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ORIGINAL PAPER

Probiotic-induced reduction of gastrointestinal oxalate absorption in healthy subjects

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Abstract Both a high dietary oxalate intake and increased intestinal absorption appear to be major causes of elevated urine oxalate, a risk factor for kidney stone formation. By favorably altering the gastrointestinal bacterial population, probiotics have the potential to lower oxalate absorption/urinary excretion. This study assessed whether a 4-wk daily consumption of a commercially available probiotic by 11 healthy volunteers (8 females, 3 males), aged 21-36 y, would decrease oxalate absorption. The study involved the ingestion of a probiotic (VSL#3®) for a 4 wk period followed by a 4 wk washout period. Oxalate load tests, providing a total of 80 mg oxalate, were conducted at baseline (pre-probiotic), and after the probiotic and washout periods. In the total subject population, mean total 22 h oxalate absorption at baseline (30.8 %) was significantly higher than after the probiotic (11.6 %) and washout (11.5 %) periods. However, four subjects identified as high oxalate absorbers at baseline had a particularly marked probiotic-induced reduction in oxalate absorption, which largely accounted for the reduction observed in the total subject population. The overall data suggested that in individuals characterized by high oxalate absorption levels, VSL#3® ingestion has the potential to reduce gastrointestinal oxalate absorption, which could decrease risk of kidney stones and other disorders related to hyperoxaluria.

Keywords Probiotic · Oxalate absorption · Kidney stones · Gastrointestinal tract

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Introduction

Oxalic acid is a toxic strong dicarboxylic acid ($pK_a^1 = 1.23$; $pK_a^2 = 3.83$) that if ingested in very high levels could lead to renal failure [1]. Oxalate (also called *ethanedioate*) is a salt of oxalic acid and is a chemical component of foods, primarily those of plant origin. Oxalate originates from dietary sources or as an end product of endogenous metabolism of ascorbate, glyoxylate and glycine [2]. Oxalic acid can combine with calcium in the urinary tract to form an insoluble salt, calcium oxalate, responsible for over 70% of diagnosed kidney stones [3]. Both a high dietary oxalate load and increased intestinal absorption appear to be major causes of hyperoxaluria, a risk factor for stone formation.

Of the total dietary oxalate ingested, about 10-15% is normally absorbed [4]. Oxalate absorption has been reported to occur throughout the gut [5, 6] and colonic absorption can account for 3-5% of dietary oxalate ingested [7]. Since oxalate is not significantly metabolized in the human body, its excretion starts almost immediately after ingestion and reaches a peak between 2 to 6 hours [8]. Once absorbed, it is rapidly taken up and cleared by the kidneys through filtration and eventually excreted in urine [9]. The mechanism of absorption in humans is not well understood, but it has been shown that rabbits' proximal colon possesses secretory as well as absorptive capacities [10].

Contribution of dietary oxalate to total urinary oxalate excretion has been estimated to be between 24% and 53% [5], but this may reach up to 67% in high absorbers [11–13].The amount of oxalate excreted in urine increases with an increase in dietary oxalate, but can be reduced by the presence of certain cations such as calcium and magnesium which can bind oxalate in the gut to form insoluble salts

thereby preventing oxalate absorption. It has therefore been suggested that reducing dietary intake [14] as well as oxalate absorption could be beneficial in individuals predisposed to hyperoxaluria [15, 16].

Probiotics are dietary supplements containing live microorganisms which have the potential to confer health benefits beyond inherent general nutrition to the host. Lactic acid bacteria are the most commonly used group of probiotic microorganisms [17]. Microorganisms that have been reported to play a role in degradation of oxalate within the gut include *Oxalobacter formigenes, Eubacterium lentum, Enterococcus faecalis,* and *Lactobacillus acidophilus* [18–20]. A non-pathogenic gram-negative anaerobic bacterium, *O. formigenes*, present in the colon of vertebrates (including humans), has well established oxalate-degrading capabilities [21]. *O. formigenes* can degrade oxalate to formate and CO_2 using two enzymes [formyl coenzyme A transferase (EC 2.8.3.16) and oxalyl-coenzyme A decarboxylase (EC 4.1.1.8)]. Lactic acid bacteria are important inhabitants of the human gastrointestinal tract and have been traditionally used as probiotics due to their reported health promoting benefits [17, 22]. Some lactic acid bacteria have been reported to express the genes encoding for oxalyl-coenzyme A decarboxylase and formyl-coenzyme A transferase, which includes strains such as *L. acidophilus, Lactobacillus gasseri* and *Bifidobacterium lactis* (Gasser AM63^T) [23–25].

It has been previously reported that lactic acid bacteria-containing probiotic cultures can reduce 24-h urinary oxalate excretion in hyperoxalurics and kidney stone formers [26, 27]. In neither of these studies were subjects specifically challenged with a high oxalate load. Thus, the objective of the present study was to determine whether a 4-wk ingestion of VSL#3®, a commercially available lactic acid bacteria-containing probiotic, by healthy non-stone formers would lead to a reduction in oxaluria after ingestion of an 80 mg oxalate dose. A secondary objective was to assess whether this effect is maintained after a further 4 wk following interruption of probiotic use. Because degree of responsiveness to the probiotic could be affected by whether subjects were colonized with *O. formigenes*, fecal samples were collected prior to study initiation for analysis of this bacterium.

Methods

VSL#3® (Probiotic)

This study used a probiotic supplement, marketed with the brand name VSL#3® (Sigma-Tau pharmaceuticals, Inc., Gaithersburg, MD, USA). It contains a freeze-dried live lactic acid bacterial culture consisting of *Streptococcus thermophilus*, three strains of *Bifidobacterium* species (*B. breve, B. longum* and *B. infantis*), and four strains of *Lactobacillus* species (*L. acidophilus, L. plantarum, L. paracasei* and *L. delbrueckii* subsp. *bulgaricus*). Each sachet of VSL#3® contains 800 billion live bacteria. The cultures used in this probiotic are generally recognized as safe by the Food and Drug Administration.

Subjects

All prospective subjects completed a pre-experimental health screening questionnaire. Eligible subjects did not have a history of kidney stones, irritable bowel disease, lower-abdominal pains or any problems associated with urine passage. Those who were taking or had taken oral antibiotics less than 4 wk before the start of this study were also not eligible. The study was initiated with 13 healthy volunteers, recruited from the student population, after obtaining informed consent in accordance with the regulations of the Institutional Review Board of the University of Wyoming (approved 11/2008). Two subjects dropped out, one due to personal reasons and the other due to the need to start antibiotic therapy, thus leaving 11 subjects (8 females, 3 males) who completed the study.

Study design

The study period lasted a total of 8 wk with an initial one-time fecal sample collection. Collected samples were immediately frozen prior to analysis for presence of *O. formigenes* by the culture method [28]. The study involved a pre- and two post-probiotic analyses of oxalate absorption. The three oxalate load tests were referred to as: baseline (the day prior to initiation of the 4-wk probiotic supplementation period), probiotic (the day immediately following the 4-wk probiotic supplementation period) and washout (the day following the 4-wk washout period). The oxalate load tests involved the analysis of urinary oxalate and creatinine pre- and post-oxalate ingestion.

Each of the three oxalate load tests involved a series of urinary oxalate analyses from timed urine samples following oral ingestion of 80 mg (10 capsules) oxalate in the form of supplemental turmeric provided in a capsulated form (Puritan's Pride, Oakdale, NY). The oxalate provided by turmeric has been demonstrated to be primarily soluble (91% of total oxalate) and relatively well absorbed [29] and was thus chosen as an ideal source of oxalate for the present study. The initial load test marked the beginning of the study which was followed by a 4-wk period of daily probiotic supplementation. The subjects took one sachet of the probiotic (VSL#3®) daily, dissolved in a glass of water after the last meal of the day. In order to track compliance, sujects were provided log sheets with dates

and corresponding check-boxes to be marked each time they consumed the probiotic.

Each subject followed a low oxalate diet on the day before the oxalate load test and for 22 h post-oxalate ingestion. A detailed list of acceptable low oxalate foods was provided to each subject and they listed all foods consumed on a separate food record form. After a minimum of 12 h overnight fast, subjects discarded the first urine discharged the morning of the load test, but noted the time it occurred. They were provided 500 ml of bottled water which they were instructed to consume immediately following the first urine discharge to ensure adequate urine production. Beverages, other than those provided as part of the low oxalate meals, were not allowed during the oxalate load test days. They were instructed to arrive in the laboratory 1 h 45 min following the first discharge so that they could be ready for the first urine sample collection. Two hours following the initial urination, a urine sample (basal) was collected. The volume of the urine sample was recorded after which 100 ml was acidified with 1 ml of 12 N HCL acid for preservation purposes. Aliquots were refrigerated for subsequent oxalate and creatinine analyses. Immediately after collecting the basal urine sample, subjects ingested 10 capsules of supplemental turmeric containing a known oxalate content (80 mg). Fifteen minutes later, they consumed a low-oxalate breakfast from a provided group of foods consisting of rice cereal with rice milk, apple juice, sausage, apple and grapes. Subjects continued drinking specified amounts of water during the oxalate load test. Urine samples were continuously collected over three 2-h intervals and processed the same way as the basal sample. A low-oxalate lunch was provided 4 h after turmeric ingestion that was comprised of hard boiled eggs, rice cakes, cheese, apple juice, and grapes. Subjects kept a detailed food record on the test day and were required to consume the same types and amounts of food from the provided meals on all 3 test days to minimize any potential confounding effects. After the third 2-h collection, subjects left the lab with acidified containers (10 ml of 6 N HCL) to collect all urine excreted up through the first urine voided the following morning. This approximately 16-h urine composite was returned to the laboratory the following day. The urine samples were designated B-2, S-2, S-4, S-6, and S-22 to correspond to the urine collection timing in hours pre- and post-oxalate ingestion.

At the end of the 4-wk probiotic period, an oxalate load test similar to the one carried out at the start of the study was conducted. Subjects continued with the study for an additional 4 wk washout period without the use of VSL#3®. At the end of this period, the final oxalate load test was conducted. Diet records were started the day before each oxalate load test and continued through 22 h post-oxalate ingestion.

An estimation of net oxalate excretion (i.e., the difference between total urinary oxalate and that portion of total urinary oxalate that can be attributed to endogenous oxalate synthesis) is required to approximate oxalate absorption. The B-2 urinary oxalate excretion on test days was considered an approximation of endogenous oxalate and rate of endogenous oxalate excretion was assumed to be constant throughout the day. For the three oxalate load tests (baseline, probiotic, and washout), total oxalate levels in the basal urine samples for each subject were averaged across all three treatments to compute the mean basal (B-2) urinary excretion. The 6- and 22-h endogenous oxalate was computed by multiplying the mean B-2 urinary oxalate by 3 and 11, respectively.

Sample analyses

Oxalate and creatinine analyses

The urine samples were analyzed for oxalate by an enzymatic method using an oxalate kit (Trinity Biotech, Berkeley Heights, New Jersey). This method is based on the oxidation of oxalate by an oxalate oxidase enzyme followed by detection of hydrogen peroxide (H_2O_2) produced during the reaction [30]. Lyophilized (control) urine samples having predetermined oxalate concentrations of between 20 and 30 mg/L were analyzed with each assay for quality control purposes. Urine samples were analyzed for creatinine according to the method developed by Lustgarten and Wenk [31]. Urinary oxalate concentrations were expressed in terms of absolute values (mg) as well as relative to creatinine concentration (mg oxalate/g creatinine) to correct for any significant variations in urine flow or errors in urine collection.

Analysis of Oxalobacter formigenes from the fecal specimens

O. formigenes was isolated and analyzed by the culture method described by Allison *et al*. [28]. Swabs were taken from thawed fecal samples and anaerobically innoculated into an anaerobic culture medium (containing 10 mM oxalate, 100 ml mineral solutions, 10 ml sodium acetate [1 M sol.], 20 ml trace minerals, 1 ml of 0.1% resazurin, 1 g yeast extract, 1.34 g sodium oxalate, 4 g sodium carbonate, 0.5 g cysteine HCL, 870 ml distilled water). The vials were incubated at 37°C for up to 2 wk, after which a calcium oxalate precipitation test was conducted [28]. Degradation of oxalate as evidenced by a marked decrease in turbidity was indicative of a positive test.

Statistical analyses

The statistical analyses made use of single-factor repeated-measures analysis of variance to test the hypothesis that average urinary oxalate excretion during the oxalate load tests were the same among baseline, probiotic, and washout periods, where both treatment and time (with time representing the different time periods of urine collection during the oxalate load tests) were entered into the model. All subjects served as their own control. Where there was a significant treatment effect as well as a treatment-by-time interaction, treatment differences at specific time points were determined using Tukey's HSD (honest significant difference) post hoc test. Statistical computations were done using the general linear model (GLM) procedure of the Statistical Analysis Software (SAS version 9.1, SAS Institute Inc., Cary, NC, USA. 2002-2003). Values of $p < 0.05$ were considered to designate statistical significance. Data are reported as means ± SD.

Results

Eleven subjects (8 females, 3 males) completed the study. The mean subject age was 24 ± 4 y (range: 21-36 y) and the mean BMI was 24 ± 6 kg/m^2 (range: 19-41 kg/m^2). The overall compliance with daily ingestion of the probiotic was good based on the returned log sheets. For the total 28 days of probiotic usage, 7 of the subjects complied fully (100%), while one subject missed taking the probiotic for five days, another missed for four days, a third missed for two days, and one missed for a day. In instances in which the probiotic was not taken, subjects fully compensated by taking two sachets the following day. No subject reported experiencing any noticeable effects from the use of this probiotic. Based on the food records kept on data collection days and previous work which quantified oxalate levels of the foods and beverages provided for breakfast and lunch [29], subjects were estimated to have consumed 11.0 ± 3.7 ($\overline{X}$ ± SD) mg of oxalate for breakfast and 19.3 ± 3.2 mg of oxalate for lunch. All subjects consumed 1-4 oz of cheese at lunch which due to its high calcium content, would have been expected to decrease oxalate absorption from this meal.

Urine volumes and creatinine excretion

Baseline, probiotic and washout urine volumes, oxalate, and creatinine excretion data are presented in Table 1. The only significant difference in urine volumes occurred for the 2-h basal (B-2) sample, collected immediately prior to ingestion of the 80 mg oxalate dose, which was significantly lower at baseline than for the probiotic and washout periods (by 56% and 58%, respectively). The low B-2 urine volume at baseline suggested that some subjects had not consumed the provided bottled water immediately following the initial urine discharge. Following this realization, it was reemphasized to subjects that the provided water must be consumed within 15 minutes following the first urine discharge on the day of the oxalate load tests which likely led to the increased B-2 urine volumes for the probiotic and washout periods. There was no overall treatment effect or treatment-by-time interaction for urinary creatinine. In light of urinary creatinine providing a rough measure of completeness of urine collection, the relative constancy in this metabolite between the three oxalate load tests suggested that the subjects complied with the urine collection procedures.

Table 1 Baseline, probiotic and washout urine volume, oxalate and creatinine excretion prior to and following administration of the oxalate load*

Parameter and time (hrs)	Treatment		
	Baseline	Probiotic	Washout
Urine volume (ml)			
B-2	126 ± 98[a]	288 ± 190[b]	298 ± 152[b]
S-2	464 ± 212	512 ± 188	528 ± 233
S-4	514 ± 228	529 ± 213	550 ± 315
S-6	207 ± 82	334 ± 231	359 ± 413
S-22	1374 ± 759	1375 ± 568	1289 ± 699
Creatinine (mg)			
B-2	110 ± 44	122 ± 60	109 ± 47
S-2	117 ± 51	105 ± 51	115 ± 51
S-4	92 ± 49	116 ± 39	112 ± 51
S-6	111 ± 47	112 ± 51	114 ± 41
S-22	860 ± 269	853 ± 276	861 ± 317
Total 24 h	1291 ± 417	1309 ± 438	1311 ± 475
Oxalate (mg)			
B-2	2.0 ± 0.9	2.0 ± 0.9	2.0 ± 0.9
S-2	4.3 ± 0.9	3.8 ± 1.7	4.3 ± 1.1
S-4	5.3 ± 2.3	5.6 ± 1.0	5.0 ± 1.4
S-6	4.1 ± 1.2	4.0 ± 1.4	3.8 ± 1.5
S-22	33.2 ± 16.2[a]	18.1 ± 5.6[b]	18.5 ± 6.6[b]
Total 6 h	13.9 ± 3.4	13.5 ± 3.3	13.0 ± 3.4
Total 22 h	47.0 ± 16.9[a]	31.6 ± 8.0[b]	31.6 ± 9.0[b]

* $\overline{X}$± *SD*; *n* = 11. B-2 refers to the 2-h baseline urine sample collected before initiation of the oxalate load test; S-2, S-4 and S-6 refer to the 2-h urine samples sequentially collected post oxalate ingestion (80 mg of oxalate in the form of supplemental turmeric); S-22 refers to the pooled urine sample that represented the approximately 16-h period initiated following S-6 up through the first urine voiding the following morning. Means within a row with different superscript letters are significantly different, $p < 0.05$ (repeated measures analysis of variance and Tukey post hoc test)

Oxalate excretion following the oxalate load test

The basal oxalate excretion (B-2) for each subject was calculated as the average of the basal samples across the three treatments (Table 1). Averaging basal oxalate over the three oxalate load test days for each subject helped negate potential bias that could result from irregularities in urine flow or oxalate excretion during these 2-h basal urine collection periods.

There was a significant overall treatment effect and treatment-by-time interaction for urinary oxalate excretion. However, this effect was only observed at the S-22 time point (from the 6th to 22nd h post-oxalate ingestion) at which time the probiotic and washout levels were significantly lower than baseline by 45% and 44%, respectively. The total oxalate excretion for the entire 22 h urine post-oxalate ingestion period, referred to as total 22 h, was also significantly higher for baseline (47.0 mg) as compared to probiotic (31.6 mg) and washout (31.6 mg) periods. There was no significant difference in oxalate excretion at any time point between the probiotic and washout periods.

There was a significant overall treatment effect as well as treatment-by-time interaction for oxalate/creatinine ratios (Table 2). However, a significant difference among treatments was only observed at the S-22 time period with baseline S-22 higher than both probiotic and washout periods.

Baseline, probiotic and washout oxalate absorption (in mg and %) from the 80 mg oxalate load were computed over 6 h and 22 h time periods (Table 3). Oxalate absorption from the low-oxalate breakfast and lunch provided during the test days was assumed to provide only a minor contribution to urinary oxalate and thus not considered in the overall absorption calculations. There was a significant treatment effect for 22 h oxalate absorption (expressed in both mg and %) with baseline higher than both the probiotic and washout periods. There were no significant differences in oxalate absorption between the probiotic and washout periods.

Table 2 Baseline, probiotic and washout oxalate/creatinine (Ox/Cr) ratios (mg/g) prior to and following administration of the oxalate load*

Time (hrs)	Treatment		
	Baseline	Probiotic	Washout
B-2	14.5 ± 4.8	19.2 ± 3.9	20.6 ± 6.0
S-2	42.2 ± 17.2	38.5 ± 15.6	41.2 ± 15.1
S-4	60.3 ± 18.9	53.2 ± 20.8	49.7 ± 20.2
S-6	40.4 ± 13.2	39.0 ± 14.3	34.1 ± 9.0
S-22	39.7 ± 17.9[a]	21.8 ± 4.3[b]	22.0 ± 4.0[b]

* $\overline{X} \pm SD$; $n = 11$. B-2 refers to the 2-h baseline urine sample collected before initiation of the oxalate load test; S-2, S-4 and S-6 refer to the 2-h urine samples sequentially collected post oxalate ingestion (80 mg of oxalate in the form of supplemental turmeric); S-22 refers to the pooled urine sample that represented the approximately 16-h period initiated following S-6 up through the first urine voiding the following morning. Means within a row with different superscript letters are significantly different, $P < 0.05$ (repeated-measures analysis of variance and Tukey post hoc test)

Oxalobacter formigenes

Analysis of *O. formigenes* was based on the culture method [28]. In the overall analysis, a positive control sample of *O. formigenes* and a bovine fecal sample (presumed to represent a second positive control) were included. As expected, both of these control samples tested positive. Analysis of subjects' fecal samples yielded only two positive results (data not shown). The oxalate absorption levels for these two subjects did not exhibit a marked change from baseline after the probiotic and washout periods which differed from the general trend observed in the other subjects (Fig. 1).

As evidenced by the data presented in Fig 1, there were 4 subjects who had oxalate absorption levels at baseline that were markedly higher than those observed for the remaining 7 subjects. Since these "high absorbers" exhibited particularly large reductions in oxalate absorption after the 28-day probiotic supplementation period, it could be argued that the overall positive response was entirely due to these 4 individuals. To test for this possibility, the study population was separated into subpopulations of high absorbers (n = 4) and normal absorbers (n = 7). The effect of probiotic supplementation on 22 h oxalate absorption was assessed within each of these subpopulations (Fig 2). Although the magnitude of the probiotic-induced reduction in oxalate absorption was markedly greater in the high absorbers, a trend was also observed in the normal absorber subpopulation, although only the baseline vs washout oxalate absorption difference reached statistical significance.

Discussion

A variety of microbes have been reported to be potentially beneficial for use as probiotics some of which include *O. formigenes* and lactic acid bacteria [32]. The probiotic used in this study contained beneficial non-pathogenic bacterial strains (*Streptococcus thermophilus*, *Bifidobacterium breve, B. longum, B. infantis*, *Lactobacillus acidophilus, L. plantarum, L. paracasei* and *L. delbrueckii* subsp. *bulgaricus*) some of which have been demonstrated to degrade oxalate [25, 27, 33]. Earlier studies conducted on VSL#3® have suggested potential medical benefits such as management of different gastrointestinal conditions, including

Table 3 Baseline, probiotic and washout oxalate absorption (mg and %) for the first 6 hrs and for the total 22 hrs following administration of the oxalate load*

Parameter	Treatment		
	Baseline	Probiotic	Washout
Total 6 h oxalate absorption (mg)	7.6 ± 4.4	7.4 ± 3.3	6.9 ± 3.4
Total 6 h oxalate absorption (%)	9.6 ± 5.4	9.2 ± 4.1	8.7 ± 4.3
Total 22 h oxalate absorption (mg)	24.6 ± 13.1[a]	9.3 ± 6.7[b]	9.2 ± 10.4[b]
Total 22 h oxalate absorption (%)	30.8 ± 16.4[a]	11.6 ± 8.4[b]	11.5 ± 13.0[b]

* $\overline{X} \pm SD$; $n = 11$. The total 6 h oxalate absorption (mg) refers to total oxalate absorbed for the first 6 h following the initiation of the oxalate load test; total 6 h oxalate absorption (%) refers to total oxalate absorbed 6 h post oxalate loading as a percentage of the ingested amount of oxalate; total 22 h oxalate absorption (mg) refers to total oxalate absorbed in the 22 h following the initiation of the oxalate load test; total 22 h oxalate absorption (%) refers to total oxalate absorbed in the 22 h following oxalate ingestion as a percentage of the ingested amount of oxalate. Means within a row with different superscript letters are significantly different, $P < 0.05$ (repeated-measures analysis of variance and Tukey post hoc test)

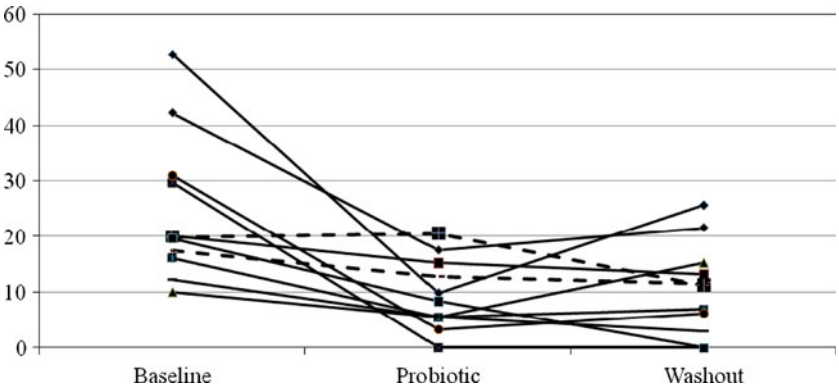

Fig. 1 Line graph showing individual 22 h oxalate absorption (mg) data from the ingested 80 mg oxalate dose for the baseline, probiotic, and washout periods. The dotted lines represent the 2 subjects who were positive for *O. formigenes*. The solid lines represent the 9 subjects who were negative for *O. formigenes*

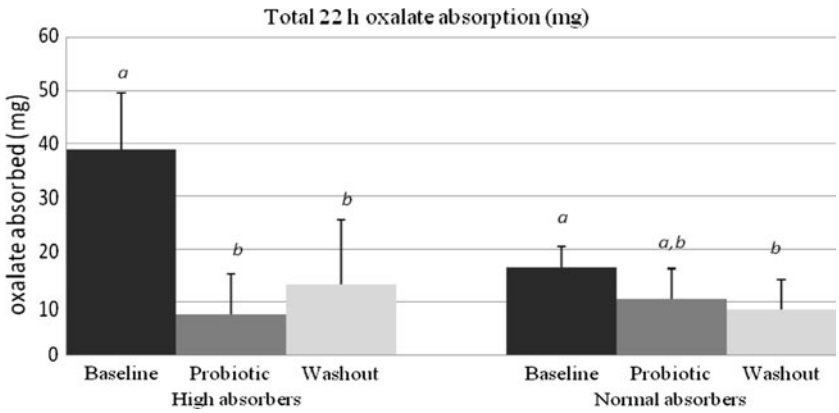

Fig. 2 Bar graph showing total 22 h oxalate absorption (mg) from the ingested 80 mg oxalate for baseline, probiotic, and washout periods for the two subpopulations (high and normal absorbers). The high absorber subpopulation refers to the 4 subjects who had high oxalate absorption (>25 mg) at baseline, while the normal absorber subpopulation refers to the remaining 7 subjects

ulcerative colitis and irritable bowel syndrome [34, 35]. The primary purpose of the present study was to assess whether a 4-wk daily ingestion of VSL#3® could lead to sufficient gut colonization to increase oxalate degradation, thereby resulting in reduced gastrointestinal oxalate absorption.

Both dietary sources as well as endogenous biosynthesis contribute to urinary oxalate [2] which is an important factor in the etiology of calcium oxalate-derived stones [36]. Factors affecting oxalate absorption include absorptive properties of the gut, gut transit time, presence of cations such as calcium and magnesium that can bind

oxalate, and presence of oxalate-degrading bacteria within the gut lumen [36, 37].

The present study assessed whether probiotic supplementation would alter gut microbial composition to the extent that there would be an impact on urinary oxalate excretion. There was no significant probiotic-induced change in oxalate excretion from 2 to 6 h post-oxalate ingestion, thus suggesting no effect of probiotic supplementation on gastric or small intestinal oxalate absorption. However, the S-22 oxalate excretion at baseline was significantly higher than after the probiotic and washout periods. The S-22 urine sample encompassed all urine collected between 6 and 22 h after oxalate ingestion, a time frame roughly expected to correspond to the period of colonic oxalate absorption. Thus, the oxalate content of this sample likely represented any colonic oxalate absorption in addition to the endogenous synthesis that occurs throughout the day. Total oxalate excretion for the entire 22 h post-oxalate ingestion period was significantly reduced by approximately 33% from baseline to the probiotic and washout periods (47.0 as compared to 31.6 and 31.6 mg, respectively). This finding in conjunction with the lack of effect for the first 6 h collection period supported the assertion that the reduction in oxalate absorption, presumably due to oxalate degradation, was limited to the distal (i.e., colonic) region of the gastrointestinal tract.

The secondary objective of this study was to determine whether any observed probiotic-induced urinary oxalate lowering effect would persist for a 4-wk washout period post-probiotic usage. The significant reduction of urinary oxalate excretion observed at washout suggested that bacterial colonization of the gut was maintained during this period. In patients with inflammatory bowel disease, fecal sample analyses indicated that *S. thermophilus, B. infantis*, and *B. breve* persisted in the gastrointestinal tract after a short period (6 days) following discontinuation of treatment with VSL#3® or yoghurt [38]. These three strains were among the cultures that made up the probiotic used in the present study.

The computation of urinary oxalate/creatinine ratios corrected for any unusual variation in urine flow or errors in urine collection. The baseline S-22 oxalate/creatinine ratio was significantly higher than after the probiotic and washout periods. This followed the same trend as that observed for oxalate excretion further supporting the assertion that probiotic supplementation could reduce colonic oxalate absorption.

Oxalate absorption has been reported to occur throughout the gut and the amount absorbed and appearing in urine following ingestion typically peaks between 2 and 6 hours [8]. In the present study, however, a different trend was observed in 4 (2 males, 2 females) of the 11 subjects. The total computed oxalate absorption up to 6 h post-oxalate load was not significantly different for these 4 subjects between baseline (7.7 mg), post-probiotic (6.7 mg) and washout (8.3 mg) periods. However, these four healthy subjects had a very high computed baseline oxalate absorption between 6 and 22 h (25, 29, 30 and 41 mg, mean = 31 mg), suggesting enhanced colonic absorption which was reduced to a mean of 2.4 and 6.1 mg after the probiotic and washout periods, respectively. Thus, their very high total oxalate absorption at baseline (38.9 mg) decreased markedly after the probiotic (7.7 mg) and washout (13.3 mg) periods. These individuals exhibited initial oxalate absorption levels well above the reported normal range of 10-15% [4] and similar to those observed in individuals with high colonic absorption secondary to intestinal disease or surgery leading to enteric hyperoxaluria [39, 40]. The finding that the probiotic was particularly effective in reducing oxalate absorption in these subjects could be expected on the basis of their very high colonic absorption levels at baseline. The remaining study subjects, termed normal absorbers, did not exhibit high oxalate absorption at baseline, but still experienced a probiotic-induced reduction in urinary oxalate between the baseline and washout periods. However, the lack of a statistically significant effect on 22 h oxalate absorption between the baseline and probiotic periods in this subpopulation suggested that normal variation in oxalate absorption and urinary excretion may have accounted for the observed trend.

It is unclear why the 4 apparently healthy individuals mentioned in the above paragraph were characterized by such high baseline oxalate absorption levels. In two previous studies that used the [$^{13}C_2$] oxalate absorption test, high oxalate absorbers were identified but none had absorption levels of greater than 22 % [41, 42]. A number of the participants in these studies were tested 3 times with the same oxalate dose, which indicated a very high intra-individual variation in oxalate absorption. It seems plausible that if the high oxalate absorbers in the present study had been tested repeatedly at baseline and computed oxalate absorption levels averaged, the very high absorption values would have been reduced.

In a previously reported study from our lab that assessed oxalate absorption from turmeric [29], scrutiny of the individual subject data indicated that only 1 of the 11 subjects had a computed oxalate absorption value greater than 22 % in response to receiving a 63 mg dose of turmeric-derived oxalate. This study used a 2 h baseline urine sample to estimate endogenous oxalate synthesis, as was done in the present study. Reliance on this estimate of endogenous oxalate synthesis combined with the assumption that endogenously derived urinary oxalate is excreted at a relatively constant rate throughout the entire urine collection period are limitations of this methodology.

The finding of high colonic oxalate absorption in apparently healthy subjects was documented in a recently reported study in which 0, 2, 4, and 8 mmol (equivalent to 0, 180, 360, and 720 mg) labeled oxalate loads were ingested by stone formers (SF) and normal individuals (N) after which their urinary and plasma oxalate levels were monitored [43]. Three subjects (two SF and one N) had markedly higher oxalate absorption from the 8 mmol load during the 8-24 h interval as compared to the remaining nine subjects (11.2% as compared to 3.1%) most likely due to high colonic absorption. Although the amount of oxalate given in the highest load (8 mmol) was approximately 9 times that used in the present study, the finding of high colonic oxalate absorption in these 3 subjects was similar to the finding in the present study for the 4 high absorbers at baseline.

Lactic acid bacteria have been previously used at high concentrations to reduce oxaluria. In one study, subjects were given $8x10^{11}$ freeze-dried cells of lactic acid bacteria consisting of five strains (*L. acidophilus, L. plantarum, L. brevis, B. infantis* and *S. thermophilus*) each day for 4 wk [26]. Subjects had a history of calcium oxalate stones and mild hyperoxaluria. Each subject's diet was the same during each urine collection period and high oxalate foods were not allowed. There was a significant reduction in oxaluria immediately after the treatment and after a month following the interruption of bacterial ingestion when compared to baseline with reported oxalate excretion levels of 55.5, 33.5 and 28.3 mg/24 h, for baseline, treatment and one month washout periods, respectively.

Another study used increasing doses of a probiotic, Oxadrop®, for 3 months in 10 patients with chronic fat malabsorption, calcium oxalate stones, and hyperoxaluria [27]. Oxadrop® consists of 4 strains (*L. acidophilus, L. brevis, S. thermophilus* and *B. infantis*) containing 2×10^{11} bacterial cells packed in 4-g sachets. The diet was not specifically controlled, but subjects were asked to consume similar foods during the two 24 h urine collection days (2 repeat 24 h urine collections) throughout the study. There was a significant reduction in urinary oxalate after probiotic use.

The above mentioned two studies [26, 27] either controlled the diet to prevent high dietary oxalate ingestion or the subjects used the same foods during each of the urine collection periods. Two possible explanations for the observed reduction in urinary oxalate excretion are as follows: Even with the omission of high oxalate foods, there may have been a reduction in the amount of dietary oxalate absorbed due to bacterial degradation. Secondly, there may have been a certain amount of bacterial degradation of the oxalate that is secreted into the proximal colon resulting from a bi-directional flux through the epithelium as has been demonstrated in animal models [10, 44]. In contrast, since the present study used a high oxalate challenge dose, the reduction in oxalate excretion could primarily be attributed to bacterial degradation of the ingested oxalate. Thus, it appears that lactic acid bacterial preparations can reduce urinary oxalate levels in two very different instances: in hyperoxaluric individuals who are not challenged with a high oxalate dose, and in normoxaluric individuals who participate in an oxalate load test.

Not all studies have demonstrated a lactic acid bacteria-induced reduction in urinary oxalate. For example, a recently reported study demonstrated no change in 24 h urinary oxalate excretion in calcium oxalate stone formers with idiopathic hyperoxaluria who received a mixture of 4 lactic acid bacterial species for 28 days [45]. The participants were older (60 ± 13 yr) than the group used in the present study. It is also important to note that participants had been advised to restrict dietary oxalate and in contrast to the present study, were not challenged with the ingestion of an oxalate load during the urine collection period. Thus, an oxalate degrading effect of the ingested bacteria would be expected to be primarily confined to oxalate secreted into the colon, a possibility alluded to in the above paragraph. Even if colonization by the provided bacterial species had occurred, oxalate secretion into the colonic lumen may not be of sufficient magnitude to allow a clinically significant amount of oxalate degradation to occur, thus explaining the lack of effect on oxalate absorption/urinary excretion.

Of the various microorganisms that have been found to inhabit the human intestinal tract, *O. formigenes* is of importance to the present study due to its ability to obligately metabolise oxalate in the colon [46, 47]. This bacterium limits the amount of dietary oxalate available for absorption within the gut and its absence has been reported to markedly influence risk of calcium oxalate stones [48, 49]. The two subjects in the present study who were positive for *O. formigenes* had baseline oxalate absorption levels of 22 and 25% which changed to 16 and 26%, respectively, after probiotic supplementation. In contrast, all four high oxalate absorbers at baseline were not colonized by *O. formigenes* and the probiotic-induced reduction in oxalate absorption was most pronounced in these individuals. These findings support the possibility that the effect of probiotic supplementation could be at least partially dependent on each individual's unique colonic microflora (e.g., lack of colonization with *O. formigenes* is associated with a greater potential for increased oxalate degradation resulting from ingestion of this probiotic).

A limitation of the current study is that the gastrointestinal microbial population of the subjects was not profiled to determine whether they were colonized, prior to or at the end of the study, by any of the bacterial strains provided in the probiotic. Such an analysis would allow an

assessment of which specific strains are most effective in colonizing the gut and are likely to be responsible for the observed reduction in oxalate absorption.

In summary, the average oxalate absorption at baseline (30.8%) was significantly higher than after the probiotic (11.6%) and washout (11.5%) periods. However, this overall reduction was primarily attributed to the marked reduction in absorption that occurred in the high oxalate absorber subpopulation. Thus, in individuals characterized by initially high oxalate absorption levels, VSL#3® ingestion appears to have the potential to reduce gastrointestinal oxalate absorption. Due to the limited number of subjects in this study, more expanded clinical studies are needed to support the use of this probiotic for the prevention of hyperoxaluria-related disorders such as kidney stones.

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