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ORIGINAL PAPER

Acute probiotic ingestion reduces 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. A number of recent studies have assessed whether daily ingestion of a probiotic containing oxalate-degrading bacteria could lead to sufficient gut colonization to increase oxalate degradation, thereby reducing urinary oxalate. In contrast, the present study assessed whether simultaneous ingestion of oxalate-degrading probiotic bacteria with a 176 mg oxalate load could lead to decreased urinary oxalate in a population of 11 healthy non-stone formers (8 females, 3 males), aged 21–45 years. The results indicated that both the single and double doses of VSL#3® probiotic solutions were effective in reducing urinary oxalate and estimated oxalate absorption with no significant difference between the two probiotic doses. The timing of the reduction in urinary oxalate suggested a small intestinal and possibly gastric reduction in oxalate absorption. Similar to what had been reported for chronic or daily probiotic ingestion, individuals characterized by high oxalate absorption were most likely to experience clinically significant reductions in urinary oxalate in response to acute probiotic ingestion.

Keywords Probiotic · Oxalate absorption · Kidney stones · Gastrointestinal tract

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Introduction

The human gastrointestinal tract (GIT) is colonized with different bacterial species that have the ability to degrade oxalate, which has led researchers to suggest a potential therapeutic use of probiotics containing oxalate-degrading bacteria for individuals with increased urinary oxalate excretion [1]. High urinary oxalate (hyperoxaluria) is considered a major risk factor for calcium oxalate kidney stone formation [2]. *Oxalobacter formigenes*, the best-known oxalate-degrading bacteria that derive metabolic energy solely from oxalate, appear to begin to colonize the colon at ages 6–8 y; however, only 60–80% of the adults test positive for these bacteria, with the use of antibiotics a possible contributing factor to this reduction [3]. Several studies have linked the absence of *O. formigenes* to higher urinary oxalate levels [4–6]. In addition, several studies have assessed the effects of oral administration of different probiotic preparations, containing bacterial species with oxalate-degrading potential, on urinary oxalate excretion [7–12]. These studies were recently reviewed and summarized by Liebman and Al-Wahsh [1]. The overall results were somewhat mixed but generally promising and the efficacy of probiotic ingestion for this purpose appeared to be primarily confined to individuals with absorptive hyperoxaluria (i.e., those characterized by higher than normal oxalate absorption in the absence of probiotic ingestion).

To our knowledge, no study, up to now, has examined the potential acute effect of consuming probiotics containing oxalate-degrading bacteria on urinary oxalate excretion. Thus, the primary objective of the present study was to assess whether the simultaneous ingestion of probiotic bacteria with an oxalate load can lead to decreased urinary oxalate, presumably due to increased oxalate degradation within the GIT during transit.

Materials and methods

VSL#3® (probiotic)

The probiotic used in this study is marketed under 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 450 billion live bacteria. The cultures used in this probiotic are generally recognized as safe (GRAS) by the Food and Drug Administration.

Subjects

Eleven subjects (3 males and 8 females) were recruited from the student population at the University of Wyoming. The subjects completed a pre-experimental health screening questionnaire and were required to not have had a history of kidney stones, irritable bowel disease, any other GIT disease, or any problems associated with urine passage. Written informed consent in accordance with the regulations of the Institutional Review Board of the University of Wyoming (approved 03/2011) was obtained from all subjects.

Study design

The study involved 3 oxalate load tests comprised of oral ingestion of 2 mmol of a sodium oxalate (NaOx) solution (176 mg oxalate) without concomitant ingestion of the probiotic, 2 mmol of NaOx plus 1 sachet of VSL#3® probiotic solution, and 2 mmol of NaOx plus 2 sachets of VSL#3® probiotic solution. The three treatments will be designated as Control, Probiotic1, and Probiotic2, respectively. The oxalate load tests were separated by at least 1 week as a washout period.

Each load test was based on the following procedures. Subjects were instructed to follow a low-oxalate diet on the day before each load test morning. 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. Subjects 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. Subjects were instructed to arrive in the laboratory 1 h 45 min following the first urine discharge so that they could be ready for the first urine sample collection. 2 h following the initial urination, a urine sample (pre-load) 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 frozen at −18°C for subsequent oxalate and creatinine analyses. Immediately after collecting the pre-load (baseline) urine sample, subjects ingested the 2 mmol of oxalate, with or without the probiotic treatments, dissolved in 240 ml of distilled, deionized water. 10 min later, they consumed a low-oxalate breakfast from a provided group of foods consisting of cold rice cereal with rice milk, apple juice, sausage, apple and grapes. Subjects continued drinking specified amounts of water during the load test. Urine samples were continuously collected over three 2-h intervals and processed the same way as the pre-load sample. A low-oxalate lunch was provided 4 h after load ingestion that comprised 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.

An estimation of net oxalate excretion (i.e., the difference between total urinary oxalate and the portion of total urinary oxalate that can be attributed to endogenous oxalate synthesis) is required to approximate oxalate absorption. The pre-load 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 oxalate load test. For the three-oxalate load tests, total oxalate levels in the pre-load urine samples for each subject were averaged across all three treatments to compute the mean pre-load urinary excretion. The 6-h endogenous oxalate was computed by multiplying the mean pre-load urinary oxalate by 3. This was subtracted from the total oxalate excreted over the 6-h post-oxalate load period and the resulting figure was divided by the amount of sodium oxalate ingested (176 mg) to estimate oxalate absorption.

Averaging pre-load oxalate across the three treatments helps to obtain the most valid estimate of endogenous oxalate excretion, which avoids a potentially significant source of error in the estimate of oxalate absorption due to an individual pre-load urinary oxalate level that is not representative of a subject's actual rate of endogenous oxalate excretion. Outlier values of this nature can most likely be attributed to methodological issues such as the inability to collect a urine sample that is truly representative of a 2-h period of endogenous oxalate synthesis due to variation in urine flow or actual urine collection timing

errors. This potential error is minimized using the average pre-load oxalate for each subject.

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 produced during the reaction. 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 [13]. 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.

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 load tests were the same among the control, probiotic1, and probiotic2 treatments, 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, treatment differences at specific time points were determined using the LSD (Fisher's least significant difference) post hoc test. Statistical computations were done using the general linear model (GLM) procedure of the statistical analysis software (SAS version 9.2, SAS Institute Inc., Cary, NC, USA. 2002–2003). Values of $p < 0.05$ were considered to designate statistical significance. Data are reported as mean ± SD.

Results

The mean age of the 11 subjects was 28 ± 8 years (range 21–45 years) and the mean BMI was 25.5 ± 5.5 kg/m^2 (range 20.5–40.0 kg/m^2). None of the subjects reported experiencing any noticeable gastrointestinal or other effects from consuming either the single or double dose of probiotic with the oxalate loads. 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 [14], subjects were estimated to have consumed 5.0 ± 1.1 mg of oxalate for breakfast and 6.1 ± 1.9 mg of oxalate for lunch. Ten of the 11 subjects consumed a minimum of a 1 oz serving of cheese at lunch which, due to its high calcium content, would have been expected to decrease oxalate absorption from this meal.

Table 1 Control, probiotic1 and probiotic2 urine creatinine and oxalate, and computed oxalate absorption during the oxalate load tests

Parameter and time (h)	Treatment		
	Control	Probiotic1	Probiotic2
Creatinine (mg)			
B-2	111 ± 38	104 ± 41	103 ± 42
S-2	119 ± 57	100 ± 42	109 ± 43
S-4	121 ± 44	102 ± 44	124 ± 40
S-6	123 ± 37	114 ± 41	100 ± 29
Oxalate (mg)			
B-2	1.5 ± 0.9	1.5 ± 0.9	1.5 ± 0.9
S-2	6.2 ± 2.4^{a}	5.4 ± 3.0$^{a, b}$	4.7 ± 2.1^{b}
S-4	7.3 ± 3.6^{a}	6.0 ± 3.0b	6.0 ± 2.8^{b}
S-6	5.1 ± 2.5^{a}	4.0 ± 2.1$^{a, b}$	3.3 ± 0.8^{b}
Oxalate/creatinine (mg/g)			
B-2	14.4 ± 4.3	14.8 ± 5.6	15.8 ± 5.4
S-2	61.0 ± 28.3^{a}	62.4 ± 45.7^{a}	43.8 ± 20.3^{b}
S-4	63.8 ± 27.2	64.2 ± 29.6	48.8 ± 21.1
S-6	42.2 ± 18.6	35.6 ± 14.8	34.4 ± 12.0
Oxalate absorption (%)	7.8 ± 4.2^{a}	6.1 ± 4.2^{b}	5.3 ± 2.6^{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 (176 mg of oxalate in the form of sodium oxalate)

Means within a row with different superscript letters are significantly different, $p < 0.05$ (repeated measures analysis of variance and LSD post hoc test)

Oxalate and creatinine excretion following the oxalate load tests

Control, probiotic1, and probiotic2 urine oxalate and creatinine excretion data are presented in Table 1. There was no overall treatment effect or treatment-by-time interaction for urinary creatinine.

The basal oxalate excretion (B-2) for each subject was calculated as the average of the basal samples across the three treatments (Table 1), the rationale for which has been previously described in the study design section. There was a significant overall treatment effect for post-oxalate load urinary oxalate excretion (control >probiotic1, probiotic2).

The LSD post hoc test indicated a greater S-2 urinary oxalate for the control compared to the probiotic2 oxalate load tests, a greater S-4 urinary oxalate for the control compared to both the probiotic1 and probiotic2 load tests, and a greater S-6 urinary oxalate for the control compared to the probiotic2 load tests (Table 1). The computed oxalate absorption during the control test (7.8%) was significantly greater than during the probiotic1 (6.1%) and probiotic2 (5.3%) tests. Oxalate absorption from the low-oxalate breakfast and lunch provided during the test days was assumed to make a negligible contribution to urinary oxalate and thus was not considered in the overall absorption calculations.

There was also a significant overall treatment effect for the post-oxalate load oxalate/creatinine ratios (control >probiotic2 with no difference between the control and probioitic1 treatments). Only at S-2 did the post hoc test indicate a significant treatment effect with the control ratio higher than the probiotic2 ratio for this time period.

Individual subject data for computed oxalate absorption values are presented in Fig. 1. The figure indicates that the two subjects with the highest oxalate absorption for the control test exhibited the greatest probiotic-induced reductions in oxalate absorption (from 18.8 to 11.4%, and from 10.1 to 1.1%, for the control and probiotic2 treatments, respectively). However, even when these subjects were removed from the statistical analysis, both probiotic treatments still led to a significantly lower mean oxalate absorption compared to control. Figure 1 also indicates that the two subjects with the lowest control oxalate absorption were least responsive to the probiotic treatments (i.e., oxalate absorption was unchanged from 3.3% for one of these subjects and in the other was only decreased from 3.4 to 2.9%).

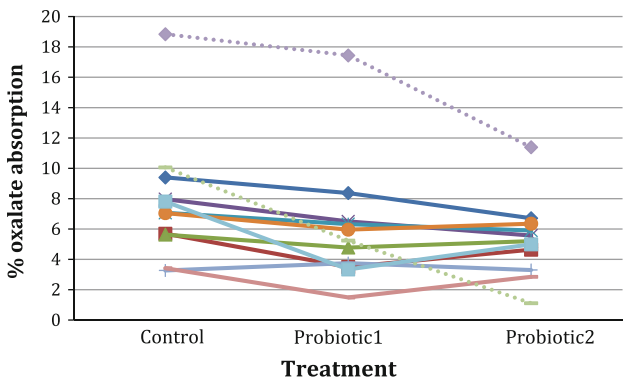

Fig. 1 Line graph showing individual percent oxalate absorption from the ingested 176 mg oxalate dose for the control, probiotic1, and probiotic2 treatments. The *dotted lines* represent the two subjects with the highest control treatment oxalate absorption

Discussion

Oxalate arises in the body from a combination of dietary sources and endogenous synthesis from precursors such as ascorbate and various amino acids [15]. Oxalate absorption has been reported to occur throughout the gut [16, 17]. Once absorbed, oxalate is not significantly metabolized in the human body but is rapidly taken up and cleared by the kidneys through filtration and eventually excreted in the urine [18]. Thus, an increase in urinary oxalate above basal levels (i.e., oxalate primarily arising from endogenous synthesis) is presumed to reflect the absorbed dietary oxalate.

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 [8, 19, 20]. Previous studies that assessed the effects of chronic oral administration of different probiotic preparations, containing bacterial species with oxalate-degrading potential, on urinary oxalate excretion [7–12] have yielded somewhat mixed but generally promising results. These studies addressed the question of whether daily ingestion of a probiotic for periods ranging from 2 to 4 week could lead to sufficient gut colonization to reduce urinary oxalate, typically attributed to increased oxalate degradation, leaving less available for absorption. In contrast, the present study addressed the question of whether simultaneous ingestion of oxalate-degrading probiotic bacteria with an oxalate load could lead to decreased urinary oxalate, presumably resulting from a higher rate of gastrointestinal oxalate degradation during transit.

The results indicated that both the single and double doses of VSL#3® probiotic solutions were effective in reducing urinary oxalate and estimated oxalate absorption with no significant difference between the two probiotic doses. The magnitude of the probiotic-induced reduction in urinary oxalate was similar among the S-2, S-4, and S-6 urine collection periods. Since oxalate absorption appears to occur throughout the entire GIT [1], the overall data suggested that the simultaneous presence of oxalate-degrading bacteria and oxalate within the upper GIT (small intestine and possibly stomach) leads to increased degradation/decreased absorption of oxalate. The finding of an acute effect of probiotic ingestion on small intestinal oxalate degradation is noteworthy but not unexpected in light of the well established ability of specific probiotic strains to improve the small intestinal lactose digestibility in lactase-deficient individuals [21].

In comparing the present results to those of the one previous probiotic study that also assessed urinary oxalate

after the ingestion of an oxalate load [11], there is a major difference in the time points at which a reduction in oxaluria was observed. In this previous study which assessed post-oxalate load urinary oxalate after a 4 week period of VSL#3® consumption, there was no probiotic-induced change in oxalate excretion from 2 to 6 h post-oxalate ingestion but rather the reduction in oxaluria was confined to a composite urine sample collected between 6 and 22 h after oxalate ingestion. Thus, it appeared that chronic VSL#3® ingestion affected only colonic oxalate absorption, which is consistent with the expectation that the provided bacterial strains would only colonize the colonic region of the GIT. In contrast, the present study demonstrated a reduction in urinary oxalate at the 2, 4 and 6 h post-oxalate ingestion time points, suggesting a small intestinal and possibly gastric reduction in oxalate absorption. A limitation of the present study was that urinary oxalate was not assessed for the remainder of the 24-h post-oxalate ingestion period, which would have allowed an assessment of whether colonic oxalate absorption was also affected.

A key point of interest was that the two subjects with the highest control treatment oxalate absorption figures exhibited the greatest probiotic-induced reduction in oxalate absorption and the opposite trend was observed for the two subjects with the lowest control oxalate absorption. In three of the six previously mentioned studies that assessed the effect of chronic probiotic ingestion on oxaluria [7, 10, 11], individual subject data indicated that having an initially high oxalate excretion, which in 2 studies was specifically attributed to enteric hyperoxaluria [10, 11], was associated with a marked reduction in urinary oxalate after a period of daily probiotic ingestion. Thus, it appears that individuals characterized by high oxalate absorption are most likely to experience clinically meaningful reductions in urinary oxalate in response to both chronic and acute probiotic ingestion.

Study limitations related to the implementation of the oxalate load tests should be acknowledged. The treatment order of control, probiotic1, and probiotic2 may have introduced some degree of bias particularly for the probiotic2 treatment. However, since there was a minimum of a 1 week washout period between oxalate load tests, it is unlikely that the acute response to the ingestion of two sachets of VSL#3® was significantly affected by the previous ingestion of one sachet of VSL#3®. In addition, because the absorption of oxalate in the form of concentrated sodium oxalate is likely to be more rapid and more efficient than that from a high oxalate-containing meal, it cannot be assumed that the magnitude of the probiotic-induced reduction in oxalate absorption presently reported would also occur with naturally occurring food-derived oxalate.

In conclusion, previous work suggests that chronic probiotic ingestion, via increased colonic colonization with oxalate-degrading bacteria, would be expected to increase oxalate degradation in the distal GIT. The present study suggests that the simultaneous ingestion of a probiotic with oxalate has the potential to increase oxalate degradation in the upper GIT. Whether the use of oxalate-degrading bacteria to allow both of these proposed mechanisms to operate could lead to more clinically significant reductions in urinary oxalate is an important question for future studies.

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