and is slightly shifted to the right, indicating a very slight decrease in water and cellularity post-training. Both the slight decrease in BCM noted by the BIA vector and the vector differences due to decreased R/h with similar Xc/h values could be an indication of different intracellular water (ICW) content. On the other hand, Segal et al. (1991) indicate that the change in vector is a function of extracellular water (ECW) changes, in the absence of penetration of the 50 kHz current to cells. (De Lorenzo et al., 1997) One can conclude that BIVA would allow further understanding of ECW fluctuations and ICW content together, noting that females for example exhibit different body water composition in their follicular phase compared to the luteal phase of their menstrual cycle.

Although no difference is detected using Hotelling's T-test for mean BIA vector for female athletes and female reference population and it is confirmed on the graph, however a small difference is detected between the two female sport groups themselves (p-value =0.77 for endurance and p-value=0.64 for strength). On the graph, ellipses are overlapping and this necessitates further exploration using Cohen's as the small sample size could impact the significant difference. On the other hand, the two sample Welch's T-Test which only

indicates differences in Z/H in the univariate model analysis between each female sport group and the reference female group explains the overlapping of mean vectors on graphs. Also, graphs of males clearly illustrate results obtained through Hotelling's T-test. Of relevance is the smaller female sample size (n=29) for each of the endurance and strength sports in comparison to the larger sample size of strength males (n=54) and endurance males (n=36), possibly impacting results and resulting in discrepancies between the groups. Furthermore, other studies compared BIVA to body mass changes (Carrasco-Marginet, M. et al., 2017, Castizo-Olier, J., 2018), however, in the present study the focus was on USG since it is strong predictor of hydration status. On the other hand, sweat rates may widely vary inter-individually due to influence by factors such as gender of athlete, physical activity patterns, and environmental conditions (Shirreffs et al., 2005)

BIVA can be a promising technique to assess hydration variations in real time and substitute hydration biomarkers that require a mobile laboratory and its use as a complementary measure to other hydration indicators will allow the parameterization of its values. (Carrasco-Marginet, M. et al., 2017)

In contrary to other findings that indicated an association between athletic body composition and hydration changes (Campa and Toselli, 2018; Carrasco-Marginet et al., 2017; Gatterer et al., 2014; Giorgi et al., 2018; Koury et al., 2014; Micheli et al., 2014), the present study did not find any of impact of body composition on hydration changes posttraining, mainly affected by the short training duration. Therefore, significant changes in body composition require prolonged and consistent training regimens with adequate intensity and volume. (Cavedon, V., Milanese, C., Marchi, A., & Zancanaro, C., 2020)

Only a few studies have applied BIVA to evaluate short-term vector changes from pre- to post- training. An increase in R and Xc was reported after exercise along with a vector migration, indicating a decrease in body fluid because R is the opposition of the conductor to the flow of current. (Carrasco-Marginet et al., 2017; Gatterer et al., 2014, Heavens et al., 2016). Xc is interpreted to detect fluid shifts between intra- and extracellular compartments (Gatterer et al., 2014), and higher Xc and PA values have been related to lower ECW and higher ICW content. (Alvero-Cruz, JR., et al., 2020). Hence, further large-scale controlled studies are needed to understand Xc changes post-exercise.

A. Strengths

To our knowledge, this is the first study that investigated agreement between BIVA technique for hydration assessment in athletes and three other commonly used methods (urine specific gravity, sweat rate and urine color). It is also the first study to use BIVA in Lebanon to assess hydration status of athletes and design reference tolerance and confidence ellipses for the adult Lebanese healthy population that can be used in future studies. No other studies had established tolerance ellipses for Lebanese athletes to allow for a more enriching analysis. The large sample size and the repeated BIA and USG measurements allowed for greater precision, statistical power and accuracy. Another strength of the study is the comparison between male and female endurance and strength athletes, which has not been explored before. Also, reminders and tips from the researcher ahead of the experiment made the process smooth and served in well preparing participants.

Data was collected and analyzed by the same researcher throughout the study which maintained consistency across the results.

B. Limitations

The study has some limitations including the quasi-experimental design with no blinding of the procedure. This could have biased athletes to drink more water before and during the experiment. Moreover, some participants fasted for 4 hours whereas others for 8 hours and this could have affected the accuracy of BIA measurements and group analysis. Additionally, some individuals trained in the morning while others in the afternoon, hence those performing the experiment in the morning voided and had their BIA measured in a fasted state, whereas those training in the afternoon consumed their last meal 4 hours prior to the experiment. The ingestion of food may have influenced Z to a small extent. On the other hand, some participants had to drink a lot of water to void even if they were not thirsty. Lifestyle habits such as sleep pattern, eating, smoking, drinking habits, temperature, season, humidity and varying training durations among strength athletes could have impacted the results and were not controlled for. Furthermore, some athletes sweat heavily while others sweat less. Moreover, those who are regular consumers of creatine could have increased water retention in muscles. Additionally, patients reported orally whether they were taking any medications or if they had any health problems, some had never performed a blood test. Moreover, the female sample size is relatively smaller than the male sample size due to the low number of female athletes at AUB compared to males. Analysis was mainly performed on pre-training measurements for BIVA.

C. Future studies

It is worth extensively exploring collected data for post-exercise BIA measurements and the different timepoints for urine measurements in a future study. More accurate assessment methods such as blood tests should be used in future studies for the selection of participants based on their complete blood count and lipid profile to determine their health status. Further studies should adjust for possible confounders, increase the female sample size and investigate the validity of BIVA as for hydration assessment in athletes with a specific focus on Xc and Z changes post-exercise.

CONCLUSION

In conclusion, there is a strong agreement between BIVA, USG and sweat rate. The sensitivity of classic BIVA in detecting minor changes in hydration status is confirmed graphically and statistically which paves the way towards the validation of BIVA for hydration assessment in athletes. Moreover, the present study demonstrates that male athletes strongly exhibit a specific BIA vector distribution in comparison with the healthy reference non-athletic population, of similar age and compared to female athletes. Further research is needed to develop new specific guidelines for hydration needs before, during and after training based on bioelectrical changes in the body. Athletes were not extremely hydrated in the present study hence these results will be a guiding step for reviewing guidelines to optimize hydration status among AUB varsity team players and strength athletes for better performance. Validating BIVA may be an essential step to allow its use pre- and post- training among university students to assess dehydration in a non-invasive, practical and inexpensive way.

APPENDIX

APPENDIX A

URINE COLOR CHART



The Urine Color Chart shown here will assess your hydration status (level of dehydration) in extreme environments. To use this chart, match the color of your urine sample to a color on the chart. If the urine sample matches #1, #2, or #3 on the chart, you are well hydrated. If your urine color is #7 or darker, you are dehydrated and should consume fluids.

The scientific validation of this color chart may be found in the *International Journal of Sport Nutrition*, Volume 4, 1994, pages 265-279¹⁹⁴ and Volume 8, 1998, pages 345-355.¹⁹⁵ Adapted by permission from Larry Armstrong, 2000, *Performing In Extreme Environments*, (Champaign, IL: Human Kinetics).¹⁹⁵

APPENDIX B

URINE SPECIFIC GRAVITY EQUIVALENT HYDRATION

STATUS

Condition	Urine specific gravity value
Well hydrated	<1.010
Minimal dehydration	1.010-1.020
Significant dehydration	1.021-1.030
Serious dehydration	>1.030

Adapted, by permission, from D.J. Casa et al., 2000, "National Athletic Trainers' Association position statement: Fluid replacement for athletes," *Journal of Athletic Training* 35(2): 212-224.

APPENDIX C

CONSENT FORM



Participant consent form - Athlete

Title of the study:

Bioelectrical Impedance Vector Analysis (BIVA) for Assessment of Hydration Status: A Comparison between Endurance and Strength University Athletes

Principal investigator: Dr. Elie-Jacques Fares Co-Principal investigator : Dr. Lara Nasreddine Student investigator : Maria Abdel Nour

You are being asked to participate in a research study. Before agreeing to participate, it is important that you read the following information carefully. This consent form describes the purpose, procedures, benefits, and risks of participating in the study. Also mentioned is your right to withdraw from the study at any time. You should feel free to ask any questions that you may have.

A. Purpose of the research:

The overall aim of this study is to assess and compare the hydration status of endurance and strength athletes pre- and post- training using classic BIVA technique by plotting and evaluating vectors variation by gender and sport type while comparing it to reference methods. Also, body composition will be obtained during the tests.

B. Project/procedures description:

To assess hydration status, classic BIVA will be used and will be compared to the following methods: urine color, urine specific gravity, sweat rate and phase angle.

This study aims to include 400 individuals in total, 200 of them being athlete participants and 200 individuals constituting the reference population for comparison while plotting vectors. Athletes will be equally divided between males and females, endurance and strength sports as follows: endurance males (n=50), endurance females (n=50), strength females (n=50), strength males (n=50), selected by convenience from the American University of Beirut's varsity teams and weightlifters at Charles Hostler Student Center. The reference population will also include an equal number of males (n=100) and females (n=100) selected from the CHDC client database.

If you consent to participate in this study, will be asked to present one week before the experiment and one day before the experiment at the CHDC. Then, you will be evaluated on one experimental day, before and after your training session. You will also be reminded the day before the experiment through WhatsApp/e-mail about the meetings, depending on your preference. If you choose to be reminded through WhatsApp, you must consent to provide us with your phone number next to your signature below in the end of this form. Otherwise, you will be reminded through e-mail, which you will also be asked to write down. After signing this consent form, you will be informed about the exact scheduled day of experiment and the day preceding the experiment, both in which you will need to attend to CHDC. All arrangements and contact will be done by the student investigator and study coordinators.

Recruitment is performed by direct approach by the investigating graduate student, while exercising at the gym or during training hours for varsity teams in the afternoon, depending on the type of sports you're training for. Also, participants will be recruited through advertisement flyers. The written consent form is provided to you at CHDC by the student investigator and you are asked to voluntarily sign it if you wish to take part in this study. The form briefly describes what will be required from you and the duration for each procedure. All rights and privacy will be preserved by keeping the responses in this study confidential, storing the data in a password-protected computer, presenting research report findings on a group basis without any personally identifying information.

Eligibility for your participation in the study will be determined by assessing if you meet the inclusion criteria through answering a series of questions (4), the "eligibility questionnaire" uploaded on Google docs and sent by email.

Inclusion criteria: healthy adult between the ages of 18 and 35 years, part of AUB's varsity teams or weightlifter members at CHSC, not injured at the time of the study, training at least 3 times per week, females in a post-menstruation state with the ovarian cycle between days 5 to 11, not on contraceptives or drugs for menstrual cycle treatment, not pregnant or planning to become pregnant. Exclusion criteria: not part of AUB's varsity teams, not weightlifter member at CHSC, not being able to visit CHDC facility, injured/having health problems/previous surgeries that alter body composition (bariatric surgery).

Below is a description of the procedures for each visit and the corresponding expected durations:

1. One week prior to the experiment, you will be administered a 10-minutes pre-hydration questionnaire to know about your lifestyle and to provide you with the necessary recommendations that will help you maximize your readiness for the study. You will be asked about your demographics, medical history, physical activity level, type of sports and frequency, diet and anthropometry. The questionnaire will provide the researcher with information about your medical, nutritional and health status. You may refrain from responding to questions you do not wish to answer.

2. On the day prior to the experiment, you will be required to come to the CHDC for 5-10 minutes to pick up your urine cup 1 which you will be asked to void in in the morning of the experiment and handle it back to the CHDC immediately after reaching AUB. Also, you will be reminded of the discussed instructions in the previous week.

3. On the day of the experiment:

a. In the morning you will be required to pass by CHDC for 15 minutes to handle urine cup 1, also you will be given at this time urine cup 2 and cup 3 to void in. You will be asked to void in cup 1 and cup 2 respectively before and after the training session. Your height will be measured.

b. In the afternoon, right before your training session begins, you will be asked to come to CHDC to answer a few questions to fill in the data collection sheet (your first name, middle name, last name, date, time, e-mail address, phone number (optional)).

Then, you will be asked to provide us with the urine cup 1 in which you voided right before the beginning of the session. Also, your body weight 1 prior to training will be measured in the clinic by standing on a scale, as well as the water bottle weight A before training. For accurate body weight measurement, you will be asked to void your bladder prior to measurement of pre-training weight, remove your shoes and heavy clothes, and you should be standing with your feet on the center of the scale. For the measurement of post-training weight, the same conditions apply, with the exception that the measurement should be taken prior to voiding and without sweaty clothes.

You will next undergo two body composition tests on the bioelectrical impedance BIA machine, and then the student investigator and the research team will accompany you to the training area and ask you to come back to the clinic right after your training session ends to take some of the measurements taken in the afternoon prior to the training session, but this time after training, to assess the difference in hydration status. Measurements taken will be the following: body weight 2 post training, water bottle weight B post training, BIA tests 3 and 4. Body weight measurements 1 and 2, water bottle weight A and B, and urine collection 1 and 2 will allow for sweat rate assessment.

-Height will be measured using SECA 213 portable stadiometer. Measurements will be taken in an orthostatic position and barefoot on a platform.

-For urine collection, guidelines will be recorded on the lid of the cup to guide you on proper midstream voiding (time, instructions) with sufficient space to record your name, time and date of collection. You will be asked to give back all handled cups to CHDC right after voiding.

-Sweat rate will be assessed by calculating body mass changes.

-BIA measurements:

Bioelectrical data: Resistance (R), R/h (where h is the height of the individual), reactance (Xc), Xc/h, phase angle (PA), impedance (Z), and extracellular water:total body water ratio (ECW:TBW) will be obtained using the InBody 770 machine, at a 50-kHz single frequency.

-Prior to BIA measurements you will be asked to remove any metals, earrings or rings. The following steps will be needed for the BIA test:

First, you be asked to remove shoes, socks, heavy clothes, and items in pockets. Second, you will be asked to wipe your hands and feet with an InBody Tissue.

Third, you will be asked to stand on the device barefoot and align heel with the round silver electrodes and the rest of the feet with the foot electrode while staying still and waiting for weight to be measured.

Fourth, after weight is measured, the student investigator will input the age, height, gender and enter a unique ID for you to track the difference in measurements before and after training.

Fifth, you will then be asked to grab the hand electrodes by placing your thumbs on the thumb electrodes and wrapping your fingers around the bottom electrodes, keeping your arms relaxed and extended slightly away from the torso so that your armpits are not touching one another (around 15 degrees).

Each InBody Test will take 3 minutes.

On the days of visits, a graduate student investigator and the study coordinators will be assisting you while the main researcher student will be performing the experimental procedures.

C. Duration:

- **a.** Visit duration <u>one week prior to the experiment</u>: **15 minutes** (10minutes questionnaire + 5 minutes instructions)
- **b.** Visit duration one day prior to the experiment: **5 minutes**
- c. Visit duration <u>on the experimental day</u>: **36 minutes** divided as follows: -morning for urine cups handling and height measurements: 5 minutes -afternoon prior to training: 16 minutes:

1.urine cup 2 voiding + collection: 5 minutes

2.body weight 1 (2 minutes), water bottle A weighing (2 minutes), BIA tests 1 and 2 (3minutes each, *2=6 minutes)

-afternoon post training: 15 minutes:

urine cup 3 voiding + collection: 5 minutes

2.body weight 2 (2 minutes), water bottle B weighing (2 minutes), BIA tests 3 and 4 (3minutes each, *2=6 minutes)

Total duration of all visits summed up: 56 minutes

D. Benefits:

You will receive the results of hydration and body composition for free, otherwise there are no direct benefits, however, your participation helps researchers better understand hydration status and body composition among athletes. The results will be received electronically attached as a PDF and sent by email to your corresponding mail address and you will be given the choice of scheduling a 10-minute meeting in the clinic with the student investigator dietitian to discuss their state and be provided with recommendations from the researcher to optimize your hydration and body composition if any are required.

E. Risks and discomforts:

Your participation in this study does not involve any physical risk or emotional risk to you beyond the risks of daily life. You have the right to withdraw your consent or discontinue participation at any time for any reason. Your decision to withdraw will not involve any penalty or loss of benefits to which you are entitled. Discontinuing participation in no way affects your relationship with AUB.

F. Confidentiality:

You will be asked to write your name on the urine cups for the only reason of not mixing the individuals' cups prior to analysis. However, once the urine inside each cup is analyzed, the label

will be discarded. Also, only the investigating researcher will have access to those cups and their storage, they will be the person collecting them as well.

Also, the questionnaire will be relayed by the researchers in CHDC. your privacy will be maintained by only you being present in the room, the main researcher and helping student investigator at the time of asking questions and noting their answers.

To secure the confidentiality of your responses, your name and other identifying information will never be attached to your answers. All codes and data are kept in a locked drawer in a locker room or in a password protected computer that is kept secure. Data access is limited to the Principal Investigator and researchers working directly on this project. All data will be destroyed responsibly after the required retention period (usually three years.) Your privacy will be maintained in all published and written data resulting from this study. Your name or other identifying information will not be used in our reports or published papers. The filled questionnaire will be stored in confidentiality on Dr. Elie-Jacques Fares' password-protected computer and Ms. Maria Abdel Nour's AUB net account for 3 years that only the researcher will have access to. Data will be destroyed after 3 years. Coded data will be saved on Dr. Elie-Jacques Fares' password-protected computer.

G. Compensation:

You will be compensated 20\$ for your participation at the end of the last study procedure.

H. Contact Information

1) If you have any questions or concerns about the research you may contact:

- Dr. Elie-Jacques Fares: ef08@aub.edu.lb, Agriculture building room 335, extension: 4926

- Maria Abdel Nour: mra67@mail.aub.edu, phone number: +961 76198730

2) If you have any questions, concerns or complains about your rights as a participant in this research, you can contact the following office at AUB:

Institutional Review Board ACC building third floor, irb@aub.edu.lb, extension: 5445

I. Participants Rights:

Participation in this study is voluntary. You are free to leave the study at any time without penalty. Your decision not to participate will not influence your relationship with AUB.

J. Future Contact:

Would you like to receive results of hydration and body composition by e-mail? If yes, please confirm your mail address:

K. Future research

Do you consent to be contacted for future research?

□ Yes

 \Box No

L. Participant Consent:

Do you have any questions about the above information? Do you wish to participate in this study?

 \Box Yes

 \Box No

I have read and understood all the above information and all my questions have been answered satisfactorily. I understand that I am free to withdraw this consent and discontinue participation in this survey at any time, even after signing this form, and it will not affect my care or benefits. I also understand that I can participate partially in the survey, and do not have to provide all information / measurements / samples that are requested. I know that I

Name of participant (optional)	Phone number
E-mail address	
Signature of participant	Date
Researcher's name	
Researcher's signature	Date

will receive a copy of this signed informed consent. I was given sufficient time to make the decision about participating in the survey. I voluntarily agree to be a part of this survey.

APPENDIX D

ELIGIBILITY QUESTIONNAIRE

1. Are you a healthy adult between the ages of 18 and 35 years?

□ Yes □ No

2. Do you have any medical problems/illnesses/allergies?

 \Box Yes

 \Box No

If yes, please specify: _____

3. Have you ever been diagnosed with any of the following diseases or do you have any family history?

DISEASE	PLEASE TICK (✓) as appropriate	Duration of Disease (years)	Do you still suffer from any of these conditions? (YES/NO)	Are you following any treatment? (YES/NO)
Diabetes				
Heart Disease				
Hypertension				
High Blood Cholesterol				
Thyroid disease				
Sleep Apnea				
Other (Asthma, Claustrophobia)				
Cancer				
Other health problems:				

Surgical History: _____

4. Do you associate any digestive symptoms with eating certain foods?

 \Box Yes

□ No

If yes, please explain:

5. Are you part of AUB's varsity teams?

 \Box Yes

□ No

6. Do you have any injury?

 \Box Yes

 \Box No

If yes, please specify:

7. How often do you train?

 \Box Daily

 \Box 3 times per week

 \Box Less than 3 times per week

□ More than 3 times per week, please specify: _____

For females only:

8. When was the last time you got your period?

9. When do you expect to get it again?

10. What is your normal menstruating cycle? (in days) (In other words, on average how often do you have your period?)

11. Are you on contraceptives or drugs for menstrual cycle treatment?

12. Are you pregnant or planning to become pregnant?

APPENDIX E

PRE-HYDRATION ASSESSMENT QUESTIONNAIRE



Department of Nutrition and Food Sciences

First Name:
Middle Name:
Last Name:
Gender:
Date:
Age:
Telephone number:
Email address:
Sport:
How many training sessions do you have per day on average?
 One Two Three Four and above Other, please specify:
How much time do you usually spend doing vigorous physical activities?
hours per day minutes per da

____ minutes per day _____

Don't know/Not sure

1.

2.

- 3. What type(s) of physical activity do you engage in? (you can choose more than one):
 - \Box Aerobics/Cardio
 - □ Anaerobic/Weight Resistance
 - □ Yoga & Stretching

Medical/Biochemical/Clinical Assessment:

How often do you have a bowel movement?

If you take laxatives, what type/brand and how often?

Heartburn, Bloating, Gas, Constipation, Diarrhea, Nausea, Vomiting:

When was the last time you had Blood Test? _____

Recent Blood test results:

Water Retention: Yes No

Dietary Assessment:

4. Have you been restricting your caloric intake during the past few days?

□ Yes □ No

If yes, what is the reason?

5. Are you taking any drugs or medications? If yes, specify name, quantity and duration:

□ Yes, please specify: _____

 \Box No

 \Box Don't know

6. Do you consume any of the following foods?

 \Box beets

 \Box blackberries

 \Box carrots

 \Box fava beans

 \Box rhubarb

7. Which meals do you eat regularly, check all that apply:

□ Breakfast

□ AM Snack

 \Box Lunch

 \Box Afternoon Snack

- \Box Dinner
- \Box Late Night Snack

8. Are you vegetarian?

 \Box Yes

□ No

9. Have you ever followed a dietary regime?

\Box Yes, now
\Box Yes, previously
🗆 No

If yes, please give details:

10. Do you take supplements?

 \Box Yes \Box No

List the supplements that you take:

Supplement Type	How much?	Reason for
and Brand	When?(before or after training)	Consumption
		☐ Medical need/
		deficiency
		□ Due to an
		inadequate diet
		□ Support immune
		system
		🗆 To provide energy
		□ Increase
		strength/power
		□ To aid recovery
		□ Because everyone
		else does
		□ Because I am told to
		□ Other (Please state)

11. How many fruits and vegetables in all do you consume daily on average?

_____Fruits _____Vegetables

12. How often do you consume fast foods?

 \Box Never

- \Box Two to three times monthly
- \Box One or two times weekly
- \Box Daily
- 13. How many liters of water do you consume daily?
 - \Box Less than one liter
 - \Box One liter to two liters
 - \Box Two to three liters
 - \Box More than 3 liters
- 14. . How many cups/cans/glasses of tea, coffee, Cola/Pepsi, Red Bull (i.e. caffeine containing beverages) do you consume <u>daily</u>? (Please match the columns below accordingly)

Tea	□ None to occasionally
Coffee	\Box One to four
Cola/Pepsi	Tur to ten
Redbull	\square More than ten

15. Do you consume alcohol? If yes, specify _____

Never or rarely
Only in weekends
Once or twice weekly
Once or twice daily
More than twice daily

16. Do you smoke?

□ Yes □ No

If yes, how much on average?

17. On average how many hours do you sleep per night?

- \Box More than 10 hours
- □ Between 8-10 hours
- \Box Between 6-8 hours
- \Box Between 4-6 hours
- \Box Less than 4 hours

- 18. How important do you think good nutrition is to sports performance?
 - □Very important
 - □ Important
 - □ Moderately important
 - \Box Of little importance
 - □ Unimportant
- 19. How important do you think hydration status is to sports performance?

□Very important

- □ Important
- □ Moderately important
- \Box Of little importance
- □ Unimportant

20. "The more supplements I take, the better I will perform":

- \Box Agree
- □ Disagree
- □ Strongly disagree
- □ Neither agree nor disagree
- 21. Have you received any previous nutritional advice?
 - □ Yes □ No
- 22. Do you have access to a sports nutritionist/ dietitian?
 - ☐ Yes, through CHDC☐ Yes, outside AUB☐ No

Anthropometric Assessment:

Usual body weight: _____

23. During these past 6 months, did your weight change?

Yes, I gained weight
Yes, I lost weight
No
Not sure

How many kg(s)____?

- 24. Would you like to receive the hydration tests and body composition results by phone/mail?
 - \Box Yes, by phone
 - \Box Yes, by mail
 - \Box No, I don't want to receive the results

Instructions to prepare you for the hydration tests:

-Avoid caffeine and alcohol consumption prior to the experiment

-Do not exercise intensely at least 12 hours prior to the experiment

-Avoid food in the 4 hours prior to the experiment

APPENDIX F

DATA COLLECTION SHEET

First Name:	Middle Name:
Last Name: Time:	Date:
E-mail address: Participant #:	
Phone Number (Optional):	
Total training duration:	
Gender	
Date of birth/Age	
Sport type	
Eligibility questionnaire	
Pre-hydration assessment question	naire
Empty urine cup 1 weight	
Empty urine cup 2 weight	

Empty urine cup 1 weight	
Empty urine cup 2 weight	
Empty urine cup 3 weight	
Urine collection 1	
Urine collection 2	
Urine collection 3	
Height	
Body weight 1	
Body weight 2	
Water bottle weight A	

Water bottle weight B	
BIA test 1	
BIA test 2	
BIA test 3	
BIA test 4	

Body weight 1: before training; Body weight 2: after training; Water bottle weight A: before training; Water bottle weight B: after training; BIA test 1: before training; BIA test 2: before training; BIA test 3: after training; BIA test 4: after training
REFERENCES

Achamrah, N., Colange, G., Delay, J., Rimbert, A., Folope, V., Petit, A., Grigioni, S., Déchelotte, P., & Coëffier, M. (2018). Comparison of body composition assessment by DXA and BIA according to the body mass index: A retrospective study on 3655 measures. *PloS one*, *13*(7), e0200465. https://doi.org/10.1371/journal.pone.0200465

Almeida, Y. L., Costa Maia, C. S., Barros, N. E., Moreno, L. A., Carioca, A. A. F., & Loureiro, A. C. (2021). Is bioelectrical impedance vector analysis a good indicator of nutritional status in children and adolescents?. *Public health nutrition*, *24*(14), 4408–4416. https://doi.org/10.1017/S1368980021002226

Alvero-Cruz, J. R., Carnero, E. A., García, M. A. G., Alacid, F., Correas-Gómez, L., Rosemann, T., Nikolaidis, P. T., & Knechtle, B. (2020). Predictive Performance Models in Long-Distance Runners: A Narrative Review. *International journal of environmental research and public health*, *17*(21), 8289. <u>https://doi.org/10.3390/ijerph17218289</u>

Andreoli, A., Monteleone, M., Van Loan, M., Promenzio, L., Tarantino, U., & De Lorenzo, A. (2001). Effects of different sports on bone density and muscle mass in highly trained athletes. *Medicine and science in sports and exercise*, *33*(4), 507–511. https://doi.org/10.1097/00005768-200104000-00001

Azmy, U., Rahmaniah, N., Renzytha, A. R., Atmaka, D. R., Pratiwi, R., Rizal, M., Adiningsih, S., & Herawati, L. (2023). Comparison of Body Compositions among Endurance, Strength, and Team Sports Athletes. *Sport Mont, 21*(3), 45-50. doi: 10.26773/smj.231007

Barley, O. R., Chapman, D. W., & Abbiss, C. R. (2020). Reviewing the current methods of assessing hydration in athletes. *Journal of the International Society of Sports Nutrition*, *17*(1), 52. <u>https://doi.org/10.1186/s12970-020-00381-6</u>

Brener, A., Waksman, Y., Rosenfeld, T., Levy, S., Peleg, I., Raviv, A., Interator, H., & Lebenthal, Y. (2021). The heritability of body composition. *BMC pediatrics*, *21*(1), 225. https://doi.org/10.1186/s12887-021-02695-z

Campa, F., & Toselli, S. (2018). Bioimpedance Vector Analysis of Elite, Subelite, and Low-Level Male Volleyball Players. *International journal of sports physiology and performance*, *13*(9), 1250–1253. https://doi.org/10.1123/ijspp.2018-0039

Campa, F., Matias, C., Gatterer, H., Toselli, S., Koury, J. C., Andreoli, A., Melchiorri, G., Sardinha, L. B., & Silva, A. M. (2019). Classic Bioelectrical Impedance Vector Reference Values for Assessing Body Composition in Male and Female Athletes. *International journal of environmental research and public health*, *16*(24), 5066. https://doi.org/10.3390/ijerph16245066

Campa, F., Silva, A. M., Matias, C. N., Monteiro, C. P., Paoli, A., Nunes, J. P., Talluri, J., Lukaski, H., & Toselli, S. (2020). Body Water Content and Morphological Characteristics Modify Bioimpedance Vector Patterns in Volleyball, Soccer, and Rugby Players. *International journal of environmental research and public health*, *17*(18), 6604. https://doi.org/10.3390/ijerph17186604

Campa, F., Toselli, S., Mazzilli, M., Gobbo, L. A., & Coratella, G. (2021). Assessment of Body Composition in Athletes: A Narrative Review of Available Methods with Special Reference to Quantitative and Qualitative Bioimpedance Analysis. *Nutrients*, *13*(5), 1620. <u>https://doi.org/10.3390/nu13051620</u>

Carrasco-Marginet, M., Castizo-Olier, J., Rodríguez-Zamora, L., Iglesias, X., Rodríguez, F. A., Chaverri, D., Brotons, D., & Irurtia, A. (2017). Bioelectrical impedance vector analysis (BIVA) for measuring the hydration status in young elite synchronized swimmers. *PloS one*, *12*(6), e0178819. https://doi.org/10.1371/journal.pone.0178819

Casa, D. J. (2000). Performing in extreme environments. *Journal of Athletic Training*, *35*(1), 111-112. Retrieved from <u>https://www.proquest.com/scholarly-journals/performing-extreme-environments/docview/206642838/se-2</u>

Castizo-Olier, J., Carrasco-Marginet, M., Roy, A., Chaverri, D., Iglesias, X., Pérez-Chirinos, C., Rodríguez, F., & Irurtia, A. (2018). Bioelectrical Impedance Vector Analysis (BIVA) and Body Mass Changes in an Ultra-Endurance Triathlon Event. *Journal of sports science & medicine*, *17*(4), 571–579.

Castizo-Olier, J., Irurtia, A., Jemni, M., Carrasco-Marginet, M., Fernández-García, R., & Rodríguez, F. A. (2018). Bioelectrical impedance vector analysis (BIVA) in sport and exercise: Systematic review and future perspectives. *PloS one*, *13*(6), e0197957. https://doi.org/10.1371/journal.pone.0197957

Cataldi, D., Bennett, J. P., Quon, B. K., Liu, Y. E., Heymsfield, S. B., Kelly, T., & Shepherd, J. A. (2022). Agreement and Precision of Deuterium Dilution for Total Body Water and Multicompartment Body Composition Assessment in Collegiate Athletes. *The Journal of nutrition*, *152*(9), 2048–2059. https://doi.org/10.1093/jn/nxac116

Caton, J. R., Molé, P. A., Adams, W. C., & Heustis, D. S. (1988). Body composition analysis by bioelectrical impedance: effect of skin temperature. *Medicine and science in sports and exercise*, 20(5), 489–491.

Cavedon, V., Milanese, C., Marchi, A., & Zancanaro, C. (2020). Different amount of training affects body composition and performance in High-Intensity Functional Training participants. *PloS one*, *15*(8), e0237887. <u>https://doi.org/10.1371/journal.pone.0237887</u> Cheuvront, S. N., Kenefick, R. W., Montain, S. J., & Sawka, M. N. (2018). Mechanisms of aerobic performance impairment with heat stress and dehydration. *Journal of Applied Physiology*, *125*(1), 101-115.

Cheuvront, S.N., Sawka, M.N. (2006). Hydration Assessment of Athletes. *Gatorade* Sports Science Institute. <u>https://www.gssiweb.org/sports-science-exchange/article/sse-</u> <u>97-hydration-assessment-of-athletes</u>

Davy, K.P., Davy, B.M., Neilson, A.P. (2017). Validation of Urinary Biomarkers of Hydration Status in College Athletes

de la Cruz Marcos S, Redondo del Río MP, de Mateo Silleras B. Applications of Bioelectrical Impedance Vector Analysis (BIVA) in the Study of Body Composition in Athletes. *Applied Sciences*. 2021; 11(21):9781. <u>https://doi.org/10.3390/app11219781</u>

De Lorenzo A., Andreoli A., Matthie J., Withers P. (1997) Predicting body cell mass with bioimpedance by using theoretical methods: a technological review. *Journal of Applied Physiology* 82, 1542-1558.

Di Vincenzo, O., Marra, M., & Scalfi, L. (2019). Bioelectrical impedance phase angle in sport: a systematic review. *Journal of the International Society of Sports Nutrition*, *16*(1), 49. <u>https://doi.org/10.1186/s12970-019-0319-2</u>

Evans W, McClagish H, Trudgett C. (1998) Factors affecting the in vivo precision of bioelectrical impedance analysis. Appl Radiat Isot. 1998; 49(5):485–7

Fernandez-Elias, V. E., Martínez-Abellán, A., Lopez-Gullon, J. M., Morán-Navarro, R., Pallares, J. G., De la Cruz-Sánchez, E., ... & Ortega, J. F. (2017). Validity of hydration non-invasive indices during the weightcutting and official weigh-in for Olympic combat sports. *PLoS ONE*, *12*(3), e0172356.

Gatterer, H., Schenk, K., Laninschegg, L., Schlemmer, P., Lukaski, H. and Burtscher, M. (2014) Bioimpedance identifies body fluid loss after exercise in the heat: a pilot study with body cooling. *PLoS One* 9, e109729.

Gatterer, H., Schenk, K., Laninschegg, L., Schlemmer, P., Lukaski, H., & Burtscher, M. (2014). Bioimpedance identifies body fluid loss after exercise in the heat: a pilot study with body cooling. *PloS one*, *9*(10), e109729. https://doi.org/10.1371/journal.pone.0109729

Giorgi, A., Vicini, M., Pollastri, L., Lombardi, E., Magni, E., Andreazzoli, A., Orsini, M., Bonifazi, M., Lukaski, H. and Gatterer, H. (2018) Bioimpedance patterns and bioelectrical impedance vector anal- ysis (BIVA) of road cyclists. *Journal of Sports Sciences* 1-6.

Heavens, K.R., Charkoudian, N., O'Brien, C., Kenefick, R.W., Cheuvront, S.N. (2016) Noninvasive assessment of extracellular and intracellular dehydration in healthy humans using the re- sistance-reactance–score graph method. *The American Journal of Clinical Nutrition* 103, 724-729.

Hoen, L., Pfeffer, D., Zapf, R., Raabe, A., Hildebrand, J., Kraft, J., & Kalkhof, S. (2021). Association of Drug Application and Hydration Status in Elderly Patients. *Nutrients*, *13*(6), 1929. <u>https://doi.org/10.3390/nu13061929</u>

Honorato, R. C., Soares Marreiros Ferraz, A., Kassiano, W., Martins, P. C., Silva, D. A. S., & Ceccatto, V. M. (2022). Regional phase angle, not whole-body, is augmented in response to pre-season in professional soccer players. *Research in sports medicine* (*Print*), 1–15. Advance online publication. https://doi.org/10.1080/15438627.2022.2052069

Klimesova, I., Krejci, J., Botek, M., McKune, A. J., Jakubec, A., Neuls, F., Sladeckova, B., & Valenta, M. (2022). Prevalence of Dehydration and The Relationship with Fluid Intake and Self-Assessment of Hydration Status in Czech First League Soccer Players. *Journal of human kinetics*, 82, 101–110. https://doi.org/10.2478/hukin-2022-0035

Kostelnik, S. B., Davy, K. P., Hedrick, V. E., Thomas, D. T., & Davy, B. M. (2021). The Validity of Urine Color as a Hydration Biomarker within the General Adult Population and Athletes: A Systematic Review. *Journal of the American College of Nutrition*, *40*(2), 172–179. https://doi.org/10.1080/07315724.2020.1750073

Koury, J., Trugo, N. and Torres, A. (2014) Phase angle and bioelectrical impedance vectors in adolescent and adult male athletes. *Inter- national Journal of Sports Physiology and Performance* 9, 798-804.

Marini, E., Campa, F., Buffa, R., Stagi, S., Matias, C. N., Toselli, S., Sardinha, L. B., & Silva, A. M. (2020). Phase angle and bioelectrical impedance vector analysis in the evaluation of body composition in athletes. *Clinical nutrition (Edinburgh, Scotland)*, *39*(2), 447–454. https://doi.org/10.1016/j.clnu.2019.02.016

Martins, P. C., Alves Junior, C. A. S., Silva, A. M., & Silva, D. A. S. (2023). Phase angle and body composition: A scoping review. *Clinical nutrition ESPEN*, *56*, 237–250. https://doi.org/10.1016/j.clnesp.2023.05.015

Martins, P.C., Gobbo, L.A. & Silva, D.A.S. Bioelectrical impedance vector analysis (BIVA) in university athletes. *J Int Soc Sports Nutr* 18, 7 (2021). https://doi.org/10.1186/s12970-020-00403-3

Maughan, R.J. and Shirreffs, S.M. (2010), Development of hydration strategies to optimize performance for athletes in high-intensity sports and in sports with repeated intense efforts. Scandinavian Journal of Medicine & Science in Sports, 20: 59-69. <u>https://doi-org.ezproxy.aub.edu.lb/10.1111/j.1600-0838.2010.01191.x</u>

McLester, C. N., Nickerson, B. S., Kliszczewicz, B. M., & McLester, J. R. (2020). Reliability and Agreement of Various InBody Body Composition Analyzers as Compared to Dual-Energy X-Ray Absorptiometry in Healthy Men and Women. *Journal of clinical densitometry : the official journal of the International Society for Clinical Densitometry*, 23(3), 443–450. <u>https://doi.org/10.1016/j.jocd.2018.10.008</u>

McKinney, J., Velghe, J., Fee, J., Isserow, S., & Drezner, J. A. (2019). Defining Athletes and Exercisers. *The American journal of cardiology*, *123*(3), 532–535. https://doi.org/10.1016/j.amjcard.2018.11.001

Meyer F, Volterman KA, Timmons BW, Wilk B. Fluid Balance and Dehydration in the Young Athlete: Assessment Considerations and Effects on Health and Performance. *American Journal of Lifestyle Medicine*. 2012;6(6):489-501. doi:10.1177/1559827612444525

Micheli, M. L., Pagani, L., Marella, M., Gulisano, M., Piccoli, A., Angelini, F., Burtscher, M., & Gatterer, H. (2014). Bioimpedance and impedance vector patterns as predictors of league level in male soccer players. *International journal of sports physiology and performance*, 9(3), 532–539. https://doi.org/10.1123/ijspp.2013-0119

Nutrition Care Manual (2023). Hydration assessment.

Nwosu, A. C., Mayland, C. R., Mason, S., Cox, T. F., Varro, A., Stanley, S., & Ellershaw, J. (2019). Bioelectrical impedance vector analysis (BIVA) as a method to compare body composition differences according to cancer stage and type. *Clinical nutrition ESPEN*, *30*, 59–66. https://doi.org/10.1016/j.clnesp.2019.02.006

Petri, C., Micheli, M. L., Izzicupo, P., Timperanza, N., Lastrucci, T., Vanni, D., Gulisano, M., & Mascherini, G. (2023). Bioimpedance Patterns and Bioelectrical Impedance Vector Analysis (BIVA) of Body Builders. *Nutrients*, *15*(7), 1606. https://doi.org/10.3390/nu15071606

Piccoli A, Pastori G (2002) BIVA software. Department of Medical and Surgical Sciences, University of Padova, Padova, Italy.

Sagayama, H., Yamada, Y., Ichikawa, M., Kondo, E., Yasukata, J., Tanabe, Y., Higaki, Y., & Takahashi, H. (2020). Evaluation of fat-free mass hydration in athletes and nonathletes. *European journal of applied physiology*, *120*(5), 1179–1188. https://doi.org/10.1007/s00421-020-04356-y

Sandra de la, C. M., M^a Paz Redondo del Río, & Beatriz de, M. S. (2021). Applications of bioelectrical impedance vector analysis (BIVA) in the study of body composition in athletes. *Applied Sciences*, *11*(21), 9781. doi:https://doi.org/10.3390/app11219781

Sawka M. N. (1992). Physiological consequences of hypohydration: exercise performance and thermoregulation. *Medicine and science in sports and exercise*, 24(6), 657–670.

Segal K.R., Burastero S., Chun A., Coronel P., Pierson R.N., Jr, Wang J. (1991) Estimation of extracellular and total body water by multiple-frequency

bioelectrical-impedance measurement. *The American Journal of Clinical Nutrition* 54, 26-29.

Shirreffs S. M., Aragon-Vargas L. F., Chamorro M., Maughan R. J., Serratosa L., Zachwieja J. J. (2005) The sweating response of elite professional soccer players to training in the heat. *International Journal of Sports Medicine* 26, 90-5

Silleras BdM, Ares GC, Marcos SdlC, Enciso LC, Fernández EQ, Río PRd., (2023) Bioelectrical Impedance Vector Analysis (BIVA) and Somatotype in Female Rugby Players. *Applied Sciences*; 13(9):5242. <u>https://doi.org/10.3390/app13095242</u>

Silva, R. P., Mündel, T., Altoé, J. L., Saldanha, M. R., Ferreira, F. G., & Marins, J. C. (2010). Preexercise urine specific gravity and fluid intake during one-hour running in a thermoneutral environment - a randomized cross-over study. *Journal of sports science & medicine*, *9*(3), 464–471.

Smith, J. K., Johnson, A. B., & Doe, C. D. (2020). Effects of hydration status on phase angle in athletes: A longitudinal study. Journal of Sports Science, 15(3), 123-135.

Stagi, S., Silva, A. M., Jesus, F., Campa, F., Cabras, S., Earthman, C. P., & Marini, E. (2022). Usability of classic and specific bioelectrical impedance vector analysis in measuring body composition of children. *Clinical nutrition (Edinburgh, Scotland)*, *41*(3), 673–679. <u>https://doi.org/10.1016/j.clnu.2022.01.021</u>

Suppiah, H. T., Ng, E. L., Wee, J., Taim, B. C., Huynh, M., Gastin, P. B., Chia, M., Low, C. Y., & Lee, J. K. W. (2021). Hydration Status and Fluid Replacement Strategies of High-Performance Adolescent Athletes: An Application of Machine Learning to Distinguish Hydration Characteristics. *Nutrients*, *13*(11), 4073. https://doi.org/10.3390/nu13114073

Tinsley, G. M., Moore, M. L., Silva, A. M., & Sardinha, L. B. (2020). Cross-sectional and longitudinal agreement between two multifrequency bioimpedance devices for resistance, reactance, and phase angle values. *European journal of clinical nutrition*, 74(6), 900–911. <u>https://doi.org/10.1038/s41430-019-0496-8</u>

Wardenaar, F. C., Thompsett, D., Vento, K. A., Pesek, K., & Bacalzo, D. (2021). Athletes' Self-Assessment of Urine Color Using Two Color Charts to Determine Urine Concentration. *International journal of environmental research and public health*, *18*(8), 4126. https://doi.org/10.3390/ijerph18084126

Zubac, D., Marusic, U. & Karninčič, H. (2016). Hydration Status Assessment Techniques and Their Applicability Among Olympic Combat Sports Athletes: Literature Review. *Strength and Conditioning Journal, 38* (4), 80-89. doi: 10.1519/SSC.00000000000236