Effect of prostaglandin inhibition on caffeine-induced hypercalciuria in healthy women

Susan J. Whiting

Division of Nutrition and Dietetics, College of Pharmacy, University of Saskatchewan, Saskatoon, Saskatchewan, Canada

Caffeine ingestion increases urinary calcium excretion. The mechanism is not known, but prostaglandin synthesis has been implicated. We hypothesized that administration of a prostaglandin inhibitor such as acetylsalicylic acid (aspirin) along with caffeine would prevent caffeine-induced hypercalciuria. We measured 3-hour excretion in fasting subjects who each randomly ingested four treatments on nonconcurrent mornings: no drug, caffeine (5 mg/kg body weight), acetylsalicylic acid (650 mg), or caffeine plus acetylsalicylic acid. In experiment 1, nine healthy premenopausal female subjects were studied; each treatment was taken with 200 ml of orange juice. Water was provided hourly to encourage urine flow. Urinary calcium excretion rose with caffeine treatment; mean 3-hour calcium (mmol/mmol creatinine) was 0.49 ± 0.07 compared with 0.23 ± 0.04 during the no-drug treatment. Acetylsalicylic acid caused a significant reduction in urinary calcium to 0.13 ± 0.02; when it was combined with caffeine, caffeine-induced calcium excretion fell significantly to 0.35 ± 0.08. Sodium excretion tended to reflect calcium excretion. Urinary prostaglandin E_2 fell significantly with acetylsalicylic acid, with and without caffeine. There were no significant changes in creatinine, water, or potassium excretion. Experiment 2 was similar, except that water was substituted for orange juice to test the possibility that acetylsalicylic acid affected elevated but not basal calcium excretion. Similar and even more pronounced results were obtained, with caffeine causing a threefold increase in urinary calcium, acetylsalicylic acid causing a decrease by half, and the combined drug treatment being greater than no drug but less than caffeine alone. Urinary phosphorus rose significantly with caffeine alone. Prostaglandin synthesis may not be directly involved in caffeine-induced hypercalciuria, as the magnitude of the caffeine-induced increase was similar when treatments given the acetylsalicylic acid were compared with those without a prostaglandin synthesis inhibitor.

Keywords: Calcium; caffeine; aspirin; prostaglandin E_2; urinary calcium; acetylsalicylic acid

Introduction

Caffeine and theophylline have been found to promote urinary calcium excretion in rats and humans.[1-3] While this finding has implications for hypercalciuria in renal calcium stone disease and for negative calcium balance in involutional osteoporosis, the mechanism for this action remains unclear. Both stimulation of prostaglandin synthesis by caffeine[1] and antagonism of adenosine receptors, as demonstrated by the alleviation of theophylline-induced calciuria with adenosine agonists,[4] have been implicated from work done in our laboratory using rats. There is evidence that methylxanthines increase prostaglandin synthesis.[5] Further, nonsteroidal antiinflammatory drugs (NSAIDs) reduce urinary calcium excretion in subjects with idiopathic hypercalciuria.[6] However, Massey and Hollingbery[7] did not find any effect on urinary calcium excretion in females given 650 mg aspirin (acetylsalicylic acid, ASA) 10 hours and 1 hour before a 3 mg/kg caffeine dose. We hypothesized that ASA given at the same time as caffeine would prevent caffeine-induced hypercalciuria if prostaglandin synthesis was indeed the mechanism for the hypercalciuria.

Address reprint requests to Dr. Susan J. Whiting, Division of Nutrition and Diuretics, College of Pharmacy, University of Saskatchewan, Saskatoon, Saskatchewan S7N 0W0, Canada.
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We examined the effect of ASA on caffeine-induced hypercalciuria using healthy premenopausal female subjects in two acute experiments; in one, orange juice was given with the test drugs while in the other, only water was provided. Evidence is presented to suggest that inhibition of prostaglandin synthesis blunts the effects of caffeine on calcium excretion but may not be directly responsible for the action of caffeine.

Materials and methods

Experiment 1

Nine female subjects between the ages of 20 to 45 years were given caffeine (5 mg/kg) with and without 650 mg ASA (Bayer Aspirin, Sterling Drug Ltd., Aurora, Ontario, Canada). Caffeine was provided as whole or half tablets containing 100 mg caffeine (WakeUps, Adrem Limited, Toronto, Ontario, Canada). The protocol was approved by the University of Saskatchewan Advisory Committee on Ethics in Human Experimentation. Subjects, who gave informed consent, were healthy and did not smoke. Oral contraceptive use was noted in three subjects. Tests were performed on nonconcurrent days once or twice a week. The subjects fasted from 9:00 PM the evening prior to their load of caffeine, ASA, caffeine plus ASA, or no drug. On each morning of the experiment, the subjects emptied their bladders and drank 250 ml of water. A fasting urine sample was obtained approximately 1 hour later. Subjects ingested 200 ml of orange juice with one of four treatments; the order was determined randomly. At 1-hour intervals for 3 hours, the subjects emptied their bladders; at each hour, the subjects drank 200 ml of distilled water to ensure adequate urine production. Both acidified and unacidified urine samples were obtained hourly.

Experiment 2

A protocol similar to experiment 1 was followed, except that distilled water was substituted for orange juice. Nine female subjects between the ages of 19 to 45 years were given caffeine (5 mg/kg) with and without 650 mg ASA. One subject was an occasional smoker and two used oral contraceptives. Three subjects from experiment 1 participated. Most subjects conducted the study at home or at their place of work after receiving instructions; only acidified urine was obtained.

Analytical procedures

The volume of the urine sample was recorded and a 50-ml aliquot, acidified with 1.0 ml of 6 M HCl, was placed in a polyethylene tube. Urine was centrifuged and refrigerated until analysis. In experiment 1, a second aliquot, unacidified, was immediately frozen for later analysis of prostaglandin E_2. Acidified urine was analyzed for calcium using a Gilford Calcium Kit (Ciba-Corning, Oberlin, OH, USA), which gave values comparable to those using a calcium analyzer (Corning 940, Corning Scientific Instruments, Medfield, MA, USA); for sodium and potassium by flame photometry (Corning 410C, Corning Medical and Scientific, Corning, NY, USA); and for creatinine and phosphorus by colorimetric methods (Sigma Kit 555 and 670, respectively, Sigma Chemicals, St. Louis, MO, USA). Prostaglandin E_2 was analyzed using an RIA kit (Prostaglandin E_2 RIA Kit, DuPont, Wilmington, DE, USA).

Statistics

Data are presented as mean plus one standard error (SE). As each subject acted as her own control, data were analzyed using repeated-measure analysis of variance using a microcomputer program[8]; if there was a significant F ($P < 0.05$), the mean values were tested using the Student-Newman-Keuls test. Bartlett's test for homogeneity was performed on the data and, when variances were not equal, a square root transformation was applied to the data.[9]

Results

Experiment 1

Fasting (i.e., hour 0) calcium to creatinine (Ca/Cr) ratios for subjects were within the expected normal range of 0.06 to 0.36 mmol/mmol (*Figure 1A*). The effects of caffeine on excretion rates of calcium, sodium, potassium, phosphorus, and creatinine are shown in *Table 1*. There was a significant increase in total 3-hour calcium excretion with caffeine administration whether ASA was present or not. Total 3-hour calcium excretion was reduced with ASA, and caffeine-induced calcium excretion was reduced when ASA was given concurrently. Drug effects on calcium excretion are shown in *Figure 2A*, in which Ca/Cr ratios show a similar pattern to total calcium excretion. Time trends are shown in *Figure 1A*. At the end of 3 hours, the effect of caffeine in both the caffeine and caffeine plus ASA groups appeared to have peaked. Calcium excretion in the ASA group tended to be in a downward direction.

Urinary sodium followed a similar pattern of excretion to that of calcium. Caffeine increased sodium excretion whether or not ASA was given; ASA alone reduced total 3-hour excretion, but values were not significantly different from the no drug treatment. Prostaglandin E_2 excretion fell significantly in both ASA groups compared with the no drug and caffeine treatment groups. There were no significant changes in total potassium, creatinine, or water excretion with any drug treatment. There was a trend in volume excreted; caffeine appeared to raise and ASA appeared to lower urinary volume. Urinary phosphorus excretion rose after caffeine ingestion and fell with aspirin ingestion so that these two groups were significantly different.

Experiment 2

Fasting values for Ca/Cr indicate that subjects again fell within the expected normal range (*Figure 1B*). The

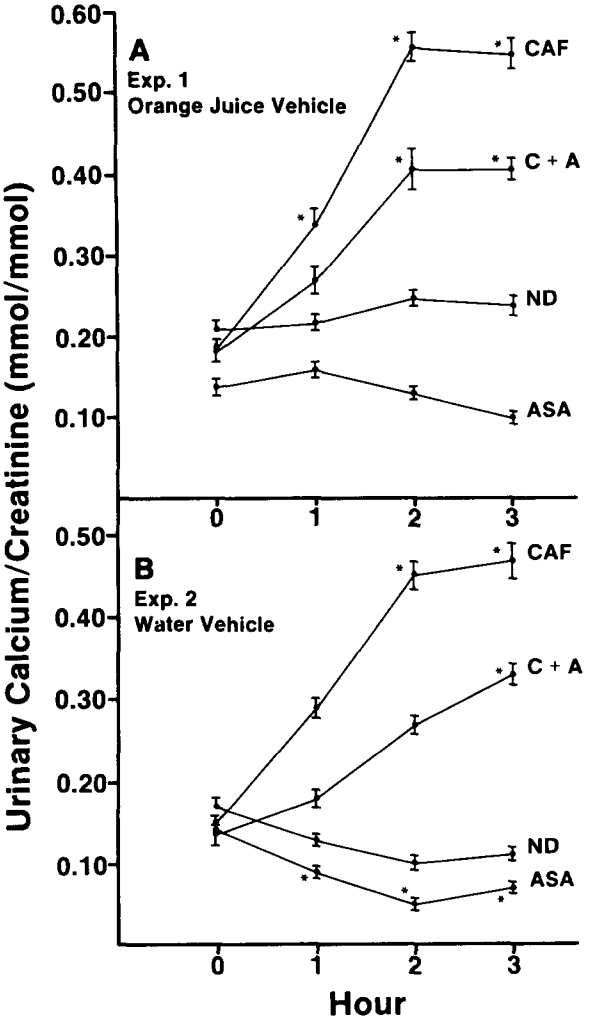

Figure 1 Time course analysis of calcium excretion (mmol/mmol creatinine) following a dose of caffeine (CAF, 5 mg/kg), acetylsalicylic acid (ASA, 650 mg), caffeine plus acetylsalicylic acid (C + A), or no drug (ND). In panel A, the treatments were given with orange juice (experiment 1); in panel B, the treatments were given with distilled water (experiment 2). Results are shown as mean ± SE values; the asterisk (*) indicates significantly different ($P < 0.05$) from hour 0.

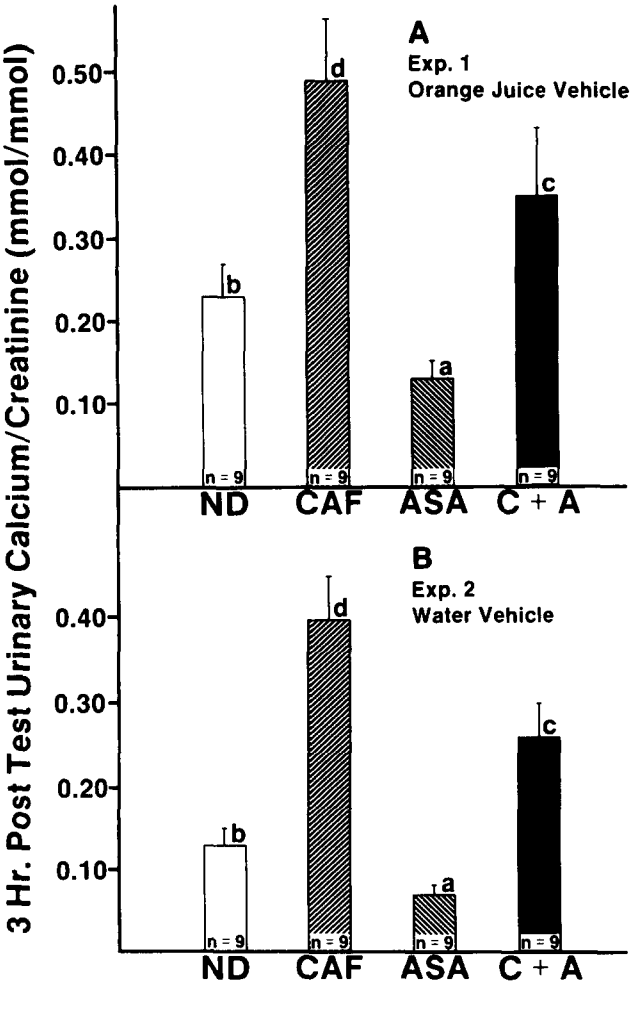

Figure 2 Three-hour posttest calcium excretion (mmol/mmol creatinine) following a dose of caffeine (CAF, 5 mg/kg), acetylsalicylic acid (ASA, 650 mg), caffeine plus acetylsalicylic acid (C + A), or no drug (ND). In panel A, the treatments were given with orange juice (experiment 1); in panel B, the treatments were given with distilled water (experient 2). Bars with different letters are significantly different ($P < 0.05$) from each other.

Table 1 Effect of caffeine (5 mg/kg) and acetylsalicylic acid (650 mg) on 3-hour urinary excretion of calcium, sodium, potassium, creatinine, volume, and prostaglandin E_2: experiment 1

	Treatment			
Measure[a]	No drug	Caffeine	ASA	Caffeine + ASA
Calcium (μmol/3 hr)	321 ± 50^{b}	664 ± 108^{d}	181 ± 32^{a}	457 ± 82^{c}
Sodium (mmol/3 hr)	16.0 ± 3.0^{a,b}	27.4 ± 5.5^{b}	8.6 ± 1.7^{a}	20.2 ± 4.0^{b}
Potassium (mmol/3 hr)	15.0 ± 1.7^{a}	12.3 ± 1.9^{a}	11.7 ± 1.8^{a}	11.2 ± 1.4^{a}
Creatinine (mmol/3 hr)	1.38 ± .09^{a}	1.35 ± .10^{a}	1.37 ± .09^{a}	1.36 ± .09^{a}
Volume (ml/3 hr)	447 ± 87^{a}	495 ± 99^{a}	315 ± 74^{a}	426 ± 72^{a}
Phosphorus (mmol/3 hr)	1.94 ± .36^{a,b}	2.44 ± .34^{b}	1.46 ± .21^{a}	2.18 ± .33^{a,b}
Prostaglandin E_2 (ng/3 hr)	12.1 ± 3.6^{a}	15.7 ± 2.8^{a}	5.6 ± 0.5^{b}	4.8 ± 0.9^{b}

[a] Mean ± SE values for nine subjects except prostaglandin E_2 (n = 7). Values in rows with different letters are significantly different, $P < 0.05$

Table 2 Effect of caffeine (5 mg/kg body weight) and acetylsalicylic acid (650 mg) on 3-hour urinary excretion of calcium, sodium, potassium, phosphorus, creatinine, and volume: experiment 2

Measure[a]	Treatment			
	No drug	Caffeine	ASA	Caffeine + ASA
Calcium (μmol/3 hr)	178 ± 50[a]	504 ± 81[c]	96 ± 29[a]	301 ± 60[a]
Sodium (mmol/3 hr)	13.2 ± 3.4[a]	28.3 ± 4.5[b]	5.2 ± 1.6[c]	15.0 ± 2.8[a]
Potassium (mmol/3 hr)	12.9 ± 2.5[a]	10.8 ± 2.0[a]	10.9 ± 1.8[a]	7.0 ± 1.5[b]
Creatinine (mmol/3 hr)	1.34 ± .17[a]	1.20 ± .09[a]	1.30 ± .13[a]	1.13 ± .12[a]
Volume (ml/3 hr)	510 ± 60[a]	678 ± 75[b]	436 ± 78[a]	536 ± 84[a]
Phosphorus (mmol/3 hr)	2.08 ± .25[a]	2.92 ± .26[b]	2.04 ± .22[a]	2.28 ± .33[a]

[a] Mean ± SE values for nine subjects. Values in rows with different letters are significantly different, $P < 0.05$

effects of caffeine on excretion rates of calcium, sodium, potassium, creatinine, volume, and phosphorus are shown in *Table 2*. There was a significant increase in total 3-hour calcium excretion with caffeine administration whether ASA was present or not. Caffeine-induced calcium excretion was significantly reduced when ASA was given concurrently. Drug effects on calcium excretion are shown in *Figure 2B;* Ca/Cr ratios indicate that ASA decreased calcium excretion significantly, as in experiment 1. Time trends are shown in *Figure 1B*. At the end of 3 hours, the effect of caffeine in both the caffeine and caffeine plus ASA treatment groups had peaked. Calcium excretion in the ASA group was on a significant downward trend.

Sodium followed a pattern of excretion similar to calcium in that caffeine significantly increased and ASA significantly reduced total 3-hour excretion. There was no significant change in total creatinine excretion with any drug treatment. Potassium excretion, with the caffeine plus ASA treatment only, fell significantly. Volume was found to significantly rise with caffeine, but not when ASA was given with caffeine. As in experiment 1, ASA tended to lower urine volume, but not significantly ($P < 0.092$). Phosphorus excretion rose significantly with caffeine treatment.

Discussion

As expected, we found a marked caffeine-induced hypercalciuria, a finding that is consistent with those from other human[2,7] and animal studies.[1] There was a higher excretion rate of urinary phosphorus with caffeine in both experiments. Other studies using human[2,7] or animal subjects[1] found no change in phosphorus excretion. Colin et al.,[10] who infused theophylline, did find a significant rise in urinary phosphorus in human subjects. It is possible that the higher phosphorus excretion rate is related to a nonspecific diuresis as phosphorus excretion was correlated to volume excretion in experiment 2 ($r = 0.64$, $P = 0.0001$), where a significant diuretic effect of caffeine was observed, and to a lesser extent in experiment 1 ($r = 0.27$, $P = 0.11$), where volume changes were not significant. Studies not finding phosphaturia also did not find diuresis,[1,7] but a study in animals finding caffeine-induced diuresis reported phosphaturia.[11]

This report is the first to document an effect of aspirin on reducing urinary calcium excretion in healthy normocalciuric subjects. These results apply to an acute situation only, as other investigators have shown that aspirin and other NSAIDs do not affect daily excretion of calcium.[12] There is controversy as to the role of prostaglandin E_2 production in the regulation of urinary calcium excretion. Because we had previously shown an inhibition by administering indomethacin to caffeine-fed rats,[1] our hypothesis was that it was involved in the caffeine-induced hypercalciuria. In a recent study,[12] healthy subjects treated with aspirin, indomethacin, or ketoprofen did not show reduction in 24-hour urinary calcium excretion but did reduce sodium excretion, suggesting no significant role of prostaglandin production in renal calcium handling. Buck,[13] on the other hand, proposed that prostaglandin E_2 production is a major determinant of hypercalciuria in renal calcium stone disease. While NSAID treatment has been advocated as a way to decrease hypercalciuria,[14] it is not clear whether prostaglandin synthesis, per se, is the cause of idiopathic hypercalciuria. Hirayama et al.[15] found a significant correlation between 24-hour prostaglandin E_2 excretion and urinary calcium excretion in male subjects with and without renal calcium stone disease. In our study, ASA ingestion with or without caffeine was followed by a significant drop in prostaglandin E_2 excretion. We did find a significant ($P < 0.02$) correlation between total 3-hour prostaglandin E_2 and calcium excretion, suggesting a role for prostaglandin E_2 production in modulating renal calcium.

This study reports a significant alleviation of caffeine-induced hypercalciuria with ASA (*Figure 2*). On the other hand, Massey and Hollingbery[7] found no effect of aspirin on caffeine-induced hypercalciuria. The difference in timing of the ASA dose likely accounts for the different response. In their study, aspirin was a pretreatment, which may have altered hepatic metabolism of the subsequent caffeine dose due to a decreased P_{450} content.[16] Prostaglandin E_2 enhances the excretion of water and sodium chloride; therefore, preventing its synthesis should result in water and sodium retention,[17] as our study reports. Additionally, we were able to show a significant decrease in prostaglandin E_2 production with ASA treatment; measure-

ment of urinary unmetabolized prostaglandins is a useful indicator of renal prostaglandin production.[17] We had to eliminate results from two of our subjects who showed up to 100-fold increases in urinary prostaglandin E_2 due to seminal fluid contamination of urine from intercourse. We did not find a significant increase in prostaglandin E_2 excretion with caffeine; in rats, we have found theophylline to increase 24-hour prostaglandin E_2 excretion* and others have found an increase after caffeine.[5] As most of the women participated at various times in their menstrual cycles, any natural cyclical variation in prostaglandin E_2 excretion would have masked changes due to caffeine.

A food effect was not observed, as both ASA and caffeine treatments were as effective with or without orange juice. An effect due to orange juice ingestion was expected since it contained (by analysis) the following: Ca, 1.5; P, 3.8; Na, 0; K, 50 mmol/l. Furthermore, orange juice contains monosaccharides and disaccharides which promote calcium absorption.[18] The orange juice did contribute to calcium excretion by raising basal (no drug) excretion; hour 0 values were not different between the two experiments, but at each hour there were significant differences ($P < 0.05$) between the no drug groups in experiments 1 and 2. That caffeine can increase basal and diet-induced urinary calcium excretion suggests that it will influence urinary calcium already increased due to other dietary factors; that ASA could influence basal calcium excretion implies that it is effective in altering calcium excretion in normocalciuric as well as hypercalciuric individuals.

While ASA treatment resulted in a blunting of caffeine-induced hypercalciuria, the magnitude of change in calcium excretion, induced by caffeine, as the percentage excreted when caffeine was not present, was similar with and without aspirin (269% and 213%, respectively, experiment 1; 371% and 308%, respectively, experiment 2). This suggests that ASA, by inhibiting prostaglandin E_2 production, has a calcium lowering effect which neither blocks nor promotes caffeine-induced hypercalciuria. Thus, the mechanism of caffeine-induced hypercalciuria appears to occur independently of prostaglandin production.

Because loss of calcium by increased urinary calcium excretion has been recently advocated as the significant factor affecting osteoporosis,[19] it is important to recognize and understand dietary factors, such as caffeine, which affect urinary calcium excretion. We have shown that in the rat, adenosine receptor antagonism may be the mechanism for caffeine-induced hypercalciuria[4]; further work is needed to determine whether adenosine receptor antagonism plays a role in caffeine-induced hypercalciuria in human subjects.

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