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MILK AND CALCIUM PREVENT GASTROINTESTINAL ABSORPTION AND URINARY EXCRETION OF OXALATE IN RATS

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1. ABSTRACT

Dietary oxalate plays a very important role in the formation of calcium oxalate stones, and dietary intake of calcium may decrease oxalate absorption and its subsequent urinary excretion. The purpose of the present study was to determine the effect on urinary oxalate excretion of an acute oral calcium load, standard milk, or high-calcium & low-fat milk followed by a dose of oxalic acid. Male Wistar rats weighing 180-200 g were divided into 7 groups of 6 rats each. All animals were fasted for about 24 hours, anesthetized, and hydrated with normal saline at 3-4 mL/hour. Then the animals were given 1 mL of normal saline {Control}, 10 mg (111.1 μ mol) of oxalic acid {Ox alone}, 2 mL of standard milk (calcium: 1.16 mg or 29 μ mol/mL) {NCa milk}, 2 mL of high-calcium & low-fat milk (calcium: 2.05 mg or 51.3 μ mol/mL) {HCa milk}, equimolar calcium (4.44 mg or 111 μ mol) followed by 10 mg of oxalic acid {Ca + Ox}, 2 mL of high-calcium & low-fat milk followed by 10 mg of oxalic acid {HCa milk + Ox}, or 2 mL of standard milk followed by 10 mg of oxalic acid {NCa milk + Ox}. All treatments were administered via a gastrostomy. Urine samples were collected by bladder puncture just before administration and at hourly intervals up to 5 hours afterwards. Urinary oxalate was measured by capillary electrophoresis, while urinary calcium, magnesium and phosphorus were measured by inductively coupled plasma spectrometry. Urinary oxalate excretion peaked at 1 hour in the Ox alone group, while it peaked at 2 or 3 hours in the Ca + Ox, HCa milk + Ox, and NCa milk + Ox groups. Urinary oxalate excretion decreased significantly when 10 mg of oxalate was administered immediately after the administration of equimolar calcium, high-calcium & low-fat milk, or standard milk. The cumulative urinary oxalate excretion over 5 hours was approximately 13.6%, 3.5%, 1.6%, and 2.4% in the Ox alone, Ca + Ox, HCa milk + Ox, and NCa milk + Ox groups, respectively. In conclusions, this study demonstrated that calcium salt, or dairy products containing calcium (especially high-calcium & low-fat milk) could decrease the gastrointestinal absorption and subsequent urinary excretion of oxalate.

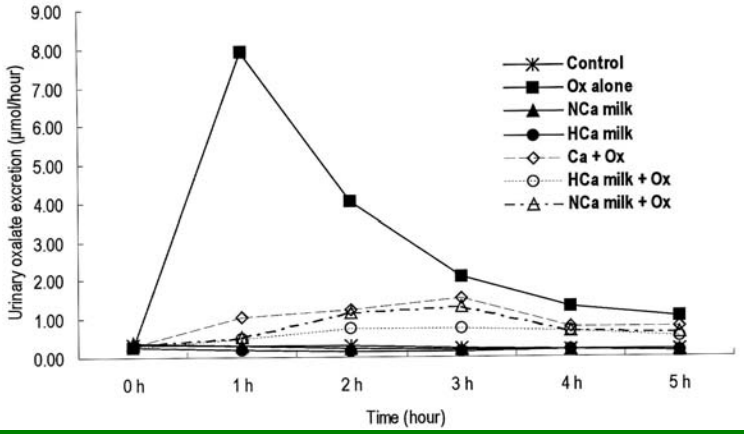
2. INTRODUCTION

Urinary stone disease has a high recurrence rate and high morbidity, afflicting approximately 10% of the population in the Western world and Japan (1-2). Calcium oxalate (CaOx) stones are the most common form of urinary stones, and either pure or mixed CaOx stones account for about 80% or more of all urinary calculi (3).

Because of the suspicion that a high-calcium diet may increase the risk of CaOx stone formation, restriction of calcium intake in stone-forming patients has long been the common clinical practice in an attempt to prevent stone recurrence (4). However, dietary calcium restriction can also cause a negative calcium balance, decreased bone mineral density, and bone loss in stone-formers, particularly in patients with idiopathic hypercalciuria (5-9). An increase of dietary calcium intake may, in fact, reduce the risk of stone formation (10-12), and may also prevent bone loss and osteoporosis in stone-formers (13-14).

Studies have shown that urinary oxalate has a greater impact than urinary calcium on the formation of calcium oxalate crystals (15-18), and mild hyperoxaluria seems to be one of the important causes of idiopathic CaOx stones (17). Total urinary oxalate excretion is determined by dietary oxalate intake, intestinal absorption, endogenous synthesis, and renal excretion (19). Although the majority of urinary oxalate is synthesized endogenously, dietary oxalate usually accounts for about 10-20% of the oxalate excreted in the urine (20). However, it has recently been reported that dietary oxalate might make a much greater contribution to urinary oxalate excretion than has been previously recognized (21).

Oxalate is mainly absorbed from the upper gastrointestinal tract, as well as from the colon (22-23). After oxalate is absorbed or synthesized, it cannot be metabolized further and the usual route of excretion is in the urine. Calcium forms a complex with oxalate in the gut to create insoluble CaOx and thereby interferes with oxalate absorption (24). Therefore, a low dietary intake of calcium will increase intestinal oxalate absorption and subsequent urinary oxalate excretion, because less calcium will be available to bind with oxalate in the gastrointestinal tract. In fact, it has been shown that addition of calcium



Various groups	Baseline oxalate excretion (μ mol)	1 hour excretion (μ mol)	2 hour excretion (μ mol)	3 hour excretion (μ mol)	4 hour excretion (μ mol)	5 hour excretion (μ mol)
Control	0.35 ±0.09	0.30 ±0.10	0.28 ±0.11	0.21 ±0.06 [1]	0.20 ±0.06 [1]	0.19 ±0.04 [1]
Ox alone	0.25 ±0.05 [2]	7.92 ±6.77 [1,2]	4.04 ±2.17 [1,2]	2.10 ±0.90 [1,2]	1.32 ±0.33 [1,2]	1.05 ±0.42 [1,2]
NCa milk	0.39 ±0.14	0.29 ±0.08 [3]	0.24 ±0.05 [1,3]	0.19 ±0.07 [1,3]	0.18 ±0.04 [1,3]	0.16 ±0.04 [1,3]
HCa milk	0.26 ±0.09	0.19 ±0.02 [2,3,4]	0.17 ±0.02 [1,2,3,4]	0.15 ±0.03 [1,2,3]	0.18 ±0.05 [3]	0.14 ±0.06 [1,3]
Ca + Ox	0.30 ±0.13	1.03 ±0.75 [1,2,3,4,5]	1.25 ±1.19 [3]	1.53 ±2.07	0.79 ±0.90	0.77 ±1.17
HCa milk + Ox	0.27 ±0.11	0.50 ±0.36 [3]	0.76 ±0.67 [3]	0.75 ±0.77 [3]	0.66 ±0.59 [3]	0.53 ±0.52
NCa milk + Ox	0.34 ±0.09	0.54 ±0.37 [3,5]	1.17 ±1.05 [3,5]	1.29 ±1.32	0.68 ±0.72	0.63 ±0.52 [5]

Data are shown as mean ± SD. [1]: P<0.05, comparison over time relative to baseline (0 hour) excretion within the group; [2]: P<0.05, when compared with control group; [3]: P<0.05, when compared with Ox alone group; [4]: P<0.05, when compared with NCa milk group; [5]: P<0.05, when compared with HCa milk group.

Figure 1. Urinary oxalate excretion after acute administration of normal saline (Control), Ox alone, NCa milk, HCa milk, Ca + Ox, HCa milk + Ox, and NCa milk + Ox via a gastrostomy in rats (n=6 per group). (Ox, 10 mg of oxalic acid; NCa milk, standard milk; HCa milk, high-calcium & low-fat milk; Ca, calcium salt equimolar to 10 mg of oxalate).

salts to oxalate-containing meals can reduce urinary oxalate excretion (14,22,25-28).

The purpose of the present study was to determine the effect on urinary oxalate excretion of an acute oral calcium load, standard milk, or high-calcium & low-fat milk, followed by a dose of oxalic acid via a gastrostomy.

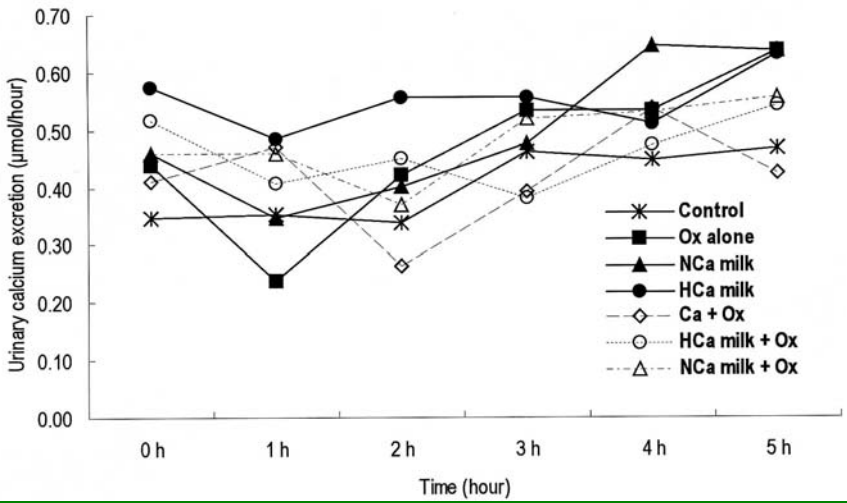
3. MATERIALS AND METHODS

Male Wistar rats weighing 180-200 g were acclimatized for one week at the University Animal Center and then were randomly divided into 7 groups of 6 rats each. All animals were fasted (with free access to drinking water) for about 24 hours before the experiment. After anesthesia was induced with intraperitoneal urethane (0.6 mg), the rats were hydrated with normal saline at 3-4 mL/hour. Then the animals were given 1 mL of normal saline {Control}, 10 mg (111.1 μ mol) of oxalic acid {Ox alone}, 2 mL of standard milk (calcium: 1.16 mg or 29 μ mol/mL) {NCa milk}, 2 mL of high-calcium & low-fat milk (calcium: 2.05 mg or 51.3 μ mol/mL) {HCa milk}, equimolar calcium (4.44 mg or 111 μ mol) followed by 10 mg of oxalic acid {Ca + Ox}, 2 mL of high-calcium & low-fat milk followed by 10 mg of oxalic acid {HCa milk + Ox}, or 2 mL of standard milk followed by 10 mg of oxalic acid {NCa milk + Ox}. All treatments were administered via a gastrostomy.

The oxalic acid solution was prepared by dissolving oxalic acid dihydrate (molecular weight: 126.07; Wako Pure Chemicals, Osaka, Japan) in 1 mL of pure water, while the equimolar calcium (4.4 mg of calcium) solution was prepared by dissolving calcium chloride dihydrate (molecular weight: 147.02; Wako Pure Chemicals) in 1 mL of pure water. Standard milk and high-calcium & low-fat milk were commercially available products, and their composition is shown in Table 1. The animals also received normal saline intravenously at a rate of 3-4 mL/hour. Urine samples were collected by bladder puncture just before administration and at hourly intervals

Table 1. Comparison of the composition of standard milk and high-calcium & low-fat milk.

Nutrients	**Standard Milk** (Morinaga Dairy Product Industries Ltd., Okinawa, Japan)	**High Calcium & Low fat Milk** (Meiji Dairy Product Industries Ltd., Okinawa, Japan)
Energy	64 kcal/100 mL	47 kcal/100 mL
Carbohydrate (Sugar)	4.9 g/100 mL	5.9 g/100 mL
Protein	3.2 g/100 mL	3.7 g/100 mL
Fat	3.4 g/100 mL	1.0 g/100 mL
Ca	116 mg/100 mL	205 mg/100 mL
Na	52 mg/100 mL	62 mg/100 mL
K	---	194 mg/100 mL
P	---	109 mg/100 mL
Vit-D3	---	51 I.U./100 mL



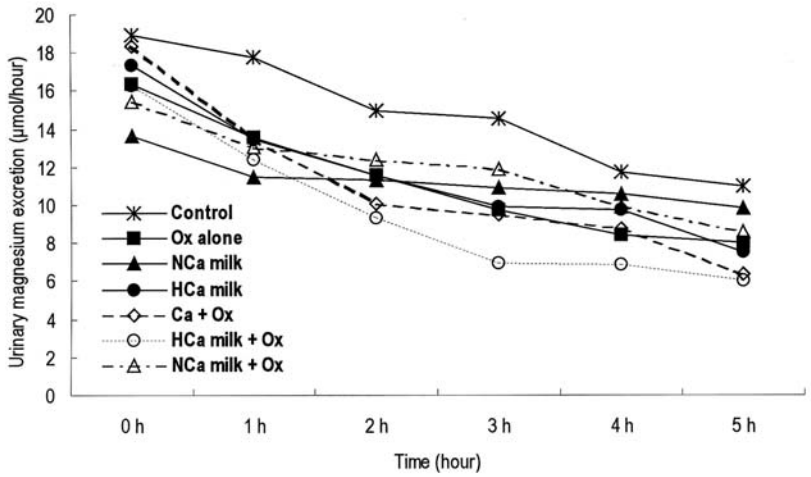
Various groups	Baseline calcium excretion (μ mol)	1 hour excretion (μ mol)	2 hour excretion (μ mol)	3 hour excretion (μ mol)	4 hour excretion (μ mol)	5 hour excretion (μ mol)
Control	0.35 ±0.08	0.35 ±0.05	0.34 ±0.09	0.46 ±0.16	0.45 ±0.12	0.47 ±0.15
Ox alone	0.44 ±0.14	0.24 ±0.19	0.42 ±0.43	0.53 ±0.69	0.53 ±0.52	0.64 ±0.61
NCa milk	0.46 ±0.25	0.35 ±0.19	0.40 ±0.24	0.48 ±0.26	0.65 ±0.61	0.64 ±0.44
HCa milk	0.57 ±0.29	0.49 ±0.10 [1,2]	0.56 ±0.11 [1]	0.56 ±0.11	0.51 ±0.18	0.63 ±0.27
Ca + Ox	0.41 ±0.11	0.47 ±0.26	0.26 ±0.13 [3]	0.39 ±0.23	0.54 ±0.32	0.42 ±0.20
HCa milk + Ox	0.52 ±0.27	0.41 ±0.14	0.45 ±0.27	0.38 ±0.12 [3]	0.47 ±0.37	0.54 ±0.35
NCa milk + Ox	0.46 ±0.18	0.46 ±0.23	0.37 ±0.25	0.52 ±0.20	0.53 ±0.21	0.56 ±0.30

Data are shown as mean ± SD. [1]: P<0.05, when compared with control group; [2]: P<0.05, when compared with Ox alone group; [3]: P<0.05, when compared with HCa milk group.

Figure 2. Urinary calcium excretion after acute administration of normal saline (Control), Ox alone, NCa milk, HCa milk, Ca + Ox, HCa milk + Ox, and NCa milk + Ox via a gastrostomy in rats (n=6 per group). (Ox, 10 mg of oxalic acid; NCa milk, standard milk; HCa milk, high-calcium & low-fat milk; Ca, calcium salt equimolar to 10 mg of oxalate).

up to 5 hours afterwards, and were immediately frozen at –80 until assay.

Thawed urine samples were acidified (to pH 2 or less) with 6 N HCl, and were filtered through a disposable 0.2 μ m Millipore filter (Millex-LG syringe-driven unit, Millipore, Bedford, MA, USA). Then the samples were diluted 20- to 40-fold with Milli-Q level pure water (Millipore Water Purification System) and injected into a capillary tube at 50 mbar (5000 pa) for 4 seconds



Various groups	Baseline magnesium excretion (μ mol)	1 hour excretion μ mol)	2 hour excretion (μ mol)	3 hour excretion (μ mol)	4 hour excretion (μ mol)	5 hour excretion (μ mol)
Control	18.93 ±7.64	17.76 ±6.10	14.97 ±3.20	14.52 ±4.01	11.75 ±2.84	10.99 ±2.09 [1]
Ox alone	16.35 ±4.82	13.58 ±3.06	11.61 ±5.38	9.77 ±4.38 [1]	8.41 ±4.09 [1]	8.03 ±4.19 [1]
NCa milk	13.61 ±7.09	11.53 ±4.89	11.30 ±2.13 [2]	10.90 ±2.34	10.54 ±2.01	9.84 ±4.97
HCa milk	17.32 ±6.56	13.50 ±2.17	11.61 ±3.29	9.88 ±2.42 [1,2]	9.78 ±4.11 [1]	7.56 ±3.50 [1]
Ca + Ox	18.37 ±7.13	13.51 ±2.97	10.05 ±2.35 [1,2]	9.51 ±4.64 [1]	8.77 ±5.15 [1]	6.37 ±3.79 [1,2]
HCa milk + Ox	16.29 ±4.99	12.37 ±2.98	9.33 ±1.92 [1,2]	6.90 ±3.65 [1,2,3]	6.89 ±2.45 [1,2,3]	6.05 ±2.53 [1,2]
NCa milk + Ox	15.43 ±6.68	13.06 ±3.45	12.38 ±2.00 [4]	11.93 ±5.59	9.94 ±4.11	8.57 ±3.46 [1]

Data are shown as mean ± SD. [1]: P<0.05, comparison over time relative to baseline (0 hour) excretion within the group; [2]: P<0.05, when compared with control group; [3]: P<0.05, when compared with NCa milk group; [4]: P<0.05, when compared with HCa milk + Ox group.

Figure 3. Urinary magnesium excretion after acute administration of normal saline (Control), Ox alone, NCa milk, HCa milk, Ca + Ox, HCa milk + Ox, and NCa milk + Ox via a gastrostomy in rats (n=6 per group). (Ox, 10 mg of oxalic acid; NCa milk, standard milk; HCa milk, high-calcium & low-fat milk; Ca, calcium salt equimolar to 10 mg of oxalate).

(approximately 20 nl), in order to measure the urinary oxalate level by capillary electrophoresis (Hewlett-Packard, Waldbronn, Germany) using a buffer (pH 7.7) for high performance capillary electrophoresis (HPCE) (Fluka, Switzerland). Urine samples were diluted 200- to 400-fold with pure water to measure urinary calcium, magnesium, and phosphorus levels by inductively coupled plasma spectrometry (ICPS-7000, Shimadzu, Kyoto, Japan).

The urinary values of oxalate, calcium, magnesium, and phosphorus at '0' hour were considered as the baseline excretion of the respective substances. In regards to the calculation of cumulative excretion of oxalate, it is to admit that although it was not possible from the present experimental model to determine the exact amount of oxalate derived from endogenous synthesis, the baseline value was considered as the level of urinary oxalate that might be coming from the endogenous sources. And, the cumulative urinary excretion of oxalate up to 5 hours after administration in each experimental group was calculated by deducing the baseline (0 h) urinary value of oxalate in the respective group. The hourly urinary excretion and the changes of urinary oxalate, calcium, magnesium, and phosphorus relative to the baseline values were compared over time using the paired t-test, and were compared between groups using multiple t-tests. Data are shown as the mean±SD, and statistical significance was set at $p<0.05$ for all comparisons.

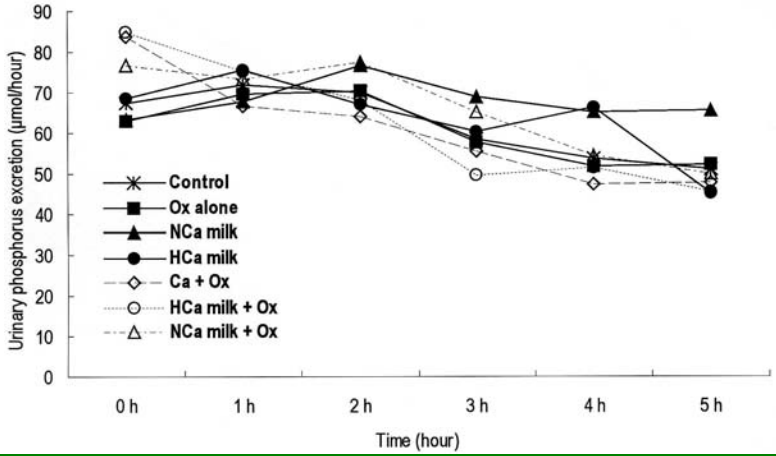
4. RESULTS

Urinary oxalate excretion peaked at 1 hour in the Ox alone group, while it peaked at 2 or 3 hours in the Ca + Ox, HCa milk + Ox, and NCa milk + Ox groups. It was significantly higher ($p<0.05$) than baseline at all times up to 5 hours in the Ox alone group, as well as at 1 hour in the Ca + Ox group (Figure 1). It was significantly lower ($p<0.05$) than baseline at 2-5 hours in the NCa milk group, as well as at 2-3 and 5 hours in the HCa milk group (figure 1).

Table 2. Cumulative urinary excretion of oxalate after acute administration of normal saline (Control), Ox alone, NCa milk, HCa milk, Ca + Ox, HCa milk + Ox, and NCa milk + Ox via a gastrostomy in rats.

Various groups of rats (n=6/group)	Baseline oxalate excretion (μ mol)	Total urinary increment of oxalate over 5 hours (μ mol)
Control	0.35±0.09	-0.59±0.05
Ox alone	0.25±0.05 [1]	15.14±2.84 (13.6±2.6%) [1]
NCa milk	0.39±0.14	-0.86±0.05 [2]
HCa milk	0.26±0.09	-0.48±0.02 [2]
Ca + Ox	0.30±0.13	3.85±0.32 (3.5±0.3%) [1,2,3,4]
HCa milk + Ox	0.27±0.11	1.83±0.12 (1.7±0.1%) [1,2,3,4,5]
NCa milk + Ox	0.34±0.09	2.62±0.34 (2.4±0.3%) [1,2,3,4]

Data are shown as mean ± SD; [1]: P<0.05, when compared with control group; [2]: P<0.05, when compared with Ox alone group; [3]: P<0.05, when compared with NCa milk group; [4]: P<0.05, when compared with HCa milk group; [5]: P<0.05, when compared with Ca + Ox group. (Ox, 10 mg of oxalic acid; NCa milk, standard milk; HCa milk, high-calcium & low-fat milk, Ca, calcium salt equimolar to 10 mg of oxalate).



Various groups	Baseline phosphorus excretion (μ mol)	1 hour excretion (μ mol)	2 hour excretion (μ mol)	3 hour excretion (μ mol)	4 hour excretion (μ mol)	5 hour excretion (μ mol)
Control	67.37 ±11.08	71.91 ±9.96	70.17 ±19.01	58.46 ±10.55	53.81 ±14.66	51.12 ±9.83 [1]
Ox alone	63.03 ±10.26	69.80 ±12.14	70.40 ±15.11	57.60 ±8.34	51.97 ±8.90	52.20 ±13.66
NCa milk	63.19 ±17.64	67.78 ±28.30	76.71 ±8.60	68.72 ±13.07	65.15 ±9.44 [2]	65.50 ±20.88
HCa milk	68.40 ±6.38	75.60 ±7.26	66.89 ±13.04	60.54 ±10.78	66.32 ±16.11	45.16 ±11.44 [1]
Ca + Ox	83.63 ±27.10	66.58 ±17.17	64.07 ±23.55	55.38 ±24.03	47.28 ±20.25 [1]	47.70 ±24.56 [1]
HCa milk + Ox	84.72 ±18.48 [2]	75.35 ±13.26	68.05 ±14.70	49.47 ±19.36 [1]	51.42 ±18.46 [1]	45.46 ±19.54 [1]
NCa milk + Ox	76.74 ±20.24	73.34 ±20.94	77.32 ±16.80	65.37 ±26.36	54.52 ±21.72	49.85 ±18.19 [1]

Data are shown as mean ± SD. [1]: P<0.05, comparison over time relative to baseline (0 hour) excretion within the group; [2]: P<0.05, when compared with Ox alone group.

Figure 4. Urinary phosphorus excretion after acute administration of normal saline (Control), Ox alone, NCa milk, HCa milk, Ca + Ox, HCa milk + Ox, and NCa milk + Ox via a gastrostomy in rats (n=6 per group). (Ox, 10 mg of oxalic acid; NCa milk, standard milk; HCa milk, high-calcium & low-fat milk; Ca, calcium salt equimolar to 10 mg of oxalate).

However, there were no significant differences in urinary oxalate excretion compared with the baseline value in the HCa milk + Ox and NCa milk + Ox groups. Urinary oxalate excretion was significantly higher (p<0.05) than in the control group at 1-5 hours in the Ox alone group, and at 1 hour in the Ca + Ox group (figure 1). However, there was no significant difference of oxalate excretion at any time between the HCa milk + Ox or NCa milk + Ox groups

and the control group. Moreover, oxalate excretion was significantly lower at 1-3 hours in the HCa milk group than in the control group (figure 1). Compared with the Ox alone group, urinary oxalate excretion was significantly lower at 1-4 hours in the HCa milk + Ox group, and at 1-2 hours in the Ca + Ox and NCa milk + Ox groups (figure 1). The cumulative urinary oxalate excretion up to 5 hours was 13.6%±2.6% (mean±SD), 3.5%±0.3%, 1.7%±0.1%, and 2.4%±0.3% of the administered dose in the Ox alone, Ca + Ox, HCa milk + Ox, and NCa milk + Ox groups, respectively (Table 2).

In the HCa milk group, urinary calcium excretion was significantly increased at 1-2 hours and at 1 hour compared with that in the Control group and the Ox alone group, respectively (figure 2). It was significantly lower at 2 hours in the Ca + Ox group than in the HCa milk group, as well as at 3 hours in the HCa milk + Ox group. However, there were no significant differences of urinary calcium excretion between baseline and other times in the experimental groups (figure 2).

Urinary magnesium excretion was significantly decreased from baseline at 3-5 hours in the Ox alone and HCa milk groups, at 2-5 hours in the Ca + Ox and HCa milk + Ox groups, and at 5 hours in the NCa milk + Ox group (figure 3). Urinary magnesium excretion was significantly lower than in the Control group at 2 hours in the NCa milk group, at 3 hours in the HCa milk group, at 2 and 5 hours in the Ca + Ox group, and at 2-5 hours in the HCa milk + Ox group (figure 3).

Urinary phosphorus excretion was significantly decreased from baseline at 5 hours in the HCa milk and NCa milk + Ox groups, at 4-5 hours in the Ca + Ox group, and at 3-5 hours in the HCa milk + Ox group, but there were no significant differences between the control and experimental groups (figure 4).

5. DISCUSSION

The findings of this study suggest that intake of calcium salt, standard milk, or high-calcium & low-fat milk along with a high-oxalate meal can significantly reduce gastrointestinal oxalate absorption and urinary excretion. Urinary oxalate excretion was significantly increased within 1 hour after administration of 10 mg of oxalic acid alone, suggesting that considerable absorption might occur in the upper gastrointestinal tract. This observation is consistent with the findings we reported previously (29), as well as, is compatible with the other reports that the small bowel was a major oxalate absorption site, although some gastric oxalate absorption might also occur (22-23,30). When calcium salts or two different kinds of milk were given immediately prior to oxalic acid, urinary oxalate excretion was significantly decreased, probably due to the well known mechanism of calcium forming a complex with oxalate and thereby preventing its absorption and urinary excretion. This result is also consistent with our previous finding that urinary oxalate excretion was markedly reduced after simultaneous administration of calcium salt and oxalic acid (28). A small decrease in oxalate excretion over time (when compared with the baseline excretion) after administration of milk alone diets was observed despite prolong fasting, however, further study is required to explain the issue. A further investigation is also required to determine whether the pH after gastric loading of oxalic acid, calcium or milk, might have any influence on oxalate absorption. The cumulative oxalate excretion in Ca + Ox was higher than the milk + Ox, suggesting that the other nutrients present in milk might also have a role in the prevention of oxalate absorption. Although from the present study, it is not possible to measure the amount of oxalate that might have absorbed from the stomach, it has been suggested that stomach is a new and powerful oxalate absorption site (30). However, in another series of our experimental model, we have found that a small amount of oxalate could be absorbed from the stomach after gastric administration and when the gastric emptying was blocked (unpublished observation). Other experimental evidence has also suggested that increased dietary intake of calcium actually reduces the risk of stone formation by preventing oxalate absorption and urinary excretion (10-12,16,26-27,31,32). These findings have superseded the previous suspicion/belief that dietary calcium may increase the risk of urinary stone formation, which has often led to restriction of calcium intake by stone-formers in order to decrease stone recurrence. In fact, a restricted or low dietary calcium intake without restricting the dietary oxalate intake by patients with hypercalciuria will actually increase the risk of stone formation or recurrent stones (11,33). Restriction of calcium intake can also cause a negative calcium balance, leading to decreased bone mineral density, bone loss, and osteoporosis, particularly in patients with idiopathic hypercalciuria (5-9). Calcium is an important nutrient for humans and it may prevent bone loss in stone-formers (13,14), as well as preventing various common disorders in women, such as osteoporosis and pre-eclampsia (34,35).

Dietary oxalate plays a very important role in the formation of calcium oxalate stones. The daily intake of oxalate ranges from 70 to 920 mg, but it is far higher among vegetarians, ranging from 80 to 2,000 mg (36). Green plants (spinach), beetroot, rhubarb, and peanuts contain large amounts of oxalate, while high oxalate concentrations can also be found in tea, coffee and cocoa (15,37). Therefore, it is recommended that tea and coffee be taken with milk rather than without milk to decrease the net oxalate content that is available for absorption (37). Substantial amounts of oxalate are also found in foods recommended as part of a balanced diet that appears to be protective against coronary heart disease (38), stroke (39), cancer (40), and diabetes (41). Oxalate may also be produced from certain precursors in the gastrointestinal tract, especially ascorbic acid, which is transformed to oxalate by the intestinal flora or by nonenzymatic degradation (30,42). About 10-20% of the oxalate excreted in the urine is usually believed to come from dietary sources (20), but it has recently been reported that dietary oxalate might make a much greater contribution to urinary oxalate than has been previously recognized (21). Urinary oxalate excretion is generally higher in stone-formers than in non-stone-formers (43). Studies have suggested that

urinary oxalate may play a greater role than urinary calcium in the formation of CaOx stones, because saturation of urine with CaOx increases more rapidly when the oxalate concentration rises than when the calcium concentration increases (15-18).

Oxalate is mainly absorbed from the upper gastrointestinal tract, but also from the colon (22-23,29,44). Absorption of oxalate occurs by passive diffusion and an active uptake mechanism in both humans and animals (42,44-45). Once oxalate is absorbed or synthesized, it cannot be further metabolized, and its usual route of excretion is in the urine. Among the various factors that may influence oxalate absorption, the dietary oxalic acid/calcium ratio is important, and rats fed various calcium & oxalate-containing diets form CaOx stones in a dependence on the dietary Ox/Ca ratio (> 1 mol/mol) (46). Dietary calcium forms a complex with oxalate in the gut and causes the excretion of insoluble CaOx in the stool, thereby preventing absorption of oxalate. Therefore, a restricted or low calcium intake will eventually allow more oxalate to be absorbed, because less calcium will be available to bind with it in the gastrointestinal tract, whereas an increase of dietary calcium will decrease oxalate absorption and its subsequent urinary excretion. We also observed this relationship in the present study. Various chronic gastrointestinal disorders, including chronic inflammatory bowel disease, ileal bypass surgery, and short bowel syndrome, can cause fat malabsorption that leads to calcium soap formation, which reduces intraluminal calcium and leaves free oxalate available for absorption. Malabsorption of bile and fatty acids increases the permeability of the colonic mucosa to oxalate (19,47). Oxalate absorption is markedly increased in patients with small bowel resection or inflammatory bowel disease providing that the colon has not been removed (20,48). Various oxalate-degrading bacteria, including Oxalobacter formigenes and Enterococcus faecalis (49), exist in the gastrointestinal tract and seem to regulate oxalate homeostasis by preventing its absorption, catabolizing free oxalate, and enhancing its secretion from the blood. Thus, the absence or decreased activity of oxalate-degrading bacteria appears to be a risk factor for hyperoxaluria.

The principal aim of this study was to determine the effect on urinary oxalate excretion of an acute oral calcium load versus standard milk or high-calcium & low-fat milk followed by a dose of oxalic acid. Although calcium salt, standard milk, and high-calcium & low-fat milk all prevented oxalate absorption and urinary excretion, it seems that high-calcium & low-fat milk might achieves the best protection against gastrointestinal oxalate absorption. The different calcium content, as well as differences of other nutrients in the two kinds of milk, might have also played a role in this regard.

6. CONCLUSIONS

This study demonstrated that the intake of calcium salt, or dairy products (as a source of calcium), especially high-calcium & low-fat milk, immediately before ingestion of oxalate could decrease the gastrointestinal absorption and subsequent urinary excretion of oxalate, and thereby may reduce the risk of urinary CaOx stones. Therefore, calcium should be consumed with every meal to achieve a balance between calcium & oxalate in the diet, and a low-calcium diet should not be recommended for patients with CaOx stones.

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