1	The influence of coffee consumption on bioelectrical impedance parameters: a randomized,
2	double-blind, cross-over trial
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4	Running title: Coffee and bioelectrical impedance
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6	João F. Mota ^{1, 2*} , Maria Cristina Gonzalez ³ , Henry Lukaski ⁴ , Gabriela L. Oto ¹ , Claire F.
7	Trottier ¹ , Jenneffer R. B. Tibaes ^{1,5} , Carla M. Prado ^{1*}
8	¹ Department of Agricultural, Food and Nutritional Science, University of Alberta, Li Ka Shing
9	Centre for Health Research Innovation, Edmonton, AB, T6G 2E1, Canada.
10	² Clinical and Sports Nutrition Research Laboratory (LABINCE), Faculty of Nutrition, Federal
11	University of Goiás, Goiânia, GO, 74.605-080, Brazil.
12	³ Postgraduate Program in Health and Behavior, Catholic University of Pelotas, Pelotas, RS,
13	96055-800, Brazil.
14	⁴ Department of Kinesiology and Public Health Education, University of North Dakota, Grand
15	Forks, ND 58202, USA.
16	⁵ Department of Food Science, Faculty of Pharmacy, Federal University of Minas Gerais, Belo
17	Horizonte, Brazil. Rua Professor Moacir Gomes de Freitas – Pampulha, Belo Horizonte – MG,
18	31270-901, Brazil.
19	*Co-corresponding authors: JF Mota and CM Prado. University of Alberta, 3158, Department of
20	Agricultural, Food and Nutritional Science, 2-004 Li Ka Shing Center for Health Research
21	Innovation, University of Alberta, Office 2-021E, Edmonton, Alberta, Canada, T6G 2E1. Email:
22	joao_mota@ufg.br, carla.prado@ualberta.ca. Phone: +1 (780) 492-7820.
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25 Abstract

26 Background: Bioelectrical impedance analysis (BIA) is a widely used method for estimating 27 body composition. Avoiding foods/beverages containing caffeine is a frequently enforced pre-28 test protocol to ensure reliability of BIA measurements. However, few studies have evaluated 29 whether this is necessary, with conflicting results. We aimed to determine whether the coffee 30 consumption differing in caffeine content influences BIA parameters in healthy adults. Methods: 31 Twenty-five healthy adults were enrolled in a randomized, double-blind cross-over trial. Three 32 amounts of caffeine were given with 200mL of coffee: 0mg (11g of decaffeinated), 200mg (5.5g 33 of caffeinated plus 5.5g of decaffeinated) and 400mg of caffeine (11g of caffeinated). BIA 34 measurements were conducted at 6 different times, and coefficient variations (CV) explored. 35 **Results:** No differences were observed for group x time interaction on impedance, resistance, or 36 reactance (p > 0.05). Values of BIA parameters increased after 30-min of coffee consumption, 37 independently of the caffeine dosage (all p < 0.001). Body fat percentage followed the same 38 pattern and increased after 45-min ($p \le 0.05$). Median CV for consecutive impedance, resistance, 39 and reactance measurements were >95%CI of expected device measurement error over 70-min, 40 without difference between groups. Urine output volume was not different between groups 41 (decaffeinated: 440.45±197.57mL; 200mg: 471.80±171.88mL; 400mg: 489.30±204.10mL, 42 p>0.05). Conclusion: Coffee consumption influenced BIA-derived results after 70-min but was 43 not related to caffeine content, likely due to water intake.

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48 Introduction

Bioelectrical impedance analysis (BIA) is a method that has been extensively used to estimate body composition¹. 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 equations².

53 To ensure the reliability of BIA measurements, several recommendations have been 54 proposed. One recommendation is to avoid caffeine consumption for 4 to 24-h prior to the test day^{3, 4}. This instruction is primarily based on the potential diuretic effect of caffeine; however, 55 56 specific mechanisms are not fully understood. Caffeine may act as an adenosine receptor 57 antagonist, increasing glomerular filtration rate by inhibiting the vasoconstriction of the afferent 58 arteriole during tubuloglomerular reflex, inhibiting sodium reabsorption by the proximal 59 convoluted tubule and also inhibiting the hepatorenal reflex via the nerve endings of space of Mall⁵. Coffee is one of the most widely consumed beverages in the world and is also a major 60 source of caffeine⁶, while theophylline and theobromine are found only in traces⁷⁻⁹. 61

62 Few studies have evaluated the impact of coffee or caffeine intake on BIA measurements, 63 and the literature is conflicting in regard to how they impact BIA parameters. Simplified and 64 evidence-based instructions can avoid unnecessary restrictions and may lead to better participant 65 compliance. Furthermore, eliminating this requirement is important for clinical settings where a 66 *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 67 68 measurements such as impedance, resistance, and reactance. We aimed to determine whether the 69 amount and time after consumption of coffee influences BIA parameters in healthy adults.

70 Materials and methods

71 Participants

72 Twenty-six healthy caffeine product consumers aged 18 to 59 years old with BMI between 18.5 and 29.9kg/m² were recruited from February to July 2019 from the local University 73 74 community. This study was approved by the University of Alberta and informed consent was 75 obtained from all participants prior to data collection. This study was registered at 76 ClinicalTrials.gov (NCT03745508). Exclusion criteria included: women who were pregnant or 77 lactating; people using certain drugs (i.e. diuretics, steroids, growth hormone) or supplements 78 that affect water balance (i.e. creatine); those who had certain medical conditions known to 79 affect muscle tissue or water balance (e.g. cardiovascular, edema, diabetes, kidney disease, liver 80 disease, chronic obstructive pulmonary disease, cancer); people with any implantable electronic 81 device (e.g. pacemaker, implanted cardiac defibrillator) or participants with hypersensitivity to 82 any of the ingredients of instant coffee. All exclusion criteria above were set to optimize BIA 83 measurements and to ensure participant safety. A total of 25 participants were eligible according 84 to the inclusion and exclusion criteria, however, two participants withdrew due to issues with 85 urine collection and one participant withdrew due to feeling sick (Figure 1).

86 Study design

This was a double-blind randomized cross-over trial. Participants (54.5% women) were allocated to ingest coffee containing either 0mg of caffeine, ~200mg of caffeine or ~400mg 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

94 medications/devices) to limit water retention caused by hormone fluctuations. Participants were 95 oriented to fast for 12-h, abstain from alcohol and exercise for 24-h, and avoid food rich in 96 caffeine (e.g. coffee, tea, chocolate, energy drinks) 12-h prior to all study visits. Participants 97 were instructed to stay well hydrated on the day before the analyses but to avoid consuming 98 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 99 100 habitual caffeine consumption on results, we divided participants into low caffeine consumers 101 (<percentile 50) and high-caffeine consumers (>percentile 50).

102 Prior to the first BIA measurement, participants were asked to void their bladder. After 103 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 104 105 provided prior to each BIA measurement. Baseline BIA measurement was obtained prior to 106 coffee consumption. Afterwards, coffee was given to the participant according to their allocated 107 intervention dose. The participant was instructed to drink the coffee within 20-min of receiving 108 it. After coffee was consumed, BIA measurements were taken at 5 different time intervals 109 separated by 20-min at 10, 30, 50, 70 and 90-min after coffee consumption. After each BIA 110 measurement, they could sit or stand up for 10-min to avoid the cumulative effect on fluid 111 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 112 113 of the coffee provided to them by the study team and to not void their bladder between BIA 114 measurements. Once all BIA measurements were completed, participants were instructed to void 115 their bladder into a labelled specimen container. Urine volume output was measured with a 116 graduated cylinder and values were expressed in total milliliters and later used to calculate 117 weight-adjusted hourly urine output¹³. The same instructions as described above were repeated
118 for all visits.

119 *Caffeine content analysis*

Nescafé Gold Instant and Roast & Ground Coffee[®] and Nescafé Gold Blend 120 Decaffeinated Instant Coffee[©] were used for treatments. Instant coffee (10 g) was added to 100 121 122 mL of hot purified water at 80°C and stirred for 1-min. After that, the mixture was centrifuged at 123 1200 g for 5-min and filtered through a cellulose syringe filter Agilent Captiva Premium Syringe 124 Filter, Regenerated Cellulose, 0.45µm, 25mm. The injection volume was 20 µL and the UV 125 detector was set at 272nm. A high-performance liquid chromatography method (HPLC, 126 Shimadzu LC-20) equipped with a photodiode array detector (Shimadzu SPD-M20A) was used 127 to determine caffeine content. Separation of compounds was carried out using Agilent Zorbax Eclipse XDB-C18 column (150 \times 4.6 mm). The calibration was done from 0.15mg/ml to 128 129 1.125mg/ml. Mobile phase was composed of water with 2% of acetic acid (solvent A) and 130 methanol with 2% acetic acid (solvent B). Solvent program was: 0.1min, 17% B; 10min, 20% B; 131 12min, 20% B; 17min, 100% B; 22min, 100% B; 23min; 17% B; 27min, 17% B. Data 132 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 133 134 regular instant coffee was 3.82±0.09mg/mL. No trace of caffeine was detected in the 135 decaffeinated instant coffee.

136 Treatment

137 The participants and researcher were blinded to the caffeine level of the provided 138 beverage. Coffee was prepared by personnel outside of the research team and was given to the 139 participant in an opaque cup with a lid. Dose 1 contained 11g of decaffeinated coffee (0mg of

140 caffeine), dose 2 5.5g of caffeinated coffee plus 5.5g of decaffeinated coffee (~200mg of 141 caffeine), and dose 3 contained 11g of caffeinated coffee (~400mg of caffeine). This approach 142 masked any flavour differences between the different caffeine concentrations. The choice of 200 mg of caffeine was based on the approximate dosage found in coffees¹⁴, and the choice of 400 143 mg as a way to explore changes with a higher dose. Instant coffees were diluted in 200ml of 144 145 boiled water. Participants were instructed to drink the coffee without adding any milk, cream, 146 sugar or sweeteners into their beverage. At the end of treatment, participants were asked to guess 147 which supplement they had received.

148 Anthropometric Assessments

149 Participants were instructed to wear light clothing and remove foot wear for all 150 measurements. Height was measured using a digital stadiometer (OuickMedical Heightronic[®] 151 235 Stadiometer, Quick Medical Inc., Snoqualmie, WA, USA) to the nearest 0.1cm. Participants 152 were asked to stand straight with their back against the stadiometer, ensuring their head is in the 153 Frankfort plane, and their shoulders, buttocks, and heels touch the wall. Body weight was 154 measured using a calibrated digital scale (Health o meter® Professional Remote Display 752KL, 155 Sunbeam Products Inc., Boca Raton, FL, USA, capacity of 227kgx0.1kg) to the nearest 0.1kg. 156 Waist circumference was recorded using a non-expandable measuring tape to the nearest 0.1cm 157 and was measured midway between the lower rib margin and the iliac crest.

158 Bioelectrical Impedance Measurements

Body composition and fluid parameters [total body water (BW), intra and extracellular water] were assessed using the Bodystat[®] 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 200kHz), resistance and reactance at 50kHz frequency. Impedance at 50kHz was used

163 for the calculation of total BW and estimations of fat and lean mass were based using proprietary 164 equations from the manufacturer. The BIA was calibrated before every test day by using a 165 manufacturer calibrator measuring impedance at each frequency and then the quality control of 166 measurement accuracy was checked. A trained research staff used an alcohol swab to cleanse the 167 areas where self-adhesive electrodes were attached on the right side of the participant's body (i.e. 168 right hand and right foot). The alcohol swab was only used once before the first measurement of 169 BIA using electrodes recommended by the company. Accuracies of the device are for impedance 170 (5 and 50kHz): $\pm 2\Omega$, impedance (100 and 200kHz): $\pm 3\Omega$, resistance (50 kHz): $\pm 2\Omega$, and 171 reactance (50kHz): $\pm 1\Omega$. Technical measurement errors [device coefficient variation (CV)] for 172 impedance, resistance and reactance were calculated based on the three baseline measurements 173 of all participants. The device CV for impedance was determined at 5kHz (2.59% 95%CI:1.81-174 3.37), 50kHz (1.99% 95%CI:1.39-2.59), 100kHz (1.91% 95%CI:1.32-2.49) and 200kHz (1.90% 175 95%CI:1.33-2.47), and at 50kHz for resistance (1.97% 95%CI:1.38-2.55) and reactance (4.08% 176 95%CI:2.95-5.22).

177 Statistical analysis

The sample size was estimated using the G*Power software (version 3.1.7), taking into consideration impedance variation after water consumption¹⁵. 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- β =0.90), a total of 11 individuals per treatment was required.

182 Statistical analyses were performed using SPSS (IBM SPSS Statistics v22.0). Data 183 distribution was evaluated using the Shapiro-Wilk test and was presented as mean±SD, median 184 (P25–P75) for habitual caffeine consumption or median (P10–P90) for CV. The CV for 185 consecutive measurements after coffee consumption was calculated using the baseline measurement as reference (CV1: between 1st and 2nd measurements, CV2: between 1st and 3nd, CV3: between 1st and 4nd, CV4: between 1st and 5nd, CV5: between 1st and 6nd) 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.

192 **Results**

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

199 No differences were observed for group x time interaction on impedance, resistance, 200 reactance, impedance standardized for height (Z/H), or phase angle (p>0.05, Figure 2). Time 201 interaction was verified for most variables, except for phase angle, after the second measurement 202 (post 30-min), independently of the caffeine dosage (all p < 0.05) which might be related to the 203 water from the coffee. As expected, the results on body fat percentage (%BF), total BW, intra 204 and extracellular water followed the same pattern (Figure 3). When participants were divided 205 according to the caffeine consumption, no group x time interactions were observed on 206 impedance, resistance, or reactance, independently of the caffeine dosage (data not shown). The 207 median CV for consecutive measurements of impedance, resistance, and reactance were higher 208 than 95%CI of the device CV over 70-min, without differences between groups (Figure 1S).

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

215 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%CI of the device CV throughout 70-min, suggesting that water intake influences BIA values only after that time point.

223 The effects of caffeine on BIA-derived %BF and BW were previously investigated in habitual caffeine users¹⁶. The authors concluded that 200mg of caffeine promoted trivial changes 224 225 on fat percentage and water parameters. However, it is important to note that each device use 226 specific formulas, which are often not disclosed by the company. The analysis of BIA crude 227 parameters seems to be more suitable since the altered parameter can be taken into account when 228 choosing the formula for body composition analysis. Our study verified that coffee consumption, 229 independent of caffeine amount, did not influence estimates of impedance, resistance, and 230 reactance values, which probably will not affect body composition analysis by different BIA-231 derived formulas.

232 Impedance is expressed by parallel-equivalent combinations of resistance and capacitance and frequently used to estimate total BW and other body compartments¹⁷. In this study, 233 234 impedance values increased in all groups after 70-min of coffee consumption (200 mL for all 235 treatments), using the 95%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 750ml 236 237 of water consumption and persisted elevated throughout a 120-min time period. The time of 238 impedance changes may be associated with the volume of water ingested by the participants. 239 However, the authors concluded that impedance changes were reflective of small variations of 240 %BF, within the imprecision of the impedance technique, and that would probably not have 241 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 application⁴. Our 242 243 study showed that the impact of water intake on BIA results is time-dependent (after 70-min); 244 therefore, fluid ingestion should be controlled for immediately prior to the test. Furthermore, 245 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 246 247 total BW providing a similar amount of water as the current study. It is important to mention that 248 procedural details were not provided in this study. As such, we do not know how long the 249 participants remained in a supine position prior to the BIA assessment. Evidence suggests that 250 impedance goes back to the initial value after lying down for 5 to 10min due to changes in the interstitial fluid^{11, 12, 18}. 251

In recreationally active adults, %BF (+1.1%) and impedance (+12 Ω) increased 20-min after 591ml of water consumption using a segmental bioelectrical impedance analysis. In the control group, which received nothing, %BF (+0.3 and +0.5%) and impedance (+7 and +11 Ω)

also increased at 40- and 60-min, respectively, compared to baseline values¹⁵. In the current 255 256 study, impedance increased higher than the device technical error in the decaf group (9.91±6.38 257 and $15.32\pm8.27\Omega$), the 200mg group (10.45±6.95 and 15.14±8.76 Ω), and in the 400mg group 258 $(12.50\pm6.49 \text{ and } 17.36\pm7.92\Omega)$ at 50- and 70-min after coffee consumption, respectively, which were similar to variations found in the control group from the Dixon's study (7 to 11Ω)¹⁵. In 259 260 addition, the authors also observed greatest impedance increases in females with lower body weight¹⁵. Interestingly, we observed the same pattern in our study and speculated two possible 261 262 explanations. Although resistance is inversely related to volume, the result was the opposite of 263 what was expected. It is known that there is a direct relationship between the concentrations of 264 ions and the electrical conductivity, and an indirect relationship exists between the ion concentration and the resistance of the solution¹⁹. Therefore, it is possible that water intake might 265 266 have altered ion status, which would directly impact resistance and impedance values over time. 267 However, to prove this effect, specific electrolytes (e.g. sodium, potassium, and chloride) in 268 plasma/ serum and urine should be determined, which was not possible for this study. 269 Nonetheless, this may not explain the findings as the volume of fluid in coffee (200 mL) is 270 insignificant relative to the estimated total BW of females (31 L) and males (39 L). In fact, the 271 relative gain in water (coffee/TBW, %) would be 0.6 and 0.5%, respectively. Thus, considering 272 the technical error of ~2%, BIA cannot technically track the increase in fluid volume from coffee 273 consumption. The second speculation would be related to a resistivity change due to chemical 274 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 consumption¹¹. In this study, urine output did not differ between groups after 120min of coffee consumption. Seal et al. $(2017)^{20}$ showed that only high caffeine content (6 mg/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 ± 12.8 vs 89.5 ± 14.8 kg), which significantly impacts the total amount of caffeine (~400mg vs ~537mg). On the other hand, low caffeine consumption (3 mg/kg of body weight, 269 ± 45 mg) did not differ from the control group (200ml of water) for cumulative urinary osmotic excretion and diuresis during the 3-h period²⁰.

285 In spite of what was discussed above, the impact of high amounts on caffeine on 286 dehydration is controversial. In another clinical trial, doses of caffeine up to 6 mg/kg of body 287 weight for 11-d had no influence on fluid, electrolyte, and renal indices of hydration in healthy males²¹. These findings were supported by a counterbalanced cross-over study which compared 288 289 the effects of coffee consumption (800mL/d) containing 4 mg/kg of body weight of caffeine 290 against water ingestion for 3-d. There were no significant differences across a wide range of 291 hematological and urinary markers of hydration status. The authors suggested that moderate consumption of coffee provided similar hydrating qualities to water²². 292

293 The absence of caffeine-induced diuresis might be explained by the habitual consumption 294 of caffeine-containing products. Clinical studies investigating the effects of caffeine on fluid 295 balance in habitual coffee drinkers (1 to 6 cups/d) concluded that caffeine did not promote diuresis²⁰⁻²³. In the present study, participants were habitual coffee drinkers and consumed 296 297 caffeine-containing products (e.g. chocolate, soda, tea). These findings are also corroborated in men who normally consumed less than 100mg/d caffeine²³. In a double-blind, randomized, 298 299 crossover trial, men ingested 5 mg/kg of body weight/d of caffeine for four consecutive days and total BW, extra and intracellular water did not differ from the control group $(maltodextrin)^{23}$. 300

301 Total BW and extracellular water were measured by deuterium oxide and sodium bromide 302 dilution, respectively, whereas intracellular water was calculated by subtracting extracellular 303 water from total BW.

304 The volume of urine output in participants who consumed caffeine was similar between the studies discussed above²¹⁻²³, except in the high caffeine content group analyzed by Seal et 305 al.²⁰. These findings might suggest that caffeine consumption up to \sim 500mg (or \sim 5 to 6 cups of 306 307 coffee) does not impact hydration in healthy adults who are habitual consumers of caffeine. A 308 review concluded that there would appear to be no clear basis for refraining from caffeinecontaining drinks in situations where fluid balance might be compromised²⁴. A meta-analysis on 309 310 caffeine-induced diuresis in healthy adults during rest and exercise showed that caffeine exerted 311 a small diuretic effect at rest; however, with a greater probability in females²⁵. Although the 312 median caffeine consumption was 300mg, the range was wide (114-741mg) and some studies 313 did not provide the relative caffeine dosages. We did not observe difference in urine output 314 between males and females in our study, suggesting further studies with a larger sample size and 315 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 frequencies²⁷.

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

amounts⁷⁻⁹. Thus, it is expected that the possible diuretic effect of coffee might be related to 324 325 caffeine. The second limitation concerns the lack of inclusion of other control groups - one 326 ingesting only water and another not consuming water prior to BIA assessment. A group 327 ingesting only water would confirm the absence of an acute diuretic effect of caffeine and the 328 effects observed in the decaf group. On the other hand, a group without consuming water prior to 329 measurement would confirm the effects found in the current study are due to water intake. 330 However, the inclusion of any of these groups would impair the double-blind nature of the study 331 design. Although, we instructed the participants to stay well hydrated before visits, measurement 332 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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341

- 342 **Conflict of Interest**
- 343 None to declare.

344

345 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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Table 1. Baseline characteristics of participants.

		All	Females	Males
		n = 22	n = 12	n = 10
	Age (y)	27 ± 6	27 ± 6	28 ± 6
	Body mass (kg)	68.5 ± 12.8	60.7 ± 9.4	78.0 ± 9.8
	Body mass index (kg/m ²)	23.7 ± 2.9	22.6 ± 3.1	25.1 ± 2.1
	Waist circumference (cm)	74.7 ± 8.5	69.4 ± 6.8	81.1 ± 5.6
	Impedance at 50kHz (Ω)	549.3 ± 92.0	624.1 ± 46.9	459.6 ± 32.3
	Resistance at 50kHz (Ω)	545.3 ± 92.3	620.4 ± 46.7	455.1 ± 32.1
	Reactance at 50kHz (Ω)	64.9 ± 6.6	66.7 ± 6.7	62.8 ± 5.7
440	Data were described by mean	\pm standard deviation		
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455	Figure	captions

- 454 **Figure 1.** Study flowchart.
- 455 Figure 2. Influence of coffee consumption with different doses of caffeine on impedance at
- 456 5kHz (A), 50kHz (B), 100kHz (C) and 200kHz (D), resistance (E), reactance (F), impedance
- 457 standardized for height in meters (Z/H) (G), and phase angle (H) after consecutive measurements
- 458 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.
- 462 **Figure 4.** Total urine output after coffee consumption with different doses of caffeine.













Minutes

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Decaf ~ 200mg ~ 400mg