Effect of Blending and the Simultaneous Ingestion of a Probiotic Containing Oxalate-Degrading Bacteria on Oxalate Absorption

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Abstract

Both a high dietary oxalate intake and increased gastrointestinal absorption can lead to elevated urinary oxalate, a risk factor for kidney stone formation. Numerous studies have assessed whether daily ingestion of a probiotic containing oxalate-degrading bacteria can reduce urinary oxalate/oxalate absorption, but it appears only one previous study assessed whether the simultaneous ingestion of oxalate-degrading probiotic bacteria consumed with an oxalate load can exert this effect. This was assessed in the present study in a population of 11 healthy non-stone formers (6 males, 5 females), aged 21 – 37 y, using the probiotic VSL#3®. A spinach-sweet potato mixture provided an oxalate dose of 534 mg and urine samples were collected for a 22 h period post-oxalate ingestion. An additional objective was to assess the effect of blending oxalate-containing foods on oxalate absorption. The overall results suggested that the spinach and sweet potato provided oxalate of low bioavailability. Changing the texture of these foods by blending did not have an effect on oxalate absorption nor was VSL#3® effective in reducing urinary oxalate levels. VSL#3® may have been more effective if the oxalate dose had been provided in a more bioavailable form leading to a higher initial oxalate absorption/urinary oxalate excretion.

Keywords: probiotic, oxalate absorption, blending, kidney stones

1. Introduction

Oxalate is a salt of oxalic acid and is present in many foods, primarily those of plant origin. Oxalate within the body originates from dietary sources or as an end product of endogenous metabolism of various precursors such as ascorbate, glyoxylate, and glycine (Noonan & Savage, 1999). Oxalic acid can combine with calcium within the urinary tract to form an insoluble salt, calcium oxalate, responsible for over 70% of diagnosed kidney stones (Johnson et al., 1979). Both a high dietary oxalate intake and increased intestinal absorption appear to be major causes of hyperoxaluria, a risk factor for stone formation.

About 2-15% of dietary oxalate is normally absorbed (Holmes, Goodman, & Assimos, 2001) and absorption appears to occur throughout the GI tract including from the stomach, small intestine, and colon (Hautmann, 1993; Binder, 1974; Hatch & Freel, 2005). Since oxalate is not significantly metabolized in the human body, absorbed oxalate is cleared by the kidneys and eventually excreted in urine (Holmes, Ambrosius, & Assimos, 2005). Urinary excretion starts soon after ingestion and has been demonstrated to reach a peak between 2 and 6 hours in healthy individuals (Prenen, Boer, & Mees, 1984; Liebman & Chai, 1997).

There are a number of factors that appear to affect the efficiency of oxalate absorption from the diet. Studies that support the assertion that the soluble oxalate content of foods is more efficiently absorbed than insoluble oxalate have been recently summarized (Liebman & Al-Wahsh, 2011). In addition, simultaneous ingestion of certain cations such as calcium and magnesium appear to lead to a reduction in oxalate absorption, most likely due to the formation of insoluble calcium or magnesium oxalate salts within the GI tract (Liebman & Chai, 1997; Liebman & Costa, 2000). An additional factor that could affect oxalate absorptive efficiency is particle size, similar to the case of an increase in blood glucose response when whole grains such as rice and rye are ground (Jenkins et al., 1986). The blending of foods disrupts their native structure and thus might be expected to exert an effect on absorptive efficiency. Thus, one objective of the present study was to compare the efficiency of oxalate absorption from the consumption of a spinach-sweet potato mixture ingested as whole foods versus consumption
of these foods blended into a smooth puree.

Probiotics contain live microorganisms, including lactic acid bacteria, which have the potential to confer health benefits to the host (Ouwehand, Salminen, & Isolauri, 2002). Since the human gastrointestinal tract is colonized with different oxalate-degrading bacterial species, there could be a potential therapeutic use of probiotics containing oxalate-degrading bacteria for individuals with hyperoxaluria (Liebman & Al-Wahsh, 2011).

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 (Ito et al., 1996; Hokama et al., 2000; Weese et al., 2004). The non-pathogenic gram-negative anaerobic bacterium, O. formigenes, which has been demonstrated to be present in the colon of some, but not all adults, has well established oxalate-degrading capabilities (Duncan et al., 2002).

Previous studies have assessed the effects of oral administration of different probiotic preparations, containing bacterial species with oxalate-degrading potential, on urinary oxalate excretion (Campieri et al., 2001; Lieske et al., 2005; Goldfarb, Modersitzki, & Asplin, 2007; Ferraz et al., 2009; Okombo & Liebman, 2010; Lieske et al., 2010; Siener et al., 2013). These studies provided daily doses of the probiotic for a number of weeks to allow potential changes in the subjects’ microflora. The overall results have been mixed as some studies demonstrated a reduction in urinary oxalate (Campieri et al., 2001; Lieske et al., 2005; Okombo & Liebman, 2010) whereas others showed no effect (Goldfarb, Modersitzki, & Asplin, 2007; Ferraz et al., 2009; Lieske et al., 2010; Siener et al., 2013). It appears only one previous study has assessed the acute effect of simultaneous ingestion of a probiotic containing oxalate-degrading bacteria on oxalate absorption, and a reduction in urinary oxalate was demonstrated (Al-Wahsh, Wu, & Liebman, 2012). Thus, an additional objective was to assess the urinary oxalate lowering potential of a probiotic containing oxalate-degrading bacteria when provided with a blended sweet potato-spinach mixture.

2. Materials and Methods

2.1 VSL#3® (Probiotic)

This study used a probiotic supplement marketed with the brand name VSL#3® (Sigma-Tau Pharmaceuticals, Inc., Gaithersburg, MD, USA) which contains 800 billion live bacteria. This bacterial culture consists 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). The cultures used in this probiotic are generally recognized as safe by the Food and Drug Administration.

2.2 Subjects

Study participants were recruited from the student population at the University of Wyoming. Prospective subjects completed a health screening questionnaire. Exclusion criteria included a history of kidney stones, irritable bowel disease, or any gastrointestinal or other metabolic disease. A written informed consent compliant with the regulations of the Institutional Review Board of the University of Wyoming (approved December 2014) was initially obtained from all subjects. A total of 12 subjects started and completed the study protocol. Subjects were provided a monetary compensation for study completion. One subject was dropped after study completion based on strong evidence of incomplete urine collection (i.e., a 3-fold variance in 24 h urinary creatinine levels among the 3 treatments). Thus, the final study population consisted of 6 men and 5 women, ranging in age from 21 to 37 (mean = 29), and ranging in BMI from 19.3 to 29.5 (mean = 24.5).

2.3 Study Design

The study involved an assessment of oxalate absorption after three treatments separated by a minimum of one week and administered as follows. The first treatment was the ingestion of two high oxalate foods (spinach and sweet potato). The second treatment was the same high oxalate foods that were blended. The third treatment was the blended high oxalate foods together with one sachet of the probiotic. The spinach (frozen) and sweet potato (canned) were purchased from the same lot prior to study initiation. The procedure for each treatment was identical. Subjects consumed a low oxalate diet on the day before each test. A detailed listing of low oxalate foods allowed and high oxalate foods prohibited was provided. All subjects recorded all foods and beverages ingested on the pre-test days.

On a test day, after a 12 h overnight fast, subjects discarded the initial morning urine sample and then drank 500 ml water to ensure adequate urine production. Two hours after initial urine discharge, another urine sample (designated baseline and referred to as B2) was collected. The volume was recorded and 100 ml was acidified with 1 ml of 12N HCl acid for preservation purposes. Immediately after collection of the B2 sample, subjects
ingested one of the test treatments which provided 534 mg total oxalate, 285 mg soluble oxalate. For the first treatment, subjects ingested a mixture of non-blended spinach (100 g) and sweet potatoes (65 g) with 250 ml of water. The spinach and sweet potato were microwaved for 1 min, stirred, and microwaved for another 30 seconds. For the second treatment, the microwaved spinach, sweet potato and water were blended (approximately 1 min) until a smooth consistency was achieved. For the third treatment, one sachet of the probiotic (VSL#3®) was also added to the blender. The spinach-sweet potato mixture was consumed within a 3-5 min period for each treatment. Two hours later, a second urine sample (designated S2) was collected after which subjects consumed a low oxalate lunch which included a chef's salad (lettuce, cucumber, red pepper, cheese, turkey and hard boiled eggs) and some combination of grapes, apples, yogurt and ice cream. Each subject consumed an identical low oxalate lunch for each of the 3 treatments. The subjects continued drinking water and urine collections were completed at 2 h intervals for the following 4 h after collection of S2. These samples were designated as S4 and S6. Thereafter, subjects were provided acidified urine collection containers and continued collecting all urine samples up through the first voiding the following morning. This composite sample, which encompassed an approximately 16 h period, was designated S22. Subjects maintained a low-oxalate diet for the remainder of the test day.

Because subjects followed a low-oxalate diet the day prior to a test day and fasted overnight, the oxalate content in B2 was assumed to be from endogenous sources (i.e., oxalate synthesis within the body). Total oxalate levels in the baseline urine sample for each subject were averaged across all three treatments to calculate the mean baseline urinary oxalate. Previous work from this lab had demonstrated that averaging baseline urinary oxalate across all treatments provided the most valid estimate of endogenous oxalate excretion. Use of urinary oxalate to approximate oxalate absorption in this study was based on the assumption that rate of endogenous oxalate excretion is relatively constant during a 24 h period. Urinary oxalate derived from oxalate absorption from the spinach/sweet potato treatments will be referred to as net oxalate excretion. Net oxalate excretion in samples S2, S4 and S6 was determined by calculating the difference between total urinary oxalate in the sample and the mean baseline urinary oxalate. Net oxalate excretion for S22 was calculated by multiplying the mean baseline urinary oxalate by 8 (due to the approximately 16 h collection period for this composite sample) and subtracting the result from the total urinary oxalate content in S22. The net urinary oxalate for S2, S4, and S6 were summed and divided by the amount of oxalate ingested (534g) to estimate 6 h percent oxalate absorption. A similar calculation, but also including S22, was made to estimate 22 h percent oxalate absorption.

2.4 Sample Analyses

Oxalate and creatinine analyses

The urine samples were analyzed for oxalate using an oxalate kit (Trinity Biotech, Berkeley Heights, New Jersey). This enzymatic method is based on the oxidation of oxalate by oxalate oxidase followed by detection of hydrogen peroxide produced during the reaction. Lyophilized (control) urine samples having predetermined oxalate concentrations were analyzed with each assay for quality control purposes. The test foods (spinach and sweet potato) were analyzed by this same procedure after extraction in acid (for total oxalate) and water (for soluble oxalate) according to a previously published extraction procedure (Okombo & Liebman, 2010). Urinary creatinine was analyzed by the picric acid method (Lustgarten & Wenk, 1972). 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.

2.5 Statistical Analysis

Single-factor repeated measures analysis of variance was used to test the hypothesis that mean urinary oxalate and creatinine excretion during the test loads were the same among treatments. Each subject served as his/her own control. LSD (Fisher's least significant difference) post hoc test was used to determine specific treatment differences in cases of significant overall treatment effects. Statistical calculations were done using the general linear model (GLM) procedure of the statistical analysis software (SAS version 9.4, SAS Institute Inc., Cary, NC, USA. 2012-2014). All p values ≤ 0.05 were considered to be statistically significant.

3. Results

All 12 subjects completed the study. As stated previously, one subject’s data was excluded from statistical analysis based on evidence of incomplete urine collection thus leaving a study population of 11. None of the subjects reported any discomfort or side effects from consuming the test foods or the low-oxalate lunches.

Table 1 shows oxalate and creatinine excretion levels as well as oxalate to creatinine ratios and percent oxalate absorption at 6 h and 22 h for the 3 treatments. There were no significant differences in urinary oxalate or
creatinine excretion levels following the three oxalate load tests. However, for all 4 urine samples collected post-oxalate ingestion, there was a moderate reduction in oxalate content from treatment 2 (blended mixture) to treatment 3 (blended mixture with probiotic).

The majority of oxalate absorption occurred during the initial 6 h post-ingestion period (i.e., 62, 77, and 78 % of total 22 h absorption within the 6 h post-ingestion period for treatments 1, 2, and 3, respectively). There was a statistically nonsignificant decrease in % oxalate absorption between treatments 2 and 3 (18 % lower at treatment 3 for both 6 h and 22 h) and the absolute changes in percent absorption were of small magnitude.

Table 1. Urinary oxalate and creatinine levels and percent oxalate absorption for the three treatments

<table>
<thead>
<tr>
<th>Parameter and sample</th>
<th>Treatment</th>
<th>1 (whole foods)</th>
<th>2 (blended foods)</th>
<th>3 (blended foods + probiotic)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Creatinine (mg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B2</td>
<td>131 ± 14</td>
<td>131 ± 17</td>
<td>131 ± 22</td>
<td></td>
</tr>
<tr>
<td>S2</td>
<td>125 ± 14</td>
<td>131 ± 17</td>
<td>121 ± 15</td>
<td></td>
</tr>
<tr>
<td>S4</td>
<td>121 ± 19</td>
<td>127 ± 17</td>
<td>132 ± 15</td>
<td></td>
</tr>
<tr>
<td>S6</td>
<td>128 ± 13</td>
<td>133 ± 13</td>
<td>145 ± 12</td>
<td></td>
</tr>
<tr>
<td>S22</td>
<td>1043 ± 113</td>
<td>1026 ± 116</td>
<td>975 ± 156</td>
<td></td>
</tr>
<tr>
<td>Oxalate (mg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B2</td>
<td>1.6 ± 0.2</td>
<td>1.7 ± 0.2</td>
<td>1.6 ± 0.4</td>
<td></td>
</tr>
<tr>
<td>S2</td>
<td>4.4 ± 0.6</td>
<td>5.4 ± 0.8</td>
<td>5.0 ± 0.8</td>
<td></td>
</tr>
<tr>
<td>S4</td>
<td>4.0 ± 0.6</td>
<td>5.3 ± 0.9</td>
<td>4.3 ± 0.6</td>
<td></td>
</tr>
<tr>
<td>S6</td>
<td>3.3 ± 0.4</td>
<td>3.0 ± 0.3</td>
<td>2.7 ± 0.3</td>
<td></td>
</tr>
<tr>
<td>S22</td>
<td>16.0 ± 1.8</td>
<td>14.3 ± 1.3</td>
<td>13.8 ± 1.9</td>
<td></td>
</tr>
<tr>
<td>Oxalate/creatinine (mg/g)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B2</td>
<td>12.5 ± 1.6</td>
<td>13.5 ± 1.1</td>
<td>12.2 ± 1.2</td>
<td></td>
</tr>
<tr>
<td>S2</td>
<td>36.6 ± 5.1</td>
<td>45.5 ± 6.5</td>
<td>42.2 ± 6.1</td>
<td></td>
</tr>
<tr>
<td>S4</td>
<td>36.1 ± 5.1</td>
<td>43.0 ± 7.0</td>
<td>32.8 ± 3.1</td>
<td></td>
</tr>
<tr>
<td>S6</td>
<td>26.9 ± 3.8a</td>
<td>23.6 ± 2.2b</td>
<td>19.1 ± 1.4b</td>
<td></td>
</tr>
<tr>
<td>S22</td>
<td>16.2 ± 1.6</td>
<td>14.6 ± 1.3</td>
<td>15.0 ± 0.9</td>
<td></td>
</tr>
<tr>
<td>Oxalate absorption (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 h</td>
<td>1.3 ± 0.2</td>
<td>1.7 ± 0.3</td>
<td>1.4 ± 0.2</td>
<td></td>
</tr>
<tr>
<td>22 h</td>
<td>2.1 ± 0.4</td>
<td>2.2 ± 0.6</td>
<td>1.8 ± 0.6</td>
<td></td>
</tr>
</tbody>
</table>

Mean ± SEM; n = 11.

B2 refers to the 2 h baseline urine sample collected before ingestion of the oxalate containing foods; S2, S4, S6 refer to the 2 h urine samples sequentially collected post oxalate ingestion (534 mg of oxalate from spinach and sweet potato mixture); S22 refers to the pooled urine sample that represented the approximately 16 h period initiated following S6 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 LSD post hoc test).

Table 1 also shows oxalate to creatinine ratios for the different urine samples. Oxalate to creatinine ratio was constant among the three treatments for B2. For S2, S4, and S22, there were no statistically significant treatment
effects. There was a significant treatment effect for S6 with treatment 1 yielding a higher value than that from treatment 3.

4. Discussion

Blending, which disrupts a food’s native structure, might be expected to lead to an increased nutrient bioavailability. However, in this study, there was no significant change in urinary oxalate between the whole food and blended food treatments. Although blending can affect the glycemic response to certain foods (Jenkins et al., 1986), there appears to be no effect on oxalate absorption.

The results indicated low percent oxalate absorption for all treatments both at 6 h and 22 h. There is some evidence that soluble oxalates are more efficiently absorbed than insoluble oxalate salts (Liebman & Al-Wahsh, 2011). A previous study (Al-Wahsh, Wu, & Liebman, 2012) in which a sodium oxalate solution (176 mg oxalate, 100% soluble oxalate) was ingested yielded a greater than 5% oxalate absorption. Approximately half of the 534 mg provided by the spinach and sweet potato was soluble. However, using real foods to provide oxalate could have led to interactions among food components that hindered oxalate absorption. Generally, the bioavailability of food oxalate is less than the bioavailability of sodium oxalate (Liebman & Al-Wahsh, 2011).

Spinach is a rich source of calcium and it appears that the insoluble oxalate fraction of spinach is primarily in the form of calcium oxalate (Prenen, 1984). Dietary calcium may complex with oxalate in the gut making it insoluble and thus unavailable for absorption. It was previously demonstrated that supplemental calcium is capable of reducing oxalate absorption by 50% (Liebman & Chai, 1997). The high calcium content of spinach likely contributed to the low oxalate absorption from the spinach/sweet potato mixtures. In a previous study which used urinary oxalate excretion to estimate absorption, spinach ingestion led to a 24 h absorption rate of 2.3%, but the study population was only 1 individual (Prenen, 1984).

Some gastrointestinal lactic acid bacteria can break down oxalate (Lewanika et al., 2007), thus potentially leading to a reduction in levels of oxalate available for absorption. Probiotics generally provide a number of Lactobacillus as well as other species, and can be included in the diet for their proposed health benefits (Ouwehand et al., 2002; Gilliland, 1990). The probiotic used in this study contained a number of oxalate-degrading bacterial strains.

The results revealed no significant effect of the probiotic in reducing urinary oxalate excretion although lower values occurred for all four post-oxalate ingestion urine samples for treatment 3 compared to treatment 2. The 18% reduction in percent oxalate absorption associated with addition of the probiotic was also not statistically significant but was consistent with the general trend suggestive of a minor effect of VSL#3® in reducing the availability of oxalate for absorption.

In the one previous study that assessed the potential of a probiotic to exert this type of acute effect, Al-Wahsh et al. (2012) showed a significant VSL#3®-induced reduction in urinary oxalate excretion/oxalate absorption. This study involved oxalate load tests comprised of oral ingestion of a sodium oxalate solution (176 mg oxalate). The study design was similar to the present study in that the same probiotic was used and urine samples were collected at B2, S2, S4, and S6, but no 22 h urine sample was collected. Both single and double doses of VSL#3® reduced urinary oxalate/oxalate absorption with no significant difference between the two doses. Prior to inclusion of the probiotic, the oxalate absorption rate was 7.8% which was reduced to 6.1 and 5.3% for the single and doubles doses, respectively. With a higher rate of initial oxalate absorption/urinary excretion, the probiotic bacteria in this study perhaps had more potential to lower urinary oxalate compared to the present study in which the initial 6 h oxalate absorption was less than 2%.

Previous studies have also evaluated the effect of chronic probiotic supplementation on urinary oxalate excretion. These studies assessed whether daily ingestion of a probiotic for at least two weeks could lead to a lowering of oxalate absorption (Campieri et al., 2001; Lieske et al., 2005; Goldfarb, Modersitzki & Asplin, 2007; Ferraz et al., 2009; Okombo & Liebman, 2010; Lieske et al., 2010; Siener et al., 2013). Although the overall results of these studies were mixed, there was some evidence that individuals characterized by high oxalate absorption were more likely to experience clinically significant reductions in urinary oxalate (Liebman & Al-Wahsh, 2011).

In conclusion, the overall results suggested that the high-oxalate foods used (spinach and sweet potato) provided oxalate of low bioavailability. Changing the texture of these foods by blending did not have an effect on oxalate absorption. Despite some evidence in the literature that VSL#3®, which consists mainly of lactic acid bacteria, can decrease the amount of oxalate available for absorption, it did not lead to a significant reduction in urinary oxalate excretion in the current study. As of the present time, simultaneous ingestion of this probiotic has only been demonstrated to significantly reduce oxalate absorption from a sodium oxalate solution (Al-Wahsh, Wu, &
Liebman, 2012). Additional studies will be required to assess whether VSL#3® ingestion can lead to clinically significant reductions in urinary oxalate after the consumption of other high oxalate-containing foods, particularly those that provide oxalate of higher bioavailability.

References


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