## The influence of coffee consumption on bioelectrical impedance parameters: a randomized, double-blind, cross-over trial

Running title: Coffee and bioelectrical impedance
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#### Abstract

Background: Bioelectrical impedance analysis (BIA) is a widely used method for estimating body composition. Avoiding foods/beverages containing caffeine is a frequently enforced pretest protocol to ensure reliability of BIA measurements. However, few studies have evaluated whether this is necessary, with conflicting results. We aimed to determine whether the coffee consumption differing in caffeine content influences BIA parameters in healthy adults. Methods: Twenty-five healthy adults were enrolled in a randomized, double-blind cross-over trial. Three amounts of caffeine were given with 200 mL of coffee: 0 mg ( 11 g of decaffeinated), 200 mg ( 5.5 g of caffeinated plus 5.5 g of decaffeinated) and 400 mg of caffeine ( 11 g of caffeinated). BIA measurements were conducted at 6 different times, and coefficient variations (CV) explored. Results: No differences were observed for group x time interaction on impedance, resistance, or reactance ( $p>0.05$ ). Values of BIA parameters increased after 30-min of coffee consumption, independently of the caffeine dosage (all $p<0.001$ ). Body fat percentage followed the same pattern and increased after $45-\min (p<0.05)$. Median CV for consecutive impedance, resistance, and reactance measurements were $>95 \% \mathrm{CI}$ of expected device measurement error over $70-\mathrm{min}$, without difference between groups. Urine output volume was not different between groups (decaffeinated: $440.45 \pm 197.57 \mathrm{~mL} ; 200 \mathrm{mg}: 471.80 \pm 171.88 \mathrm{~mL} ; 400 \mathrm{mg}: 489.30 \pm 204.10 \mathrm{~mL}$, $p>0.05$ ). Conclusion: Coffee consumption influenced BIA-derived results after 70-min but was not related to caffeine content, likely due to water intake.


## Introduction

Bioelectrical impedance analysis (BIA) is a method that has been extensively used to estimate body composition ${ }^{1}$. It measures the body response of impedance and its components to the passage of an electric current. From these measurements, fluids volumes or body composition can be estimated, based on biophysical models or multiple regression equations ${ }^{2}$.

To ensure the reliability of BIA measurements, several recommendations have been proposed. One recommendation is to avoid caffeine consumption for 4 to $24-\mathrm{h}$ prior to the test day ${ }^{3,4}$. This instruction is primarily based on the potential diuretic effect of caffeine; however, specific mechanisms are not fully understood. Caffeine may act as an adenosine receptor antagonist, increasing glomerular filtration rate by inhibiting the vasoconstriction of the afferent arteriole during tubuloglomerular reflex, inhibiting sodium reabsorption by the proximal convoluted tubule and also inhibiting the hepatorenal reflex via the nerve endings of space of Mall ${ }^{5}$. Coffee is one of the most widely consumed beverages in the world and is also a major source of caffeine ${ }^{6}$, while theophylline and theobromine are found only in traces ${ }^{7-9}$.

Few studies have evaluated the impact of coffee or caffeine intake on BIA measurements, and the literature is conflicting in regard to how they impact BIA parameters. Simplified and evidence-based instructions can avoid unnecessary restrictions and may lead to better participant compliance. Furthermore, eliminating this requirement is important for clinical settings where $a$ priori test preparation may be unfeasible. To the best of our knowledge, this study is the first to explore the effect of coffee consumption (with two different amounts of caffeine) on BIA measurements such as impedance, resistance, and reactance. We aimed to determine whether the amount and time after consumption of coffee influences BIA parameters in healthy adults.

## Materials and methods

## Participants

Twenty-six healthy caffeine product consumers aged 18 to 59 years old with BMI between 18.5 and $29.9 \mathrm{~kg} / \mathrm{m}^{2}$ were recruited from February to July 2019 from the local University community. This study was approved by the University of Alberta and informed consent was obtained from all participants prior to data collection. This study was registered at ClinicalTrials.gov (NCT03745508). Exclusion criteria included: women who were pregnant or lactating; people using certain drugs (i.e. diuretics, steroids, growth hormone) or supplements that affect water balance (i.e. creatine); those who had certain medical conditions known to affect muscle tissue or water balance (e.g. cardiovascular, edema, diabetes, kidney disease, liver disease, chronic obstructive pulmonary disease, cancer); people with any implantable electronic device (e.g. pacemaker, implanted cardiac defibrillator) or participants with hypersensitivity to any of the ingredients of instant coffee. All exclusion criteria above were set to optimize BIA measurements and to ensure participant safety. A total of 25 participants were eligible according to the inclusion and exclusion criteria, however, two participants withdrew due to issues with urine collection and one participant withdrew due to feeling sick (Figure 1).

## Study design

This was a double-blind randomized cross-over trial. Participants ( $54.5 \%$ women) were allocated to ingest coffee containing either 0 mg of caffeine, $\sim 200 \mathrm{mg}$ of caffeine or $\sim 400 \mathrm{mg}$ of caffeine during the first session and repeated the alternative supplementation procedures with a washout period of 24-h (Figure 1). The order of the interventions was randomized using a random-number-generating software system by a researcher not related to data collection. For females, the tests were conducted within the follicular phase of their menstrual cycle and within 14-d of each other where a regular menstrual cycle was not present (i.e. certain birth control
medications/devices) to limit water retention caused by hormone fluctuations. Participants were oriented to fast for $12-\mathrm{h}$, abstain from alcohol and exercise for $24-\mathrm{h}$, and avoid food rich in caffeine (e.g. coffee, tea, chocolate, energy drinks) 12-h prior to all study visits. Participants were instructed to stay well hydrated on the day before the analyses but to avoid consuming water prior to the testing period of their study visit. The habitual caffeine consumption was assessed by the questionnaire proposed by Irons et al. ${ }^{10}$. To analyze the possible effect of habitual caffeine consumption on results, we divided participants into low caffeine consumers ( $\leq$ percentile 50) and high-caffeine consumers ( $>$ percentile 50).

Prior to the first BIA measurement, participants were asked to void their bladder. After that, participants were instructed to lie down in a supine position for 10 -min to control for fluctuations in fluid distribution due to change in body position ${ }^{11,}$. 12 . This instruction was provided prior to each BIA measurement. Baseline BIA measurement was obtained prior to coffee consumption. Afterwards, coffee was given to the participant according to their allocated intervention dose. The participant was instructed to drink the coffee within 20-min of receiving it. After coffee was consumed, BIA measurements were taken at 5 different time intervals separated by $20-\mathrm{min}$ at $10,30,50,70$ and $90-\mathrm{min}$ after coffee consumption. After each BIA measurement, they could sit or stand up for 10-min to avoid the cumulative effect on fluid distribution that can be caused when in the supine position for an extended period of time (and its potential impact on BIA results) ${ }^{11,12}$. They were instructed to not drink or eat anything outside of the coffee provided to them by the study team and to not void their bladder between BIA measurements. Once all BIA measurements were completed, participants were instructed to void their bladder into a labelled specimen container. Urine volume output was measured with a graduated cylinder and values were expressed in total milliliters and later used to calculate
weight-adjusted hourly urine output ${ }^{13}$. The same instructions as described above were repeated for all visits.

## Caffeine content analysis

Nescafé Gold Instant and Roast \& Ground Coffee ${ }^{\odot}$ and Nescafé Gold Blend Decaffeinated Instant Coffee ${ }^{\odot}$ were used for treatments. Instant coffee ( 10 g ) was added to 100 mL of hot purified water at $80^{\circ} \mathrm{C}$ and stirred for $1-\mathrm{min}$. After that, the mixture was centrifuged at 1200 g for 5-min and filtered through a cellulose syringe filter Agilent Captiva Premium Syringe Filter, Regenerated Cellulose, $0.45 \mu \mathrm{~m}, 25 \mathrm{~mm}$. The injection volume was $20 \mu \mathrm{~L}$ and the UV detector was set at 272 nm . A high-performance liquid chromatography method (HPLC, Shimadzu LC-20) equipped with a photodiode array detector (Shimadzu SPD-M20A) was used to determine caffeine content. Separation of compounds was carried out using Agilent Zorbax Eclipse XDB-C18 column ( $150 \times 4.6 \mathrm{~mm}$ ). The calibration was done from $0.15 \mathrm{mg} / \mathrm{ml}$ to $1.125 \mathrm{mg} / \mathrm{ml}$. Mobile phase was composed of water with $2 \%$ of acetic acid (solvent A) and methanol with $2 \%$ acetic acid (solvent B). Solvent program was: $0.1 \mathrm{~min}, 17 \%$ B; $10 \mathrm{~min}, 20 \%$ B; $12 \mathrm{~min}, 20 \% \mathrm{~B} ; 17 \mathrm{~min}, 100 \% \mathrm{~B} ; 22 \mathrm{~min}, 100 \% \mathrm{~B} ; 23 \mathrm{~min} ; 17 \% \mathrm{~B} ; 27 \mathrm{~min}, 17 \%$ B. Data acquisition and analysis was performed using EZ-Start software. Samples were measured in triplicate and the coefficient of variation was $2.4 \%$. The amount of caffeine detected in the regular instant coffee was $3.82 \pm 0.09 \mathrm{mg} / \mathrm{mL}$. No trace of caffeine was detected in the decaffeinated instant coffee.

## Treatment

The participants and researcher were blinded to the caffeine level of the provided beverage. Coffee was prepared by personnel outside of the research team and was given to the participant in an opaque cup with a lid. Dose 1 contained 11 g of decaffeinated coffee ( 0 mg of
caffeine), dose 25.5 g of caffeinated coffee plus 5.5 g of decaffeinated coffee $(\sim 200 \mathrm{mg}$ of caffeine), and dose 3 contained 11 g of caffeinated coffee ( $\sim 400 \mathrm{mg}$ of caffeine). This approach masked any flavour differences between the different caffeine concentrations. The choice of 200 mg of caffeine was based on the approximate dosage found in coffees ${ }^{14}$, and the choice of 400 mg as a way to explore changes with a higher dose. Instant coffees were diluted in 200 ml of boiled water. Participants were instructed to drink the coffee without adding any milk, cream, sugar or sweeteners into their beverage. At the end of treatment, participants were asked to guess which supplement they had received.

## Anthropometric Assessments

Participants were instructed to wear light clothing and remove foot wear for all measurements. Height was measured using a digital stadiometer (QuickMedical Heightronic ${ }^{\circledR}$ 235 Stadiometer, Quick Medical Inc., Snoqualmie, WA, USA) to the nearest 0.1 cm . Participants were asked to stand straight with their back against the stadiometer, ensuring their head is in the Frankfort plane, and their shoulders, buttocks, and heels touch the wall. Body weight was measured using a calibrated digital scale (Health o meter ${ }^{\circledR}$ Professional Remote Display 752KL, Sunbeam Products Inc., Boca Raton, FL, USA, capacity of 227 kgx 0.1 kg ) to the nearest 0.1 kg . Waist circumference was recorded using a non-expandable measuring tape to the nearest 0.1 cm and was measured midway between the lower rib margin and the iliac crest.

## Bioelectrical Impedance Measurements

Body composition and fluid parameters [total body water (BW), intra and extracellular water] were assessed using the Bodystat ${ }^{\circledR}$ QuadScan 4000 (Bodystat Ltd, Isle of Man, British Isles, UK), a multi-frequency BIA technology that records impedance at four frequencies $(5,50$, 100 and 200 kHz ), resistance and reactance at 50 kHz frequency. Impedance at 50 kHz was used
for the calculation of total BW and estimations of fat and lean mass were based using proprietary equations from the manufacturer. The BIA was calibrated before every test day by using a manufacturer calibrator measuring impedance at each frequency and then the quality control of measurement accuracy was checked. A trained research staff used an alcohol swab to cleanse the areas where self-adhesive electrodes were attached on the right side of the participant's body (i.e. right hand and right foot). The alcohol swab was only used once before the first measurement of BIA using electrodes recommended by the company. Accuracies of the device are for impedance ( 5 and 50 kHz ): $\pm 2 \Omega$, impedance ( 100 and 200 kHz ): $\pm 3 \Omega$, resistance $(50 \mathrm{kHz}$ ): $\pm 2 \Omega$, and reactance $(50 \mathrm{kHz}): \pm 1 \Omega$. Technical measurement errors [device coefficient variation (CV)] for impedance, resistance and reactance were calculated based on the three baseline measurements of all participants. The device CV for impedance was determined at 5 kHz ( $2.59 \% 95 \% \mathrm{CI}: 1.81-$ $3.37), 50 \mathrm{kHz}(1.99 \% 95 \% \mathrm{CI}: 1.39-2.59), 100 \mathrm{kHz}(1.91 \% 95 \% \mathrm{CI}: 1.32-2.49)$ and $200 \mathrm{kHz}(1.90 \%$ $95 \% \mathrm{CI}: 1.33-2.47$ ), and at 50 kHz for resistance ( $1.97 \% 95 \% \mathrm{CI}: 1.38-2.55$ ) and reactance ( $4.08 \%$ 95\%CI:2.95-5.22).

## Statistical analysis

The sample size was estimated using the $G^{*}$ Power software (version 3.1.7), taking into consideration impedance variation after water consumption ${ }^{15}$. The value found as reference (effect size of 1.10) showed that with a level of significance of $5 \%$ and statistical power of $90 \%$ (power1- $\beta=0.90$ ), a total of 11 individuals per treatment was required.

Statistical analyses were performed using SPSS (IBM SPSS Statistics v22.0). Data distribution was evaluated using the Shapiro-Wilk test and was presented as mean $\pm$ SD, median (P25-P75) for habitual caffeine consumption or median (P10-P90) for CV. The CV for consecutive measurements after coffee consumption was calculated using the baseline
measurement as reference (CV1: between $1^{\text {st }}$ and $2^{\text {nd }}$ measurements, CV2: between $1^{\text {st }}$ and $3^{\text {nd }}$, CV3: between $1^{\text {st }}$ and $4^{\text {nd }}$, CV4: between $1^{\text {st }}$ and $5^{\text {nd }}, \mathrm{CV} 5$ : between $1^{\text {st }}$ and $6^{\text {nd }}$ ) and compared to the device CV. Repeated measures analysis of variance (adjusted by sex, age and device CV) were performed to explore differences in BIA measurements among different testing conditions and time points. Spearman's rank correlations were also performed between BIA parameters and urine output. The level of significance for all analysis was set at $p<0.05$.

## Results

Table 1 shows the physical characteristics of the participants. A total of 22 participants were screened, $54.5 \%$ were women $(\mathrm{n}=12)$. The caffeine habitual consumption was estimated at $206.50(119.31-280.49) \mathrm{mg} / \mathrm{d}$. The relative consumption of caffeine was $6.04 \pm 0.97 \mathrm{mg} / \mathrm{kg}$ body weight for the 400 mg group and $3.02 \pm 0.48 \mathrm{mg} / \mathrm{kg}$ body weight for the 200 mg group. Only $15.2 \%$ of answers from participants were positive for identification of which dose was administered.

No differences were observed for group x time interaction on impedance, resistance, reactance, impedance standardized for height $(\mathrm{Z} / \mathrm{H})$, or phase angle ( $p>0.05$, Figure 2). Time interaction was verified for most variables, except for phase angle, after the second measurement (post $30-\mathrm{min}$ ), independently of the caffeine dosage (all $p<0.05$ ) which might be related to the water from the coffee. As expected, the results on body fat percentage ( $\% \mathrm{BF}$ ), total BW , intra and extracellular water followed the same pattern (Figure 3). When participants were divided according to the caffeine consumption, no group x time interactions were observed on impedance, resistance, or reactance, independently of the caffeine dosage (data not shown). The median CV for consecutive measurements of impedance, resistance, and reactance were higher than $95 \% \mathrm{CI}$ of the device CV over $70-\mathrm{min}$, without differences between groups (Figure 1 S ).

Total urine output volume also did not differ between groups after treatment (decaf: $440.45 \pm 197.57 \mathrm{~mL} ; \sim 200 \mathrm{mg}: 471.80 \pm 171.88 \mathrm{~mL} ; \sim 400 \mathrm{mg}: 489.30 \pm 204.10 \mathrm{~mL}$, Figure 4) even when adjusted by weight and time (decaf: $4.53 \pm 2.33 \mathrm{~mL} ; \sim 200 \mathrm{mg}: 4.86 \pm 2.21 \mathrm{~mL} ; \sim 400 \mathrm{mg}$ : $5.10 \pm 2.54 \mathrm{~mL}, p=0.720)$. Urine output was not correlated with changes in impedance at $5 \mathrm{kHz}(\mathrm{r}=$ $-0.019, p=0.879), 50 \mathrm{kHz}(\mathrm{r}=-0.089, p=0.477), 100 \mathrm{kHz}(\mathrm{r}=0.156, p=0.211)$ and $200 \mathrm{kHz}(\mathrm{r}=$ $0.131, p=0.296)$, resistance $(\mathrm{r}=-0.083, p=0.508)$, and reactance $(\mathrm{r}=-0.118, p=0.344)$.

## Discussion

This is the first trial testing the effects of different amounts of caffeine from coffee on BIA measurements. This study demonstrated that coffee consumption did not influence urine output and estimates of impedance, resistance, and reactance of the BIA over a short time period. While time interactions for consecutive BIA measurements were observed after ingestion of coffee, they were not correlated with the amount of caffeine. Additionally, the CV for BIA parameters did not exceed $95 \% \mathrm{CI}$ of the device CV throughout 70-min, suggesting that water intake influences BIA values only after that time point.

The effects of caffeine on BIA-derived $\% \mathrm{BF}$ and BW were previously investigated in habitual caffeine users ${ }^{16}$. The authors concluded that 200 mg of caffeine promoted trivial changes on fat percentage and water parameters. However, it is important to note that each device use specific formulas, which are often not disclosed by the company. The analysis of BIA crude parameters seems to be more suitable since the altered parameter can be taken into account when choosing the formula for body composition analysis. Our study verified that coffee consumption, independent of caffeine amount, did not influence estimates of impedance, resistance, and reactance values, which probably will not affect body composition analysis by different BIAderived formulas.

Impedance is expressed by parallel-equivalent combinations of resistance and capacitance and frequently used to estimate total BW and other body compartments ${ }^{17}$. In this study, impedance values increased in all groups after 70-min of coffee consumption ( 200 mL for all treatments), using the $95 \% \mathrm{CI}$ of device CV as a comparison. Our findings were consistent with those found by Androutsos et al. $(2015)^{4}$, where impedance increased immediately after 750 ml of water consumption and persisted elevated throughout a $120-\mathrm{min}$ time period. The time of impedance changes may be associated with the volume of water ingested by the participants. However, the authors concluded that impedance changes were reflective of small variations of $\% \mathrm{BF}$, within the imprecision of the impedance technique, and that would probably not have clinical significance. Thus, it is suggested that body composition analysis by BIA does not require strict adherence to fasting, which increases the opportunities for clinical application ${ }^{4}$. Our study showed that the impact of water intake on BIA results is time-dependent (after 70-min); therefore, fluid ingestion should be controlled for immediately prior to the test. Furthermore, recommendations to perform the BIA test after 2-h of food or drink ingestion should be reconsidered as it may not be suitable. Williamson et al. (2018) ${ }^{16}$ did not observe differences in total BW providing a similar amount of water as the current study. It is important to mention that procedural details were not provided in this study. As such, we do not know how long the participants remained in a supine position prior to the BIA assessment. Evidence suggests that impedance goes back to the initial value after lying down for 5 to 10 min due to changes in the interstitial fluid ${ }^{11,12,18}$.

In recreationally active adults, $\% \mathrm{BF}(+1.1 \%)$ and impedance $(+12 \Omega)$ increased $20-\mathrm{min}$ after 591 ml of water consumption using a segmental bioelectrical impedance analysis. In the control group, which received nothing, $\% \mathrm{BF}(+0.3$ and $+0.5 \%)$ and impedance ( +7 and $+11 \Omega$ )
also increased at $40-$ and $60-\mathrm{min}$, respectively, compared to baseline values ${ }^{15}$. In the current study, impedance increased higher than the device technical error in the decaf group $(9.91 \pm 6.38$ and $15.32 \pm 8.27 \Omega$ ), the 200 mg group ( $10.45 \pm 6.95$ and $15.14 \pm 8.76 \Omega$ ), and in the 400 mg group (12.50 $\pm 6.49$ and $17.36 \pm 7.92 \Omega$ ) at $50-$ and $70-\mathrm{min}$ after coffee consumption, respectively, which were similar to variations found in the control group from the Dixon's study (7 to $11 \Omega)^{15}$. In addition, the authors also observed greatest impedance increases in females with lower body weight ${ }^{15}$. Interestingly, we observed the same pattern in our study and speculated two possible explanations. Although resistance is inversely related to volume, the result was the opposite of what was expected. It is known that there is a direct relationship between the concentrations of ions and the electrical conductivity, and an indirect relationship exists between the ion concentration and the resistance of the solution ${ }^{19}$. Therefore, it is possible that water intake might have altered ion status, which would directly impact resistance and impedance values over time. However, to prove this effect, specific electrolytes (e.g. sodium, potassium, and chloride) in plasma/ serum and urine should be determined, which was not possible for this study. Nonetheless, this may not explain the findings as the volume of fluid in coffee ( 200 mL ) is insignificant relative to the estimated total BW of females (31 L) and males (39 L). In fact, the relative gain in water (coffee/TBW, \%) would be 0.6 and $0.5 \%$, respectively. Thus, considering the technical error of $\sim 2 \%$, BIA cannot technically track the increase in fluid volume from coffee consumption. The second speculation would be related to a resistivity change due to chemical components of coffee. Unfortunately, we did not have a control treatment (water alone).

The recommendation to avoid coffee consumption before BIA assessment is based mainly on the possibility of dehydration due to caffeine consumption ${ }^{11}$. In this study, urine output did not differ between groups after 120 min of coffee consumption. Seal et al. (2017) ${ }^{20}$
showed that only high caffeine content ( $6 \mathrm{mg} / \mathrm{kg}$ of body weight) induced an acute diuretic effect at rest. Considering the caffeine content for our sample, the amount would be slightly higher than 400 mg of caffeine; however, the average body weight in our study was quite lower than that reported in Seal's study ( $68.5 \pm 12.8$ vs $89.5 \pm 14.8 \mathrm{~kg}$ ), which significantly impacts the total amount of caffeine ( $\sim 400 \mathrm{mg}$ vs $\sim 537 \mathrm{mg}$ ). On the other hand, low caffeine consumption ( $3 \mathrm{mg} / \mathrm{kg}$ of body weight, $269 \pm 45 \mathrm{mg}$ ) did not differ from the control group ( 200 ml of water) for cumulative urinary osmotic excretion and diuresis during the 3-h period ${ }^{20}$.

In spite of what was discussed above, the impact of high amounts on caffeine on dehydration is controversial. In another clinical trial, doses of caffeine up to $6 \mathrm{mg} / \mathrm{kg}$ of body weight for 11-d had no influence on fluid, electrolyte, and renal indices of hydration in healthy males ${ }^{21}$. These findings were supported by a counterbalanced cross-over study which compared the effects of coffee consumption ( $800 \mathrm{~mL} / \mathrm{d}$ ) containing $4 \mathrm{mg} / \mathrm{kg}$ of body weight of caffeine against water ingestion for 3-d. There were no significant differences across a wide range of hematological and urinary markers of hydration status. The authors suggested that moderate consumption of coffee provided similar hydrating qualities to water ${ }^{22}$.

The absence of caffeine-induced diuresis might be explained by the habitual consumption of caffeine-containing products. Clinical studies investigating the effects of caffeine on fluid balance in habitual coffee drinkers ( 1 to $6 \mathrm{cups} / \mathrm{d}$ ) concluded that caffeine did not promote diuresis ${ }^{20-23}$. In the present study, participants were habitual coffee drinkers and consumed caffeine-containing products (e.g. chocolate, soda, tea). These findings are also corroborated in men who normally consumed less than $100 \mathrm{mg} /$ d caffeine $^{23}$. In a double-blind, randomized, crossover trial, men ingested $5 \mathrm{mg} / \mathrm{kg}$ of body weight $/ \mathrm{d}$ of caffeine for four consecutive days and total BW, extra and intracellular water did not differ from the control group (maltodextrin) ${ }^{23}$.

Total BW and extracellular water were measured by deuterium oxide and sodium bromide dilution, respectively, whereas intracellular water was calculated by subtracting extracellular water from total BW.

The volume of urine output in participants who consumed caffeine was similar between the studies discussed above ${ }^{21-23}$, except in the high caffeine content group analyzed by Seal et al. ${ }^{20}$. These findings might suggest that caffeine consumption up to $\sim 500 \mathrm{mg}$ (or $\sim 5$ to 6 cups of coffee) does not impact hydration in healthy adults who are habitual consumers of caffeine. A review concluded that there would appear to be no clear basis for refraining from caffeinecontaining drinks in situations where fluid balance might be compromised ${ }^{24}$. A meta-analysis on caffeine-induced diuresis in healthy adults during rest and exercise showed that caffeine exerted a small diuretic effect at rest; however, with a greater probability in females ${ }^{25}$. Although the median caffeine consumption was 300 mg , the range was wide ( $114-741 \mathrm{mg}$ ) and some studies did not provide the relative caffeine dosages. We did not observe difference in urine output between males and females in our study, suggesting further studies with a larger sample size and different amounts of caffeine or coffee are needed to confirm these findings.

The device CV for impedance and resistance in the current study was corroborated by another study. Using the same device as in the current trial, the authors found that the betweenday device CV for impedance was $0.9 \%-1.8 \%{ }^{26}$ in healthy adults of Asian ethnicity. In healthy subjects assessed by an eight-point tactile-electrode impedance method, the between-day device CV for resistance was $<2.8 \%$ for all segments and frequencies ${ }^{27}$.

This study has potential limitations. We did not quantify the amount of methylxanthines present in the coffee. However, it has been previously described that caffeine is the main methylxanthine in coffee and that theophylline and theobromine are found only in trace
amounts ${ }^{7-9}$. Thus, it is expected that the possible diuretic effect of coffee might be related to caffeine. The second limitation concerns the lack of inclusion of other control groups - one ingesting only water and another not consuming water prior to BIA assessment. A group ingesting only water would confirm the absence of an acute diuretic effect of caffeine and the effects observed in the decaf group. On the other hand, a group without consuming water prior to measurement would confirm the effects found in the current study are due to water intake. However, the inclusion of any of these groups would impair the double-blind nature of the study design. Although, we instructed the participants to stay well hydrated before visits, measurement of urine gravity would have been important to estimate hydration state.

Coffee consumption influenced impedance, resistance and reactance BIA-derived results, which were not related to the caffeine content. There was a probable influence of water intake on BIA parameters after 70-min. Additionally, coffee consumption did not exhibit a diuretic effect. Further studies are needed to corroborate the findings of the present study.

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## Conflict of Interest

None to declare.

## Author Contributions

JFM, CT, and CMP: designed the research; JFM and JRBT: product acquisition; GLO and CT: performed data collection; JFM: performed the statistical analyses; HL, JFM and MCG: analyzed and interpreted the data; JFM and JRBT: wrote the first draft of the manuscript; CMP, CT, GLO, HL and MCG: reviewed the manuscript, contributed to the discussion. All authors were involved in editing the manuscript and read and approved the final manuscript.

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## References

1. Lee SY, Gallagher D. Assessment methods in human body composition. Curr Opin Clin Nutr Metab Care. 2008; 11: 566-72.
2. Lukaski HC, Vega Diaz N, Talluri A, Nescolarde L. Classification of Hydration in Clinical Conditions: Indirect and Direct Approaches Using Bioimpedance. Nutrients. 2019; 11(4).
3. Bera TK. Bioelectrical Impedance Methods for Noninvasive Health Monitoring: A Review. J Med Eng. 2014; 2014: 381251.
4. Androutsos O, Gerasimidis K, Karanikolou A, Reilly JJ, Edwards CA. Impact of eating and drinking on body composition measurements by bioelectrical impedance. J Hum Nutr Diet. 2015; 28: 165-71.
5. Marx B, Scuvee E, Scuvee-Moreau J, Seutin V, Jouret F. Mechanisms of caffeine-induced diuresis. Med Sci. 2016; 32: 485-90.
6. Reyes CM, Cornelis MC. Caffeine in the Diet: Country-Level Consumption and Guidelines. Nutrients. 2018; 10(11).
7. Blauch JL, Tarka Jr. SM. HPLC Determination of Caffeine and Theobromine in Coffee, Tea, and Instant Hot Cocoa Mixes. J Food Sci. 1983; 48: 745-7.
8. Stavric B. Methylxanthines: toxicity to humans. 1. Theophylline. Food Chem Toxicol. 1988; 26: 541-65.
9. Hackett J, Telepchak MJ, Coyer MJ. Analysis of total caffeine and other xanthines in specialty coffees using mixed mode solid-phase extraction and liquid chromatography-diodearray detection after microwave digestion. J Anal Toxicol. 2008; 32: 695-701.
10. Irons JGB, Bassett DT, Prendergast CO, Landrum RE, Heinz AJ. Development and Initial Validation of the Caffeine Consumption Questionnaire-Revised. J Caffeine Res. 2016; 6: 205.
11. Earthman CP. Body Composition Tools for Assessment of Adult Malnutrition at the Bedside: A Tutorial on Research Considerations and Clinical Applications. JPEN J Parenter Enteral Nutr. 2015; 39: 787-822.
12. Price KL, Earthman CP. Update on body composition tools in clinical settings: computed tomography, ultrasound, and bioimpedance applications for assessment and monitoring. Eur $J$ Clin Nutr. 2019; 73: 187-93.
13. Kellum JA, Lameire N. Diagnosis, evaluation, and management of acute kidney injury: a KDIGO summary (Part 1). Crit Care. 2013; 17: 204.
14. McCusker RR, Goldberger BA, Cone EJ. Caffeine content of specialty coffees. J Anal Toxicol. 2003; 27: 520-2.
15. Dixon CB, Ramos L, Fitzgerald E, Reppert D, Andreacci JL. The effect of acute fluid consumption on measures of impedance and percent body fat estimated using segmental bioelectrical impedance analysis. Eur J Clin Nutr. 2009; 63: 1115-22.
16. Williamson CM, Nickerson BS, Bechke EE, McLester CN, Kliszczewicz BM. Influence of acute consumption of caffeine vs. placebo over Bia-derived measurements of body composition: a randomized, double-blind, crossover design. J Int Soc Sports Nutr. 2018; 15: 7.
17. Foster KR, Lukaski HC. Whole-body impedance--what does it measure? Am J Clin Nutr. 1996; 64: 388s-96s.
18. Kushner RF, Gudivaka R, Schoeller DA. Clinical characteristics influencing bioelectrical impedance analysis measurements. Am J Clin Nutr. 1996; 64: 423s-7s.
19. Dehghan M, Merchant AT. Is bioelectrical impedance accurate for use in large epidemiological studies? Nutr J. 2008; 7: 26.
20. Seal AD, Bardis CN, Gavrieli A, Grigorakis P, Adams JD, Arnaoutis G et al. Coffee with High but Not Low Caffeine Content Augments Fluid and Electrolyte Excretion at Rest. Front Nutr. 2017; 4: 40.
21. Armstrong LE, Pumerantz AC, Roti MW, Judelson DA, Watson G, Dias JC et al. Fluid, electrolyte, and renal indices of hydration during 11 days of controlled caffeine consumption. Int J Sport Nutr Exerc Metab. 2005; 15: 252-65.
22. Killer SC, Blannin AK, Jeukendrup AE. No evidence of dehydration with moderate daily coffee intake: a counterbalanced cross-over study in a free-living population. PLoS One. 2014; 9(1): e84154.
23. Silva AM, Judice PB, Matias CN, Santos DA, Magalhaes JP, St-Onge MP et al. Total body water and its compartments are not affected by ingesting a moderate dose of caffeine in healthy young adult males. Appl Physiol Nutr Metab. 2013; 38: 626-32.
24. Maughan RJ, Griffin J. Caffeine ingestion and fluid balance: a review. J Hum Nutr Diet. 2003; 16: 411-20.
25. Zhang Y, Coca A, Casa DJ, Antonio J, Green JM, Bishop PA. Caffeine and diuresis during rest and exercise: A meta-analysis. J Sci Med Sport. 2015; 18: 569-74.
26. Lu HK, Chiang LM, Chen YY, Chuang CL, Chen KT, Dwyer GB et al. Hand-to-Hand Model for Bioelectrical Impedance Analysis to Estimate Fat Free Mass in a Healthy Population. Nutrients. 2016; 8(10).
27. Bedogni G, Malavolti M, Severi S, Poli M, Mussi C, Fantuzzi AL et al. Accuracy of an eight-point tactile-electrode impedance method in the assessment of total body water. Eur J Clin Nutr. 2002; 56: 1143-8.

Table 1. Baseline characteristics of participants.

|  | All | Females | Males |
| :--- | :---: | :---: | :---: |
|  | $\mathrm{n}=22$ | $\mathrm{n}=12$ | $\mathrm{n}=10$ |
| Age $(\mathrm{y})$ | $27 \pm 6$ | $27 \pm 6$ | $28 \pm 6$ |
| Body mass $(\mathrm{kg})$ | $68.5 \pm 12.8$ | $60.7 \pm 9.4$ | $78.0 \pm 9.8$ |
| Body mass index $\left(\mathrm{kg} / \mathrm{m}^{2}\right)$ | $23.7 \pm 2.9$ | $22.6 \pm 3.1$ | $25.1 \pm 2.1$ |
| Waist circumference $(\mathrm{cm})$ | $74.7 \pm 8.5$ | $69.4 \pm 6.8$ | $81.1 \pm 5.6$ |
| Impedance at $50 \mathrm{kHz}(\Omega)$ | $549.3 \pm 92.0$ | $624.1 \pm 46.9$ | $459.6 \pm 32.3$ |
| Resistance at $50 \mathrm{kHz}(\Omega)$ | $545.3 \pm 92.3$ | $620.4 \pm 46.7$ | $455.1 \pm 32.1$ |
| Reactance at $50 \mathrm{kHz}(\Omega)$ | $64.9 \pm 6.6$ | $66.7 \pm 6.7$ | $62.8 \pm 5.7$ |

Data were described by mean $\pm$ standard deviation.

## Figure captions

Figure 1. Study flowchart.
Figure 2. Influence of coffee consumption with different doses of caffeine on impedance at $5 \mathrm{kHz}(\mathrm{A}), 50 \mathrm{kHz}(\mathrm{B}), 100 \mathrm{kHz}(\mathrm{C})$ and $200 \mathrm{kHz}(\mathrm{D})$, resistance (E), reactance (F), impedance standardized for height in meters $(\mathrm{Z} / \mathrm{H})(\mathrm{G})$, and phase angle $(\mathrm{H})$ after consecutive measurements of bioelectrical impedance analysis. ${ }^{*} p<0.05$ for time interaction.

Figure 3. Influence of coffee consumption with different doses of caffeine on body fat percentage (A), total body water (B), intra (C) and extracellular water (D). ${ }^{*} p<0.05$ for time interaction.

Figure 4. Total urine output after coffee consumption with different doses of caffeine.


| Analyzed $(\mathrm{n}=22)$ |
| :--- |
| Withdrew $(\mathrm{n}=3)$ |
| - Feeling sick $(\mathrm{n}=1)$ |
| - Issues collecting urine |
| sample $(\mathrm{n}=2)$ |



Decaff

- ~ 200 mg

ـ $\sim 400 \mathrm{mg}$



