

Calciuric Response to Acute Mate Tea Load is Inversely Associated with Habitual Mate Consumption and Dietary Caffeine in Young Adult Women

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Abstract Yerba mate (*Ilex paraguariensis*) tea is a caffeine-containing beverage habitually consumed in South-America and increasingly consumed worldwide. Mate consumption could adversely affect calcium homeostasis through caffeine-mediated increased urinary calcium loss. The aim of this study was to assess the change of urinary calcium in response to an acute mate tea load and to examine associations with habitual mate consumption, dietary caffeine, dietary calcium and bone mass status, in young adult women (20-37y, n=30). The women participated in two acute load tests, mate-tea (~300 mg caffeine) and water, in a cross-over-designed study with 7d wash-out period. Each woman was her own control. Calcium was measured in 4h urine post-load (UCa). Habitual mate consumption, and dietary caffeine and calcium, were assessed by questionnaires. Bone mineral density (BMD) at total body, femur and lumbar spine was measured by dual-X-ray-absorptiometry. Habitual mate and dietary caffeine median intakes were 293 mL/d and 207 mg/d, respectively. Mean dietary calcium was 1103 mg/d. Median BMD z-scores were within the normal range. UCa increased with acute mate load compared to water (median increase 15.9 mg, paired t-test, p<0.001). Increased UCa with acute mate load (Δ UCa) correlated directly with dietary calcium (r=0.556, p=0.001) and trochanter BMD z-score (r=0.380, p=0.042), and inversely with habitual mate intake (r=-0.524, p=0.003) and total dietary caffeine (r=-0.445, p=0.014). Δ UCa was higher in women with habitual mate intake <250 mL/d compared to \geq 250 mL/d, and in those with total dietary caffeine <150 mg/d compared to \geq 150 mg/d, adjusting for dietary calcium and trochanter BMD z-score (multifactor ANOVA, p<0.05). Habitual mate consumption and total dietary caffeine appeared to attenuate the calciuric response to the acute mate tea load in the women regardless of dietary calcium and bone mass status. This suggests physiologic adaptation to chronic caffeine exposure in women which habitually consume mate.

Keywords: calcium, caffeine, *Ilex paraguariensis*, bone mineral density, adult women

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1. Introduction

Yerba mate (*Ilex paraguariensis*) tea (mate for short) is a caffeine-containing beverage habitually consumed in Uruguay, Argentina, Paraguay and southern Brazil [1,2], and increasingly consumed in other countries worldwide [3,4]. Uruguay has the highest *per capita* consumption ranging from 6 to 8 kg dry yerba mate/person/year [2]. Consumption of mate provides a sensation of well-being and improves mood and alertness probably due to synergistic effects of caffeine, theobromine, L-theanine and polyphenols [3]. Other potential health benefits of mate components include antimicrobial, antioxidant and anti-inflammatory properties associated with anti-obesity, anti-diabetes, cardiovascular and neuro-protective effects [4].

In contrast to possible health benefits, mate consumption could, in theory, adversely affect calcium balance and bone mass status due to caffeine [5], since the caffeine content of mate is similar to that of other major dietary sources such as brewed coffee and tea [6]. Caffeine intake is known to induce diuresis and increase excretion of calcium in urine [7,8,9] which could result in negative calcium balance, reduced bone mineral density and increased fracture risk particularly in older women [5]. However, the potential negative effects of mate consumption on bone mass are not supported by the limited evidence available. A retrospective cross-sectional study of postmenopausal women in Argentina indicated that those who drank mate at least 1 liter per day during 4 years or more had higher bone mineral density at trabecular bone sites compared to matched women who did not drink mate [10]. A study in rats testing the

interaction between mate and calcium intake on histomorphometric and biomechanical bone properties did not find negative effects of mate on bone [11]. There are no human studies testing the effect of mate as a dietary source of caffeine on calcium homeostasis.

Different human studies on the effects of caffeine on calcium homeostasis and bone mass have produced inconsistent results probably because of differences among studies regarding risk factors for osteoporosis such as age, sex, genotype, hormonal status, cigarette smoking, physical activity, and particularly dietary calcium intake [12,13]. Since bone turnover contributes to support calcium balance [14], bone mass status could also interfere on the effect of caffeine on calcium homeostasis. Moreover, the dose and period of caffeine exposure are important factors to consider in relation to the effect of caffeine on calcium homeostasis due to the physiologic capacity for adaptation to chronic caffeine intake and development of tolerance [15,16]. Therefore, dietary calcium, bone mass status, and habitual caffeine intake should be considered when assessing the effect of mate intake on urinary calcium loss.

The aim of this study was to assess the change of urinary calcium excretion in response to an acute mate tea load and to examine associations with habitual mate and total caffeine intake, dietary calcium and bone mass status, in young adult women.

2. Methods

2.1. Subjects and Study Design

Thirty young adult women (20-37y) apparently healthy, non-smokers, non-obese ($BMI < 30\text{kg/m}^2$), non-pregnant, non-breastfeeding, and with no history of bone pathologies, participated in the study after informed consent. Exclusion criteria were daily consumption of more than 3 cups of coffee (450 mL), 4 cups of tea (600 mL), or 700 mL of cola-beverages. The women were asked to maintain their dietary and lifestyle habits during the study.

Each woman participated in two acute load tests, mate tea intake (~300 mg caffeine) and water intake (no caffeine), in a cross-over designed study in which each woman was her own control, with 7 days wash-out period between tests. The tests were scheduled 7-12 days after the last menstrual period. Prior to the acute tests, the participating woman fasted for 08 to 10 hours, drank water (150 mL) early in the morning, and emptied her bladder. The corresponding acute test beverage (mate or water, 420 mL; random order) was then ingested together with a light snack consisting of cream crackers (30 g). All the urine produced during the following 4 hours was collected into an appropriate plastic container for measurement of calcium excretion. No other foods or beverages were ingested during this period. The change in calcium excretion in the 4h urine of the acute mate test in comparison with the water test (control) was used as the response variable of the study.

Prior to participation in the acute tests, information on habitual dietary intake and habitual consumption of caffeine-containing beverages was obtained. Following the acute tests, bone mass status was assessed.

The sample size of the study was estimated to be sufficient to detect a difference of 10 mg calcium in 4h urine, between the acute mate and water tests, assuming a 95% CI and 80% statistical power. The estimation was based on results from previous studies testing urinary calcium in response to acute dietary caffeine load in adult women [7,8,9].

The study protocol was approved by the Ethical Committee for Human Research, Escuela de Nutrición, Universidad de la República, Uruguay (identification number: 360193).

2.2. Habitual Nutrient and Caffeine Intake

Information on habitual dietary intake was obtained by a food frequency questionnaire consisting of 114 food items referring to consumption during the previous month. The size of portions consumed was identified from pictures of commonly used home food measures. Daily nutrient intake from the habitual diet was estimated using the program *Food Processor* (ESHA Research, version 11.4.548) adapted to locally available foods.

Information on habitual consumption of mate and other caffeine-containing beverages (coffee, tea, and cola soft drinks) was obtained using a separate questionnaire that included frequency of consumption in the last month, serving size, and form of preparation when applicable. For mate, information on the period (years) elapsed since the beginning of habitual intake was also obtained. Total habitual caffeine intake from these sources was estimated based on the daily amounts consumed, and the caffeine content of these beverages reported in published studies. For mate, the caffeine content used was 370 mg/L considering the habitual form of preparation in Uruguay with 50 g of dry yerba mate product in one liter of water [17,18,19]. For coffee, the caffeine content used was 21 mg/g and 380 mg/L, for instant and filtered coffee, respectively [20]; for cola soft drinks, 112mg/L [21]; and, for tea, 125mg/L [22].

2.3. Anthropometric Measurements

Standing height and body weight were measured with light clothing early in the morning on each of the two acute test days, using a stadiometer (SECA) and a calibrated electronic scale (SECA), respectively. Average height and body weight were used for calculation of the body mass index (kg/m^2).

2.4. Mate Tea Preparation for the Acute Load Tests

The dry yerba mate used for the preparation of the infusion for the acute tests was a commercial brand purchased in the local market. The product was stored in a dry and dark place at room temperature until use. The same lot of the yerba mate product was used throughout the study. Similarly, the same lot of bottled commercial water was used in the acute tests during the study.

The mate infusion was prepared on the same day of the acute load test immediately prior to consumption. Briefly, dry yerba mate (30g) was added to a beaker placed on a heating plate containing 500 mL of bottled commercial

water at 80°C, and stirred with a magnetic rod for exactly 3 minutes. Then, it was filtered through layers of cotton gauze directly into an appropriate drinking glass. The weight of the filtered infusion was recorded. The infusion was allowed to cool down to 40-50°C before consumption. In the water acute load test, the same weight of the bottled commercial water was used. Weight of the mate infusion consumed in the acute load test was 418 ± 5.6 g.

The caffeine content of the mate infusion was analyzed by high-performance liquid chromatography using the equipment Agilent 1200 (*Agilent Technologies*), reversed-phase C18 analytical column Luna C18 (*Phenomenex*), formic acid in ultra-pure water and methanol as mobile phase and 274nm detection wavelength, as previously described [23]. Caffeine content of 3 independent samples of the infusion analyzed in duplicate was 707 ± 10.4 mg/L. Based on this result and the amount of mate infusion consumed in the tests, caffeine intake from the mate infusion in the acute load tests was 296 ± 3.8 mg.

2.5. Urine Processing and Laboratory Analyses

The urine samples were processed immediately after completion of the tests. The volume of all the urine produced during the 4 hours following the acute tests was determined by the weight of the total urine and measurement of the urine density. Urine aliquots were acidified with HCl (final concentration 0.01M) and kept frozen at -20°C until analyzed. Calcium in urine was measured by the method with arsenazo III (Wiener Lab, Argentina), and creatinine in urine by the kinetic method with picric acid (Wiener Lab, Argentina). Results of calcium excretion in 4h urine were expressed as total amount (mg), and as calcium:creatinine molar ratio [(calcium (mg) / 40.078) / (creatinine (mg) / 113.12)]. The change in calcium excretion in 4h urine of the acute mate test in comparison with the water test was calculated by difference.

2.6. Bone Mass Measurements

Total body, total femur, femoral neck, trochanter, and lumbar spine (L2-L4) bone mineral density (BMD) were measured by dual-X-ray-absorptiometry, using the equipment GE Healthcare (*Lunar Prodigy Advance*). Results were expressed as *z* score values obtained by comparison with the age- and gender-matched reference database included in the equipment software.

2.7. Statistical Analyses

Description of continuous variables are presented as mean and standard deviation, and/or median, minimum and maximum, as appropriate. Variables were tested for normal distribution by the Shapiro Wilk test. Comparison of calcium and other measurements in 4h urine between the acute tests (mate and water) was done by paired *t*-test for variables with normal distribution, and by Wilcoxon test for variables with no normal distribution. Associations between the change in urinary calcium in response to the acute mate test and the variables related to habitual diet,

mate intake, anthropometric measurements and bone mass status, were assessed by Pearson or Spearman correlations, for normally or non-normally distributed variables, respectively. The change in calcium excretion in 4h urine in response to the acute mate intake was compared according to tertiles of the variables related to habitual intake (mate, total caffeine, calcium) and bone mass status (BMD *z*-scores), by one-way ANOVA using Multiple Rank Analysis as post-test. For habitual mate and total caffeine intakes, comparisons were also done by multifactor ANOVA followed by Multiple Rank Analysis adjusting for significant covariates. $P < 0.05$ was considered significant. Statistical analyzes were carried out with Statgraphics software (Centurion version XVI.II).

3. Results

Information on age, body mass index, habitual dietary nutrient and caffeine intake, and bone mass status of the women participating in the study are presented in Table 1. Information on bone mass status is given for 29 women as one of the participants did not show up for DXA measurements. Women were young adults (median age 21y) and had an average body mass index (23.0 kg/m^2) within the normal range, with 6 women in the overweight range [24]. Most women ($n=27$) were habitual mate consumers, although the daily amount consumed and the period since starting consumption varied considerably between women. Median amount of mate consumed was 293 mL/d, with some women reaching up to 2000 mL/d. Median period of time since beginning of mate consumption was 6.5 years ranging up to 22 years. Median total dietary caffeine intake from caffeine-containing beverages was 207 mg/d ranging up to 867 mg/d. Mate intake had the largest contribution (72%) to total dietary caffeine intake, followed by coffee (24%), tea (3%), and cola beverages (1%). Other potential sources of dietary caffeine, such as chocolate and energizing beverages, were not present in the habitual diet or made negligible contributions to total caffeine intake. The average dietary intakes of energy (1908 kcal/d), protein (95g/d), and calcium (1103mg/d) were according to the reference dietary intake for adult women [25,26,27], with large interindividual variability for calcium intakes (279–1907 mg/d). Some women ($n=11$) had dietary calcium intake lower than 1000 mg/d. Median bone mineral density *z* scores were within the normal range (-1 to +1) for total body and specific bone sites although some women had *z* scores in the range indicative of osteopenia (> -2 to < -1) at trabecular bone sites [trochanter, $n=7$; total femur, $n=2$; lumbar spine (L2-L4), $n=1$].

Results on volume, creatinine and calcium content of 4h urine in response to the acute load tests with mate and water are shown in Table 2. Creatinine excretion did not change between tests ($p=0.64$) but urinary volume and calcium excretion increased with the acute mate load compared to water ($p=0.033$ and $p<0.0001$, respectively). Median increase in the 4h urinary volume was 40 mL (1.1fold) ($p=0.033$). Median increase in calcium excretion in 4h urine was 15.9 mg (2.5fold) when expressed in absolute amount, and 0.19 (2.4 fold) when expressed as calcium:creatinine molar ratio ($P<0.0001$).

Table 1. Characteristics of the women studied

Characteristic [#]	Mean ± SD	Median (min-max)
Age (y)	23 ± 4	21 (20 - 37)
Body mass index (kg/m ²)	23 ± 2	23 (19 -29)
Habitual mate intake*		
Amount (mL/d)	488 ± 489	293 (0 - 2000)
Period (y)	7 ± 6	6 (0 - 20)
Habitual caffeine dietary intake*		
Total amount (mg/d)	251 ± 197	207 (18 - 867)
Dietary sources (mg/d)		
Mate	181 ± 190	122 (0 - 740)
Coffee	61 ± 54	45 (0 - 211)
Tea	7 ± 8	4 (0-31)
Cola beverages	3 ± 7	0 (0-32)
Habitual nutrient dietary intake **		
Energy (kcal/d)	1908 ± 402	1908 (875 - 2848)
Protein (g/d)	95 ± 23	94 (38 -137)
Calcium (mg/d)	1103 ± 389	1052 (259 - 1907)
Bone mineral density z score		
Total body	0.38 ± 0.72	0.20 (-1.20 - 1.70)
Total femur	0.11 ± 0.78	-0.11 (-1.40 - 1.70)
Femoral neck	-0.08 ± 0.67	-0.20 (-1.00 - 1.80)
Trochanter	-0.37 ± 0.83	-0.40 (-1.80 - 1.50)
Lumbar spine (L2-L4)	-0.08 ± 0.75	0.00 (-1.90 - 1.40)

[#] n=30 for all variables except bone mineral density (n=29).

*Survey questionnaire. Five women did not consume mate

** Food frequency questionnaire.

Table 2. Urinary volume, creatinine and calcium in response to the acute tests with mate and water (n=30)¹

Measurement in 4h urine	Acute test		P paired test
	Water	Mate	
Volume (mL)	750 (479 - 1409)	790 (476 - 1756)	0.033 ³
Creatinine (mg)	218 ± 34	216 ± 37	0.64 ⁴
Calcium (mg)	10.7 (7.4-19.3)	26.6 (6.7 - 62.8)	<0.000 ³
Ca:creatinine molar ratio ²	0.14 (0.03 - 0.58)	0.33 (0.13 - 0.73)	<0.000 ³

¹Values are expressed as median (min - max) except for creatinine. Creatinine was expressed as mean ± SD. ²Calculated as [(Calcium (mg)/40.078) / (Creatinine (mg) / 113.12)] ³Wilcoxon test. ⁴Paired t test.

Table 4. Urinary calcium response to the acute mate load in relation to tertiles of habitual mate, total caffeine and calcium intakes, and trochanter BMD z score

Independent variable [#]	Independent variable tertiles			p ²
	Change in 4h urine calcium (mg) ¹			
Habitual mate intake	<250 mL/d 19.8 ± 2.4 ^a	250-499 mL/d 13.4 ± 2.3 ^{ab}	≥ 500 mL/d 10.8 ± 2.2 ^b	0.030
Period of habitual mate intake	<4y 15.7 ± 2.4	4 -9.9 y 16.9 ± 2.4	≥ 10y 10.6 ± 2.4	ns
Total habitual caffeine intake	< 150 mg/d 19.8 ± 2.2 ^a	150-299 mg/d 12.7 ± 2.1 ^b	≥ 300 mg/d 10.4 ± 2.4 ^b	0.018
Habitual calcium intake	< 970 mg/d 8.9 ± 2.2 ^a	970- 1224 mg/d 16.2 ± 2.2 ^b	≥ 1225 mg/d 18.0 ± 2.2 ^b	0.018
BMD trochanter z score	≤-0.90 8.0 ± 2.1 ^a	-0.80 - 0 19.7 ± 2.1 ^b	≥ 0.10 15.2 ± 2.2 ^b	0.002

[#] n=30 for all variables except BMD trochanter z score (n=29). Values are mean ± SE. ¹ The change in urinary calcium was calculated as calcium in 4h urine after the acute test with mate minus calcium in 4h urine after the acute test with water, expressed in mg. ²One-way ANOVA followed by Multiple Rank Analysis; ns, not significant (p>0.05). Different superscript letters in the same row indicate significant difference in the change in 4h urine calcium between tertiles of the independent variable (p<0.05); ns, not significant (p>0.05).

Correlations between the change in calcium excretion (mg) in 4h urine in response to the acute mate load and indicators of habitual dietary intake and bone mass status are shown in Table 3. Negative correlations were found with habitual volume (mL) of daily mate intake (r=-0.524, p=0.003), period (y) since beginning of habitual mate intake (r=-0.367, p=0.046), and habitual total caffeine dietary intake (mg) (r=-0.445, p=0.014). Positive correlations were found with dietary calcium intake (mg) (r=0.556, p=0.001), and trochanter BMD z score (r=0.380, p=0.042). No significant correlations were observed with dietary protein intake and BMD z score values at total bone or other bone sites. Moreover, there was no significant correlation (Pearson) between BMI and the change in urinary Ca in response to the acute test with mate (r=-0.297, p=0.11). Similar statistical significance was obtained for correlations and comparisons done with the change in urinary calcium excretion expressed as calcium:creatinine molar ratios (results not shown). Therefore, further statistical analyses were done using calcium excretion expressed in mg.

Table 3. Correlations between habitual mate intake, dietary nutrient intakes and bone mass status, and the urinary calcium response¹ to the acute test with mate

Variable [#] related to change in 4h urine Ca (mg)	r	p
Mate intake (mL/d) ²	-0.524	0.003
Period of mate intake (y) ²	-0.367	0.046
Caffeine total intake (mg/d) ²	-0.445	0.014
Calcium intake (mg/d) ³	0.556	0.001
Protein intake (g/d) ³	0.236	ns
BMD total body (z score) ³	0.015	ns
BMD total femur (z score) ³	0.231	ns
BMD femoral neck (z score) ³	0.021	ns
BMD trochanter (z-score) ²	0.380	0.042
BMD L2-L4 (z score) ³	0.194	ns

[#] n=30 for all variables except BMD z scores (n=29). ¹ Change in 4h urine was calculated as calcium in 4h urine after the acute test with mate minus calcium in 4h urine after the acute test with water, expressed in mg. ²Spearman correlations. ³Pearson correlations. BMD, bone mineral density; r, correlation coefficient; p, probability of significance; ns, not significant (p>0.05).

The change in calcium excretion in 4h urine in response to the acute mate load test according to tertiles of habitual volume and period of mate intake, dietary intake of total caffeine and calcium, and trochanter BMD z score was analyzed using univariate analysis (Table 4). There was a decrease in the urinary calcium response with increasing daily volume of habitual mate intake and increasing total amount of daily habitual caffeine intake ($p < 0.05$). The urinary calcium response was higher in the women with habitual mate intake < 250 mL/d compared to those with mate intake ≥ 500 mL/d (mean response 19.8 mg and 10.8 mg, respectively; $p = 0.030$). Also, the urinary calcium response was higher in women with habitual caffeine intake < 150 mg/d compared to those with habitual caffeine intake ≥ 150 mg/d (mean response 19.8 mg compared to 12.7-10.4 mg, respectively; $p = 0.018$). There was no difference in urinary calcium response by tertiles of period of mate intake ($p = 0.18$). In contrast, there was an increase in the urinary calcium response with increasing habitual calcium intake, and with increasing BMD z score values at the trochanter ($P < 0.02$). Urinary calcium response was lower (mean 8.9 mg) in women with dietary calcium intake < 970 mg/d compared to those with higher calcium intakes (mean urinary calcium response 16.2-18.0 mg) ($P = 0.018$). Similarly, urinary calcium response was lower (mean 8.0 mg) in women with BMD z score at the trochanter ≤ -0.9 compared to those with higher z scores (mean urinary calcium response 19.7-15.2 mg) ($p = 0.002$).

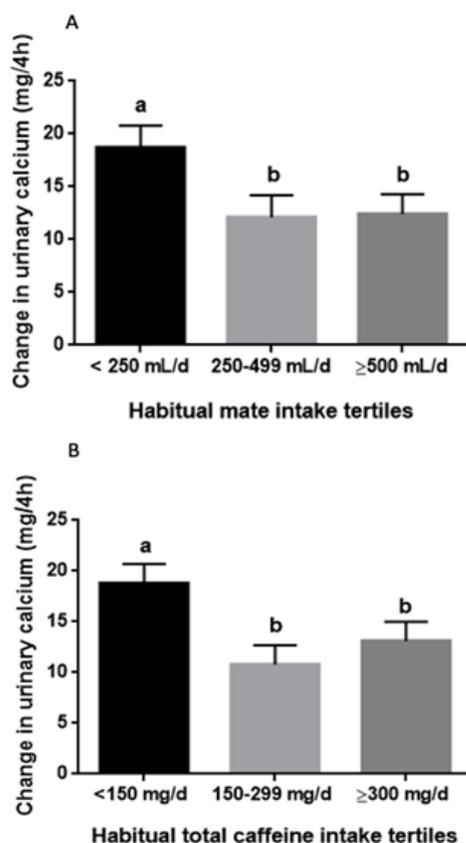


Figure 1. Change in 4h urine calcium after the acute mate load in relation to tertiles of habitual mate intake (mL/d) (A), and total habitual caffeine intake (mg/d) (B). Values shown are mean \pm SE using calcium intake and BMD trochanter z score as covariates (total $n = 29$). In each graph, different superscript letters indicate significant difference between groups (Multifactor ANOVA followed by Multiple Rank Test, $p < 0.05$)

The change in calcium excretion in 4h urine in response to the acute mate load test was re-examined according to tertiles of habitual amount of mate intake and habitual total caffeine intake by multivariate analysis, using dietary calcium intake and BMD z score at the trochanter analysis as co-variables (Figure 1). The results indicated that the urinary calcium response to the acute mate load was higher in women with habitual mate intake < 250 mL/d compared to those with mate intake ≥ 250 mL/d, and in women with total habitual caffeine intake < 150 mg/d compared to those with habitual caffeine intake ≥ 150 mg/d, when adjusting for dietary calcium intake and BMD z score at the trochanter ($p < 0.05$).

4. Discussion

The potential effects of mate consumption on calcium homeostasis and calcium balance in humans are largely unknown. In the present crossover-designed study, an acute mate tea load providing about 300 mg caffeine increased calcium excretion in 4h urine post-test compared to water in young adult women. This increase was associated directly with dietary calcium intake and bone mass status, and inversely with habitual mate and total caffeine intakes. The increased urinary calcium excretion in response to acute mate intake was of smaller magnitude in women habitually consuming daily ≥ 250 mL mate or ≥ 150 mg total caffeine compared to those with lower habitual mate and caffeine intakes, adjusting for dietary calcium and bone mass status. These results indicate that habitual intake of mate and total dietary caffeine appeared to attenuate the calciuric effect of mate consumption in the women studied.

The increased urinary calcium excretion after acute mate intake observed in our study was an expected response since previous studies in adult women have shown that a dietary caffeine load acutely increases calcium loss in urine [8,9]. The acute intake of a caffeinated cola beverage (77.5 mg caffeine) increased calcium excretion in 5h urine from 0.523 to 0.712 nmol (1.4fold) [8], whereas the acute intake of coffee (caffeine load 5 mg/kg body weight) increased calcium excretion in 4h urine from 0.2 to 0.5 mmol/mmol creatinine (2.5fold) [9]. In our study, the caffeine load was 300 mg and calcium excretion increased 2.5fold in 4h urine. The effect of caffeine on renal handling of calcium has been explained by antagonism of adenosine receptors, with caffeine and particularly its metabolite paraxanthine competitively blocking the action of endogenous adenosine in the kidney glomerular epithelial cells [12].

The increase in urinary volume following the acute mate intake in our study was also an expected response consistent with previous studies [7,9], explained by the diuretic effect of caffeine due to increasing renal blood flow and decreasing tubular reabsorption of sodium [7]. Also consistent with previous studies [7,8,9], urinary creatinine excretion did not change with the acute mate load suggesting that the glomerular filtration rate is possibly not affected by mate intake.

The urinary calcium response to the acute mate test of our study was positively (directly) associated with

habitual dietary calcium intake and bone mass status as indicated by BMD z score at the trochanter, a trabecular bone site. The increase in 4h urine calcium with the mate tea load was about half in the women with calcium intake <970 mg/d compared to that in women with higher dietary calcium intakes ($p=0.018$). Also, it was about half in women with BMD z score at the trochanter ≤ -0.90 compared to that of women with higher trochanter BMD z scores ($p=0.002$). These results suggest that calcium loss in the urine in response to the caffeine challenge was maintained low when dietary calcium intake was lower than recommended [27] and when bone mass status approached osteopenia. This is consistent with the tightly controlled physiologic mechanisms to maintain calcium homeostasis and conserve body calcium under different conditions of calcium intake and bone calcium needs [14]. Moreover, our results indicate that the caffeine challenge provided by the acute mate test did not prevent the physiologic urine calcium conservation mechanism to occur in the women studied.

The increased calcium in 4h urine in response to the mate load test was negatively related to habitual mate and total caffeine intakes, and these inverse relationships were maintained after adjusting for the positively related factors dietary calcium and bone mass status. In the adjusted analyses, the increase in 4h urine calcium in response to the acute mate load was about half in the women with habitual mate intake ≥ 250 mL/d compared to that in women with lower mate intake ($p<0.05$). Also, it was about half in women with habitual total caffeine intake ≥ 150 mg/d compared to that of women with lower total caffeine intake ($p<0.05$). These results are consistent with the recognized metabolic adaptation and development of tolerance to chronic caffeine intake [15,16,28,29] that appear to occur through upregulation of adenosine receptors following repeated exposure to caffeine [30]. Evidence of development of tolerance with increasing dosage of caffeine has been obtained in human studies for blood pressure [28,29], cerebral blood flow [15] and ergogenic effects [16]. In our study, the period of habitual mate intake was not a significant factor affecting the urinary calcium response to acute mate intake probably because this period of time was considerably long for most women, in the range of years, and the available evidence indicates that adaptation to continuous caffeine exposure occurs more rapidly, in the range of days or weeks [15,16,28,29].

Several studies indicate that there is a limit of the physiologic capacity for adaptation to increased caffeine exposure since the effects may not be completely compensated by the development of tolerance [15,28,29]. Consistent with this concept, in our study the 4h urinary calcium response to the acute mate intake was not different between women with habitual mate intake 250-499 mL/d and ≥ 500 mL/d, neither between women with habitual total caffeine intakes 150-299 mg/d and ≥ 300 mg/d. Therefore, increasing habitual mate intake above 250 mL/d or total habitual caffeine intake above 150 mg/d did not further reduce the magnitude of the acute calciuric response. These results suggest that there is a limit for the physiologic capacity of the renal glomerular epithelial cells to adapt to chronic caffeine exposure.

Results from our study indicate that even with previous adaptation to caffeine exposure, acute mate intake temporarily increased calcium loss in the urine of the young adult women, and this could eventually impair calcium balance and bone mass status. However, the increased calcium loss in urine was of small magnitude (~ 15 mg calcium in 4h) and it could be compensated over 24 h resulting in no change in daily urinary calcium excretion, as observed in previous studies in adult women with adequate calcium intake [12,14]. Consistent with this interpretation, animal and human studies have described beneficial rather than detrimental effects of mate consumption on bone mass [10,11], similarly as described for tea consumption [31]. Yerba mate contains flavonoids, caffeoylquinic acids and other polyphenolic compounds [4] that appear to favor bone health [31,32]. However, the potential contribution of habitual mate consumption to bone mass status of young adult women is not known.

A limitation of our study was the acute response design, thus results should be interpreted with caution and may not be extrapolated to situations of habitual consumption. Moreover, the significant associations observed between the calciuric response to the acute mate tea load and habitual dietary intakes and bone mass status do not imply causal relationships between these variables. On the other hand, these associations might be useful for designing further investigations. Other limitations of our study were lack of information on vitamin D status and on serum levels of parathyroid hormone, both directly regulating calcium homeostasis [14]. Strengths of our study were the use of a cross-over design in which each woman was her own control, and the fact of have taken into account potential confounding factors such as dietary calcium intake and bone mass status when examining the associations between mate consumption and urinary calcium loss.

In conclusion, results from our study indicate that habitual consumption of mate and total dietary caffeine appear to attenuate the calciuric response to mate intake in young adult women, consistent with the physiologic capacity of adaptation to chronic caffeine exposure. The acute urinary calcium response to the mate tea load was of smaller magnitude in women habitually consuming ≥ 250 mL/d mate or ≥ 150 mg/d total dietary caffeine, compared to those with lower habitual intakes, irrespective of dietary calcium and bone mass status. The long-term effects of habitual mate consumption on calcium homeostasis and bone mass status of young adult women need further investigation.

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List of Abbreviations

BMD, bone mineral density

DXA, dual-X-ray-absorptiometry

Statement of Competing Interests

The authors have no competing interests.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author (CMD), upon reasonable request.

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