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DIETARY OXALATE AND ITS INTESTINAL ABSORPTION

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Abstract

Dietary oxalate is currently believed to make only a minor contribution (< 20%) to urinary oxalate excretion. A recent prospective study of stone disease suggested that dietary oxalate may be a significant risk factor. This observation led us to re-evaluate the contribution of dietary oxalate to urinary oxalate excretion. Previous studies have been hampered by inaccurate food composition tables for oxalate and inadequate methods for studying intestinal oxalate absorption. This evidence as well as factors that modify oxalate absorption are reviewed. New approaches to measure food oxalate and intestinal oxalate absorption have been examined. Capillary electrophoresis appears to be well suited for the analysis of the oxalate content of food. Two individuals consumed an oxalate-free formula diet for 7 days. This diet decreased urinary oxalate excretion by an average of 67% (18.6 mg per 24 hours) compared to oxalate excretion on self-selected diets. The absence of detectable oxalate in feces by day 6 of the diet suggested that the intestinal absorption was minimal. However, an effect of the formula diet on endogenous oxalate synthesis cannot be excluded. Restoring oxalate to the formula diet increased urinary oxalate excretion and illustrates that this experimental protocol may be well-suited for studying oxalate absorption and factors that modify it. Our results suggest that the intestinal absorption of dietary oxalate makes a substantial contribution to urinary oxalate excretion and that this absorption can be modified by decreasing oxalate intake or increasing the intakes of calcium, magnesium, and fiber.

Key Words: Dietary oxalate, intestinal oxalate absorption, urinary oxalate excretion, oxalate-free diet, oxalate analysis.

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Introduction

Oxalate is ubiquitous in the plant kingdom and is consumed in normal human diets as a component of nuts, fruits, vegetables and grains [25]. Oxalate forms an insoluble salt with Ca^{2+} which may lead to nephrolithiasis or deposition of crystals in tissues. Human tissues have limited mechanisms to degrade oxalate. Fortunately, only a fraction of the oxalate ingested is absorbed into the body in normal individuals. The remainder is either broken down by intestinal bacteria or excreted in feces. This is clearly not the case in individuals with enteric hyperoxaluria where either resection or bypass of a segment of the bowel or small bowel dysfunction causes hyperabsorption of dietary oxalate in the colon [15, 21]. This hyperabsorption is believed to result from a changed colonic milieu due to malabsorption of fat and bile salts in the upper intestinal tract [21]. An alteration in intestinal flora has also been proposed as a contributing factor [2].

Dietary oxalate is usually cited as contributing 10-20% of the oxalate excreted in urine in normal individuals [25, 51], while the remainder is derived from endogenous synthesis. However, as we shall document below, the amount of oxalate that is ingested and the fraction that is absorbed is uncertain, making these factors of unknown significance in calcium oxalate stone disease. The thesis will be developed that the intake of oxalate is underestimated because of inaccurate techniques used to assay food oxalate, and that absorption is underestimated because of the difficulty in assessing uptake in the large intestine. This thesis is supported by a recent prospective study of health professionals which showed that a low dietary intake of Ca^{2+} was a prominent risk factor for stone formation [12]. The most likely explanation for this observation is that increasing dietary Ca^{2+} intake decreases oxalate absorption. This finding argues that increases in urinary oxalate may have larger effects on stone risk than increases in urinary Ca^{2+} despite the higher urinary Ca^{2+} excretion produced by a higher Ca^{2+} intake [37]. This effect of dietary Ca^{2+} would also suggest that intestinal oxalate absorption may be a more significant risk factor than previously recog-

nized. Individuals with an apparent diet-induced hyperoxaluria have been identified in a calcium stone-forming population [20].

Dietary Oxalate Intake

One of the first estimates of the dietary intake of oxalate was by Archer *et al.* [3] who examined the diets of 6 individuals in 1957 and reported intakes to be an average of 920 mg/day. Zaremski and Hodgkinson [52] estimated the intake of oxalate in English diets to be 70-150 mg/day using a revised analytical procedure for oxalate determination in food. Hodgkinson [25] estimated that a derived typical diet contained 130 mg of oxalate, a level consistent with their previously reported results and a widely cited value.

Although there are many reported values for the oxalate content of certain foodstuffs (reviewed in [25, 34, 52]), the values reported by Zaremski and Hodgkinson [52] and Kasidas and Rose [34] are the most comprehensive and the most widely used. These values figure prominently (for instance, in Massey *et al.* [43] and Ney *et al.* [45]), and are used by health professionals in offering dietary advice to Ca oxalate stone-formers. The validity of the compositional data in advising stone patients is, of course, no greater than the accuracy of the methods used to obtain them. Only multi-stepped and indirect methods have been previously used in these compositional assays and they are subject to interferences and inaccuracies. For example, the method used by Hodgkinson and Zaremski [26] involved an ether extract of an acidified sample followed by a precipitation of oxalate with Ca. Both the ether extraction and precipitation are unlikely to be quantitative and the amount extracted may vary widely in different foods. The reduction of oxalate to glycolate is also a critical step in this procedure and an incomplete reduction will underestimate oxalate [49]. The method developed by Kasidas and Rose [34] was an enzyme-linked assay that utilized oxalate oxidase. Inhibitors of the enzyme have to be removed for assays of urinary oxalate with oxalate oxidase [49]. Such inhibitors are also likely to occur in foods and without any pre-purification steps to remove inhibitors from food extracts, an oxalate oxidase-based procedure is likely to underestimate oxalate.

Other sources for the oxalate content of foods include a 1984 USDA publication [24] reporting values for many vegetables which has been used by some investigators (e.g., by Massey *et al.* [43]). The methodology employed to assay oxalate content was not stated, and hence, the usefulness of these data is uncertain. Significant amounts of oxalate, 100 mg/100 g, were reported in most vegetables including asparagus, beans, broccoli, brussels sprouts, cabbage, carrots, cauliflower, celery,

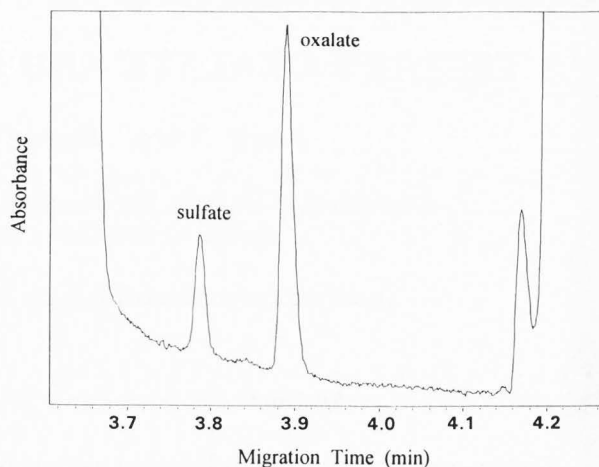


Figure 1. The analysis of oxalate in Bran Flakes (from Post, Kraft Foods, White Plains, NY) by capillary electrophoresis. The sample was homogenized in 9 volumes of 1 M H_3PO_4 using a Polytron homogenizer and diluted 1/100 for analysis as described for urinary oxalate. The large peak before sulfate is chloride, and the phosphate peak has been cut-off at the end of the profile.

chives, eggplant, garlic, lettuce, parsley, sweet potato, and turnips, as well as in the more familiar values for spinach and beet. These values were at least one order of magnitude higher than previous estimates and more recent ones we have obtained using capillary electrophoresis. Based on these limitations, it is clear that the actual amount of oxalate in normal diets is still uncertain.

We have adapted a method developed for the assay of oxalate in urine [28] to measure the oxalate content of foods. The method relies on high performance capillary electrophoresis (CE) with the measurement of anions by indirect ultra-violet (UV) absorbance detection. A Waters (Milford, MA) Quanta 4000 CE was used and the data obtained were analyzed on a microcomputer using Millennium® (Waters) software. Anions are separated on a 60 cm x 75 μm fused silica capillary column using 10 mM sodium chromate and 0.5 mM tetradecyltrimethylammonium bromide (a modifier of electro-osmotic flow) as the electrolyte. Oxalate had a migration time of 4 minutes using a constant current of 25 amps. Because much of the oxalate in many plant species is present as crystalline calcium oxalate [38], procedures that dissolve this oxalate have to be employed. Samples were prepared by homogenization (10% w/v) in 1 M H_3PO_4 using a Polytron® homogenizer (Brinkmann Instruments, Westbury, NY), heating at 55°C for 1 hour and clarified by centrifugation and filtration. Increasing the acid concentration, the temperature, the time of

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Table 1. The oxalate content of foods in mg per 100 g.

Food	Colorimetric Assay [52]	Enzymatic Assay [34]	Gas Chromatography [8, 9]	USDA Handbook #8-11 [24]	CE
Spinach	780	750	618	970	645
Celery	17.5	20		190	61.2
Tomato	5.3	2		50	5.7
Carrots	22.7	4		500	9.6
Chocolate	124	117	140		
Corn Flakes ^{®1}	5	2			1.9
Weetabix		3.2			76.1
Tea	7 ^a	5.5 ^b	280 ^a		7.5 ^a
V-8 [®] juice ²			5.2		5.8
Orange juice		0.25	0.4		0.4

^a2 minute infusion of 2 g tea in 100 ml of boiled water

^b2 min infusion of 1 g tea in 100 ml of boiled water.

¹Kellogg Co., Battle Creek, MI;

²Campbell Soup Co., Camden, NJ.

incubation, or re-extraction of the pellet did not increase the yield of oxalate. As an example, the analysis of Bran Flakes breakfast cereal is shown in Figure 1. The Bran Flakes contained 141 mg oxalate/100 g. Repetitive analyses of this product showed that intra-assay variability (CV) was 2.4% and the inter-assay variability, 3.3%. The recovery of 20 mg added oxalate was $92 \pm 3\%$. These results indicate that for the analysis of Bran Flakes the assay is both accurate and reproducible.

Oxalate values reported for some commonly consumed food items using several different methods are listed in Table 1 and compared with values obtained by CE. As the oxalate level in plants may vary depending on the particular genetic strain of the plant or the growth conditions for the plant [38], some of the variability between results could be due to strain or growth differences. Similar arguments may apply to manufactured products such as bread, Weetabix, and chocolate which may vary in processing as well as in ingredients. The overall trend observed in Table 1 was for food oxalate estimates to be lower using colorimetric and enzymatic procedures than using CE. This underestimation particularly applied to the cereal grains and their products. As the current food pyramid for daily nutrition recommended by the USDA calls for the consumption of 8 servings of cereal products per day, such products could be important sources of oxalate in an American diet. Seeds of all description appear to be rich sources of oxalate. Costello *et al.* [11] reported that sesame seeds contained 2800 mg/100 g and, as such, must be the richest source of oxalate yet identified. The discovery that the

protein, germin, which is important in seed germination, is actually an oxalate oxidase suggests an important biological role for oxalate in plants [36]. The bran portion of wheat seeds is enriched with oxalate and CE analysis indicates that it contains 524 mg/100 g. In view of the number of bran-enriched foods currently available in the supermarket, bran is becoming an important source of oxalate in the diet. The results clearly indicate that a reliable method for oxalate analysis of foods is required, particularly if intestinal oxalate absorption is a significant factor in calcium oxalate stone disease. Furthermore, it is possible that absorption can be substantially modified with prudent dietary choices.

Measurement of the Intestinal Absorption of Oxalate

Three methods have been used to measure the amount of ingested oxalate absorbed in the intestine. There are problems associated with each of these methods creating much doubt about the validity of current estimates of oxalate absorption. The methods will be referred to as the **isotopic method**, the **load method**, and the **daily excretion method**, and are reviewed below.

Isotopic method

On the surface, this method would appear to be the most straightforward. ¹⁴C-oxalate is ingested orally and the ¹⁴C-oxalate appearing in urine is used as an index of the amount of oxalate absorbed. The two major problems with this procedure involve the way the dose is administered and the mixing of the isotopic dose with un-

labelled oxalate in the large intestine. The way the isotope has been administered has varied from providing it in the fasting state with 8 mg of sodium oxalate [10, 50] to including it with complex meals [16, 39]. In some studies, the isotope was included in a formula diet [16, 48]. The critical factor in the method of administering the isotope is whether the isotope is free, complexed or crystalline when it reaches absorption sites. Evidence from absorption studies indicates that a much larger uptake of ^{14}C -oxalate occurs when the isotope is given in the fasting state as sodium oxalate, compared with co-administration with any nutrients. In the study by Chadwick *et al.* [10], $29 \pm 4\%$ of the fasting, sodium oxalate dose was absorbed in 36 hours in normal subjects compared with $6.6 \pm 0.9\%$ when the isotopic dose was given with food. This larger uptake of sodium oxalate in the fasting state strongly implies that absorption of oxalate occurs as the free, charged anion and not as a neutral complex when bound to Ca or Mg. This is further supported by the evidence reviewed below that the intake of Ca, Mg and other ions suppresses intestinal oxalate absorption. Thus, the absorption of ^{14}C -oxalate from the administered dose depends on how much has crystallized, how much has complexed with Ca and Mg, and how much is free. This is further complicated by the changing milieu of the intestinal contents. Questions that arise include how much of the crystalline Ca oxalate that would undoubtedly form in the formula diets dissolves in the stomach, and how much would re-crystallize when the pH increases in the small intestine? How do intestinal excretions and absorptions influence the solubility of oxalate? Erickson *et al.* [16] have shown, for instance, that a hyperabsorption of Ca in the intestine increases oxalate absorption.

The second complication arises when the administered dose reaches the large intestine where substantial mixing with previously ingested food will occur. The amount of previously ingested Ca, Mg, oxalate, phosphorus and other ions will affect the distribution of label into crystalline, complexed and free forms. Thus, the dietary intake in the 24-48 hours prior to the study will influence the absorption of the dose. This mixing of intestinal contents coupled with the long transit time in the large intestine makes the study of oxalate absorption in the large intestine using the isotopic method very difficult.

Not surprisingly in view of these complications, large ranges in the percentages of administered doses absorbed have been reported. The largest amount of ^{14}C -oxalate reported to be absorbed in normal subjects was by Chadwick *et al.* [10] who found an average of 29% absorbed (range 8-55%) in 36 hours when given with sodium oxalate in the fasted state. This absorption would presumably have increased further with longer collec-

tions. Most studies [10, 16, 39, 48, 50] report an absorption of 6-12% in 24 hours for ^{14}C -oxalate ingested with food or formula under normal conditions. Prenen *et al.* [46] reported, however, that absorption was low, 1.7-2.6% over 96 hours, when the isotope was given with oxalate-rich foods. This low absorption may be related to the incorporation of the isotope into crystals before reaching an absorption site. An absorption of 6-12% of dietary oxalate would contribute 7.8-15.6 mg oxalate to a 24 hour urine sample if dietary oxalate is an average of 130 mg/day, and 12-24 mg if it is 200 mg/day. Even the lowest figure, 7.8 mg, would represent a contribution of > 25% to the average excretion of 29 mg/day in 101 individuals consuming self-selected diets [29].

Load method

This method has been used primarily to obtain data on the bioavailability of oxalate in food and to study modification of oxalate absorption in the small intestine. A baseline urine collection is obtained, which in one study was 6-12 hours after a meal [4], 9-12 hours in another [9], and 11-14 hours in the most recent study [8]. This baseline oxalate value was used to examine changes following either a sodium oxalate load or ingestion of a food item of known oxalate content. Food provided post-load was an oxalate-free formula diet. The increase in urinary oxalate excretion over the next 8 hours [8, 9] or 48 hours [4] was determined and used to calculate the oxalate absorbed. For a sodium oxalate load the amount absorbed was 9% over a 48 hour period [4]. For the 8 hour post-load evaluations, absorption ranged from 43% for pecan oxalate to zero for cranberry juice oxalate [8, 9].

The main weakness in this experimental approach is the reliance on a pre-load urine collection as the baseline value. This value may change over time due to fluctuations in endogenous synthesis and to differences in the colonic absorption of oxalate which will also contribute to urinary oxalate during the experimental period. The amount of oxalate available for absorption may depend on the food ingested 12-60 hours prior to the studies. Such factors may account for the unrealistically high absorption of oxalate from pecans.

Daily excretion method

The basis for this method is the equilibration of individuals on two controlled diets with a different oxalate content, the collection of 24 hour urine samples after equilibration, and calculating the amount of oxalate absorbed from the differences in dietary oxalate and the amount of urinary oxalate excreted. Criteria that have to be fulfilled for this method to be valid are (a) that equilibration to the diets has occurred, (b) that urinary oxalate excretion on the diets is constant (i.e., endoge-

nous synthesis does not fluctuate), and (c) that the diet is well controlled. Four studies [15, 17, 41, 53], two from the same laboratory, have utilized this approach. Only criterion (b) was satisfied and then only in two of the studies [41, 53]. The variability obtained in these studies, however, was less than what we have observed in individuals on a diet controlled in magnesium, fiber, sodium, potassium and protein [30]. It is difficult to know whether the difference in the results is due to differences in the diets, differences in the study participants, or differences in the methods for oxalate determination. Equilibration to the high oxalate diets was not achieved in any of these studies and the mean excretions on the first 3 or 4 days of the diet were used. Finch *et al.* [17] showed that equilibration on a diet was not obtained until days 6 or 7, which is similar to what we show below. Despite recognizing this time required for equilibration, Finch *et al.* [17] used shorter study periods. Experimental details of the diets were scant in all studies making it impossible to evaluate criterion (c). Those details supplied suggested that variable menus were used rather than the same menu each day. Variability in the menus is important as the magnesium and fiber content of these diets were not controlled and could have varied and thereby influenced the results. The ranges in oxalate absorption obtained in these studies were 2.6-4.1% [53], 0.1-13.6% [41], 5-7% [15], and 1.3-22.2% [17]. This large range in values is consistent with methodological problems being associated with the studies.

Sites of Intestinal Absorption of Oxalate

Oxalate absorption can occur by paracellular fluxes along the entire gastrointestinal tract [7]. To date, an active uptake mechanism has been identified only in the colon of experimental animals but may also occur in man [21, 22]. The amount of oxalate absorbed in each intestinal segment will depend on the amount of oxalate solubilized, transit times, and the absorptive properties of the luminal epithelial cells. In most studies, an initial peak of oxalate absorption occurs in 2-5 hours [16, 40, 46, 48, 50] which is very similar to the absorption of calcium [14]. The timing of this peak indicates that it is occurring in the small intestine. Barilla *et al.* [4] have argued that this absorption site is in the proximal section of the small intestine based on observations that the initial peak of absorption was still observed in individuals with the distal portion of the small intestine resected. It has been ascertained that the bulk of paracellular calcium absorption in the small intestine occurs in the ileum, principally because the transit time is longest in this segment [15]. If oxalate absorption in the small intestine occurs predominantly by paracellular routes, the bulk of

this absorption may also occur in the ileum. Confirmation that the ileum is an important absorption site for oxalate in humans is warranted as well as a comprehensive study of its absorptive properties. It has been suggested that the stomach is an important absorption site [23], but the nature of the protocol in blocking gastric emptying and the use of patients who had been on long-term gastric tube feeding, indicates that the significance of this absorptive site should be investigated by other approaches in normal individuals. Some absorption does occur in the first hour and dissolution of food-borne crystalline oxalate in gastric juices could be time dependent leaving the question of the extent of stomach oxalate absorption open.

Because of the difficulty in measuring absorption processes in the large intestine in humans, there is a lack of direct evidence showing that the large intestine is a significant site of absorption in normal individuals. There is substantial indirect evidence described below, however, based on studies with individuals with bowel resections, the kinetics of ^{14}C -oxalate absorption, and isolated rabbit intestinal segments. In individuals with enteric hyperoxaluria secondary to intestinal disease or intestinal surgery, the colon is clearly the site of hyperabsorption of intestinal oxalate [13, 44]. As patients who had resection of the proximal ascending colon developed hyperoxaluria, the distal colon was implicated in the hyperabsorption [13]. These studies do not exclude a role for the proximal colon in hyperabsorption of oxalate, however. In several studies where an oral dose of ^{14}C -oxalate was given to normal individuals, the percentage of the dose absorbed after 8 hours ranged from 0.4-10.1% with the bulk of the studies in the 7% range [27, 39, 40, 48, 50]. Based on estimates of the transit of food through the intestinal tract [47], some of this absorption may occur in the proximal part of the large intestine. Experiments with segments from the large intestine of rabbits have indicated that net oxalate absorption by active transport can be detected only in the distal colon [21, 21]. The relative contributions of active and paracellular transport to intestinal oxalate absorption along the human intestinal tract are yet to be determined.

Modification of Absorption

Several factors have been identified that modify intestinal oxalate absorption. They include Ca, Mg and fiber. Other compounds that bind to Ca, Mg, or oxalate, such as phosphate and phytate, could also modify oxalate absorption but have not been studied as yet. The extent of bacterial degradation of oxalate is another factor that may be potentially important. Ca and Mg decrease oxalate absorption presumably by binding to oxalate and decreasing its "free" level. Fiber decreases

oxalate absorption apparently by decreasing transit times, but binding of oxalate or changes in the solution properties of stool oxalate have to be considered. A 12% difference in urinary oxalate excretion was observed in individuals consuming diets of similar oxalate content but differing in fiber content [35]. The effects of bacterial degradation of intestinal oxalate on the absorption of intestinal oxalate have not yet been studied. The foregoing study on dietary fiber showed from stool analyses that 30-40% of the ingested oxalate was degraded in the intestine. *Oxalobacter formigines*, which can utilize only oxalate as a carbon source, is a normal component of the intestinal flora of most, but not all, individuals [2]. The rate of oxalate degradation in fecal suspensions (mean 0.2 mg/g/hr) suggests that intestinal bacteria have the capacity to completely degrade all fecal oxalate. However, the fecal excretion of the bulk of the ingested oxalate [35] indicates that degradation in the colon is not complete. This suppression may result, for instance, from the low amount of free oxalate in intestinal fluid due to the complexing of oxalate with Ca or Mg and its crystallization. The low concentration of free oxalate is likely to be a significant factor as the reported Michaelis constant (K_m) for oxalate utilization by *Oxalobacter* is 20 mM [1].

The extent that dietary Ca can modify intestinal oxalate absorption was dramatically shown by Zarembski and Hodgkinson [53]. Decreasing calcium intake from 900 mg per day in 13 stone patients to 150 mg per day increased oxalate excretion by an average of 56%. Oxalate excretion on the low calcium diet was increased further to 86% above the normal calcium diet by including 1.5 g of ethylenediaminetetraacetic acid (EDTA) in the diet. Several other studies have confirmed the observations that calcium restriction in the diet increases urinary oxalate excretion by 16-33% [5, 33, 41] in both normal and stone-forming individuals. By corollary, supplementation of the diets of stone-formers with calcium lactate has been reported to decrease oxalate excretion by 40% [32]. Increasing dietary magnesium also decreases oxalate absorption [6]. Urinary magnesium also increases with magnesium supplementation providing an additional benefit by increasing an inhibitor of calcium oxalate crystallization. In a more controlled study using formula diets, Barilla *et al.* [4] showed that a combination of Ca and Mg decreased oxalate absorption detected in both 8 hour and 47 hour urine collections.

Attempts to modify intestinal oxalate absorption by lowering the dietary intake of oxalate [41, 42] have not been very successful. Such studies are hampered by incomplete and inaccurate data for the oxalate content of foods. The lack of control of dietary magnesium and fiber, and an inadequate equilibration of subjects to the diet may have also contributed to the results.

Table 2. Oxalate-free formula diet.

Substance	g/l
sodium caseinates	37.2
corn oil	37.2
corn syrup	101.5
sucrose	43.5
α -cellulose	12.7
sodium citrate.2H ₂ O	1.32
potassium citrate	1.37
potassium chloride	1.63
choline chloride	0.43
magnesium chloride.6H ₂ O	1.42
calcium phosphate	0.81
sodium chloride	0.47
potassium phosphate (monobasic)	0.34
vitamin/trace mineral mixture	0.61
lecithin	0.74
vanilla extract	0.37
calories	1060

Response to an Oxalate-Free Diet

We have examined the response of two individuals to an oxalate-free diet using the daily excretion method to investigate the absorption of dietary oxalate. A polymeric formula diet that contained no oxalate was used to obtain complete control of the diet. A number of commercially available formula products were initially evaluated for their suitability. All formulae examined (liquid and spray-dried) contained some oxalate and, when used as a complete source of nutrition, these products would provide 10 mg oxalate per day. The source of oxalate may have been derived from ascorbic acid breakdown as 30% of the vitamin is lost during processing (B.D. Travis, Ross Labs., personal communication). The constant formulation of the diet also eliminates potential confounding influences of dietary factors that may modulate endogenous oxalate synthesis. Dietary glycolate, estimated to be 33 mg/day, has been proposed to contribute to endogenous oxalate synthesis [19]. This contribution is likely to be minimal, however, if man is similar to the guinea pig where we calculated the turnover of glycolate to be 25 g/day/kg liver [31].

When oxalate is cleared from the gut, all of the oxalate in urinary excretions will be derived from endogenous synthesis. Twenty-four hour urine collections from individuals at this stage will serve as a baseline value for

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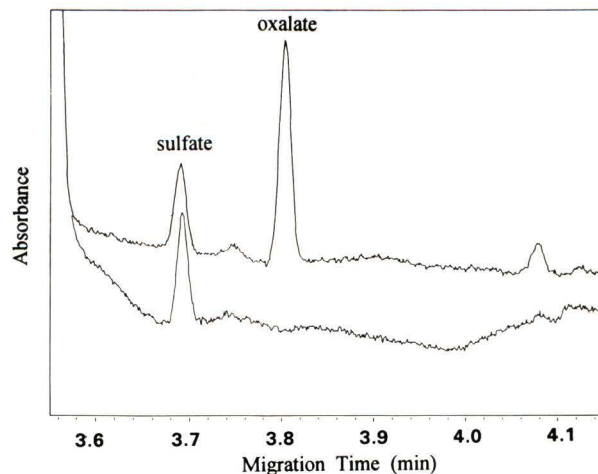
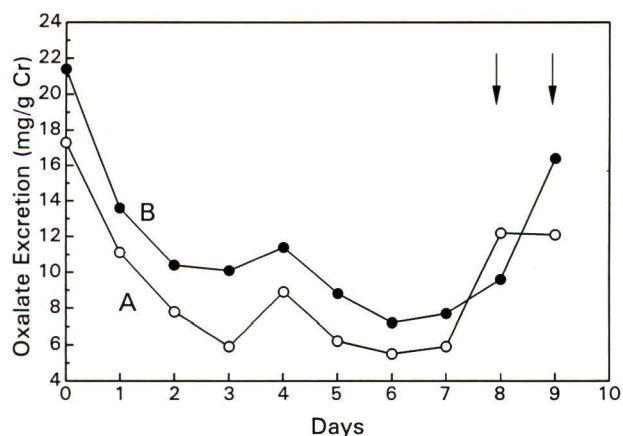


Figure 2. Changes in urinary oxalate/creatinine excretion in two individuals consuming an oxalate-free formula diet. The formula diet documented in Table 2 was ingested for 7 days followed by oxalate supplementation for the 2 days denoted by arrows. It was consumed 6 times daily beginning at 7:00 AM at 2.5 hourly intervals. Caloric requirements of the individuals were assessed by their age (A 47, B 70), sex (male), weight (A 77 kg, B 65 kg), body frame, and level of activity, and were 2500 for A and 1800 for B. Oxalate was measured by CE [28] and creatinine as previously described [29] in 24 hour urine collections.

Figure 3. The analysis of oxalate in fecal samples. The upper chromatogram shows the analysis of oxalate in feces from individual A while consuming a self-selected diet. For analysis, 0.5 g of feces was mixed with 4.5 ml of 1 M H_3PO_4 and incubated at 55°C for 1 hour. Particulate matter was removed from a 1 ml aliquot by centrifugation in a microfuge for 2 minutes at 15,000 g. The supernatant was diluted 1/100 for CE analysis as described for urinary oxalate [28]. The lower chromatogram shows the analysis of oxalate in a stool sample from individual B after consumption of the oxalate-free diet for 6 days.

studying the effects of adding oxalate and modifiers of oxalate absorption back to the diet. The number of collections required to accurately reflect excretion levels will depend on the variability in the endogenous synthesis of oxalate. The formulation of a diet used for testing in two individuals is shown in Table 2. Sodium caseinate was used as the source of protein. Corn oil, corn syrup, sucrose and vanilla extract were purchased at a local supermarket. α -Cellulose (Avicel® PH101) was obtained from FMC (Philadelphia, PA) and added at 12.5 g/l. All other additions were of food grade quality. A multi-vitamin/mineral supplement (Centrum Silver®; Lederle, Lederle, NY) was crushed and added to the formula with the Ca and Mg content of the supplement taken into consideration.

Two male individuals consumed this formula diet for 7 days. On self-selected diets, individual A excreted a mean of 29.7 mg/day and individual B 26.7 mg/day based on four 24 hour urine collections. The changes observed in urinary oxalate excretion are shown in Figure 2. By days 6 and 7, urinary oxalate had declined to a mean of 10.5 mg in A and 8.6 in B, a mean decrease of 18.6 mg. Stool samples were monitored by a CE analysis to follow changes in fecal oxalate excretion. The analysis of feces obtained before ingestion of the

formula diet is shown in Figure 3. Fecal oxalate could be detected on day 4 of the formula diet in both individuals (25 g/g in A and 42 g/g in B) but in neither individual at day 6. Based on a signal to noise ratio of 3:1, these assays indicated that the fecal samples contained < 10 g/g fecal wet weight. An analysis of feces collected on day 6 is shown in Figure 3. These results showed that it takes at least 5 and possibly even 6 days to reduce intestinal oxalate to below detectable levels in the gut. Urinary oxalate declined by 65.1% in A and 69.1% in B when comparing mean excretions on days 6 and 7 of the formula diet with their mean excretions on self-selected diets. Thus, dietary sources of oxalate may have contributed the majority of the oxalate excreted by these two individuals on self-selected diets. However, it remains possible that endogenous synthesis is suppressed by the composition of the formula or by the six times a day formula ingestion. It was estimated from recorded dietary intakes that both these individuals consumed 200 mg oxalate/day on their self-selected diets based on the preliminary CE analyses of the majority of the foods ingested. Thus, the mean amount absorbed, 18.6 mg, is close to the average of 10% absorption detected in ^{14}C -oxalate absorption studies reviewed above.

On days 8 and 9, the formula was supplemented with sodium oxalate (AR grade; Fisher, Pittsburgh, PA) equivalent to 200 mg oxalate per 2500 kcal. This addition of oxalate to the diet increased urinary oxalate excretion of individual A to 70% and B to 76% of the levels obtained on self-selected diets. The restoration of urinary oxalate excretion to near levels on self-selected diets by adding an amount of oxalate to the formula equivalent to the normal intake suggests that the formula diet was not drastically altering endogenous synthesis, although the possibility that the formula diet has modified the permeability properties of the intestinal epithelium has to also be considered. On day two of the oxalate-containing diet, the increment in urinary oxalate excretion would be equivalent to an absorption of 6.1% in A and 5.1% in B. A period longer than two days may have been required to re-equilibrate the gut with oxalate and maximize oxalate absorption so these values most likely underestimate absorption. A factor that may cause enhanced oxalate absorption on self-selected diets is the ingestion of oxalate and cations that modify absorption at different times during the day. We believe that this experimental system can be manipulated not only to study the timing of ingestion of nutrients but also to study, with some precision, the effects of dietary oxalate, Ca, Mg, and fiber on oxalate absorption.

The contribution of dietary oxalate to urinary oxalate excretion in these two individuals is large because they have a low rate of endogenous oxalate synthesis. This endogenous synthesis is apparently genetically determined due to the presence of two co-dominant alleles of a gene that regulates oxalate synthesis [16; Holmes RP, Goodman HO, Assimos DG, manuscript in preparation]. These alleles create 3 classes of oxalate excretors, low (49% of the Caucasian population), intermediate (46%), and high (5%) excretors. The high oxalate excretors (hyperoxaluric) have the highest risk of stone disease with intermediate excretors having approximately twice the risk of low excretors [18]. The proportional contribution of dietary oxalate to urinary oxalate excretion obviously differs between these classes. Whereas, we have shown experimentally above that dietary oxalate may contribute 67% to urinary oxalate excretion by low oxalate excretors, the contributions are estimated to be 49% for intermediate excretors and 32% for high excretors, based on the mean oxalate excretion of these excretory classes. The assumption was made that an average of 19 mg of oxalate, the mean absorption of the two individuals studied, was absorbed from the diet by each excretory class. A more precise estimate of the contribution of dietary oxalate to urinary oxalate excretion in each excretory class will depend on determining whether the formula diet modifies endogenous oxalate synthesis and in accurately classifying individuals into excretory

groups.

Conclusions

(1). The amount of oxalate ingested in typical western diets is not known with certainty due to incomplete and inaccurate food composition tables for oxalate. What is regarded today as a healthy diet rich in whole grain products, fruits and vegetables may contain close to 200 mg of oxalate per day. Ingestion of significant amounts of wheat bran-enriched products would increase the intake of oxalate further, as would the ingestion of spinach, beets, peanuts, and chocolate. A less healthy diet (rich in animal protein, fat and refined sugars) may result in an intake of less than 100 mg of oxalate.

(2). The amount of ingested oxalate that is absorbed in the intestine is also not known with certainty. It appears to range between 5 and 15% depending on the co-ingestion of Ca, Mg and fiber. This level of absorption would contribute 10-30 mg with an intake of 200 mg of oxalate per day.

(3). The use of oxalate-free formula diets will make it possible to study, in more detail, intestinal oxalate absorption and factors that modify it. Preliminary studies suggest that ingestion of such diets for 6 days will be required to eliminate detectable oxalate from the intestinal tract.

(4). The use of formula diets to study oxalate absorption and its modification should make it possible to confirm whether lowering the intake of oxalate and increasing the intakes of Ca, Mg and fiber are viable strategies for decreasing urinary oxalate excretion in calcium oxalate stone-formers.

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Discussion with Reviewers

P.O. Schwillle: How did the authors handle the ascorbate or ascorbic acid content of both traditional food and the formula diet, viz., the non-enzymatic conversion to oxalate at luminal pH of the gut? In other words, to deduce that changes in urinary oxalate after a prior stabilization period (food) or eating formula reflect endogenous oxalate production may be not warranted in my view. To be valid, at least substances known to influence oxalate biosynthesis need proper control, too. Also, pre-existent calcium oxalate crystals adherent to tissue(s) can undergo dissolution under chosen conditions, thereby mirroring changes in oxalate biosynthesis. **Authors.** We do not believe that significant, if any, conversion of ascorbate to oxalate occurs in the gut. The initial acidification of food ingested in the stomach, the subsequent decrease in luminal O₂, and the creation of a strong reducing environment in the intestinal tract will limit oxidative processes. The failure of large oral doses of ascorbate to increase urinary oxalate excretion is further evidence against this intestinal conversion occurring. With our experimental protocol, this question could be easily addressed by comparing formula diets with and without ascorbate.

An examination of dietary components or conditions that influence oxalate biosynthesis is a feature that can be explored with the use of oxalate-free formula diets. If, for instance, the hypothesis is proposed that saturated fat increases endogenous synthesis the formula can be readily modified to directly address this question. This aspect is discussed below in answer to a question more directly asked by Dr. Messa.

The dissolution of pre-existent calcium oxalate crystals could be making a minor contribution to the oxalate excreted on formula diets, but the emphasis is on minor as we observed a decrease in oxalate excretion, and not an increase.

P. Messa: The only small objection is that the experimental part relies on the observation of only two sub-

jects, the informative value would certainly be greater if the number of observations were to be increased.

Authors: We agree that it would be ideal to study more than two individuals and will do so when we obtain sufficient funding. Our emphasis at the moment is to use shorter periods of formula ingestion to decrease the dietary influences on urinary excretions and thereby detect more readily, genetic differences between individuals.

P. Messa: What influence might dietary factors have on the endogenous production of oxalate, especially with the substantial modifications to the diet in this study?

Authors: The percentage of calories from protein (14.0%), fat (31.5%), and carbohydrate (54.5%) in the formula diet are all within normal limits. The composition of each macronutrient differs from that of a normal diet, however, with a single source of protein, a polyunsaturated fat source low in saturated fat, and a high sucrose level. Such differences in composition are not known to influence endogenous synthesis, but they cannot as yet be discounted. Studies testing whether such factors affect endogenous synthesis could be tested in formula diets to identify foods that increase or decrease oxalate excretion.

J.F. Costello: The content of oxalate precursors (e.g., glycolate, ascorbate, etc.) in the self-selected (SS) and formula diets or their possible contribution to urinary oxalate excretion, via endogenous metabolism, is very important as the large decrease in oxalate excretion observed on the oxalate-free formula diet could, in large part, be due to a lack of oxalate precursors in this diet (see, Harris and Richardson [19], they conclude that > 5% of urinary oxalate comes from dietary glycolate).

L.C. Cao: Considering that some oxalate precursors (e.g., glycolate) are present especially in foods of vegetable origin and are well absorbed and, at least partially, converted into oxalate [19], and that only oxalate, but not glycolate in foods was defined in the present study (see Table 1), do you not think that dietary oxalate precursors should be considered for stone-former's diet management?

Authors: As stated in the text, it is possible that some immediate precursors of oxalate in its synthetic pathway could be removed by the formula diet and contribute to a decreased oxalate excretion. However, we do not believe, that the studies of Harris and Richardson [19] show that the amounts of glycolate encountered in normal human diets would be converted to significant amounts of oxalate. They used extremely large doses of glycolate in rat feeding studies. The lowest dose they used was 0.5 mmole/kg rat weight. This dose is equivalent to 150 moles per 300 g rat. If this is all absorbed, as stated, it is equivalent to 150 μ moles entering a blood volume of \sim 20 mls. Assuming that the peak blood

concentration is about 10% of this (to account for urinary losses and tissue uptake during absorption), the peak concentration would be 1.5 mM in plasma. This figure is in contrast to the normal circulating glycolate concentration of 5-10 μ M. With such high concentrations, it is not surprising that some enters the liver and is converted to oxalate. To extrapolate from metabolism in rats at such high doses of glycolate to what may occur in humans is not warranted. Under normal conditions, the liver presumably excretes glycolate and does not take it up from the blood. We have not been able to get HepG2 cells (derived from a human hepatoma) to take up significant glycolate when exposed to 1 mM. We did not present these arguments in the text as they are distracting. As stated in the text, based on our calculations in guinea pigs, using the measured intracellular concentration of glycolate, the amount of glycolate oxidase, and the properties of glycolate oxidase, the daily turnover of glycolate is \sim 25 g/kg liver. The entry into such a pool of 33 mg of glycolate, estimated to be the mean dietary intake in man, would be insignificant.

J.F. Costello: Was the Ca, Mg, and fiber content of the SS-diets determined, and if so how do they compare with these components in the formula diet? As the authors correctly point out, these components alter oxalate absorption.

Authors: The Ca and Mg contents of the self-selected diets were measured but not the fiber content. The Ca content of self-selected diets was \sim 80% of the formula diet in A and \sim 130% in B. Mg contents were in the same area but we did not have complete Mg contents of all foods ingested. The individuals did not have abnormal eating habits that would have caused an unusually high oxalate absorption. Their oxalate intakes (estimated to be \sim 200 mg in the text) may be higher than average due to the ingestion by both of bran-enriched breakfast cereal.

J.F. Costello: How was the sodium oxalate added to the formula diet? Does this oxalate absorption study not have the same deficiencies as outlined for the isotopic method?

Authors: The sodium oxalate added to the formula diet would be expected to form calcium oxalate crystals due to the supersaturation of the formula with Ca (10 mM) and oxalate (0.9 mM). The free oxalate would be expected to be that available for absorption. The amount of free oxalate in the gut may change due to secretions and absorptions of ions during transit. The changes, although complex, are all subject to testing under precisely controlled dietary conditions when individuals are equilibrated to the formula diet. Gut contents could be sampled at various intestinal sites to determine changes in free oxalate and how variable these changes are between

and within individuals. The points raised in the text with regard formula diets are complexities, not necessarily deficiencies.

J.F. Costello: The conclusion that the formula diet does not drastically alter endogenous synthesis appears unsubstantiated. Absorption of oxalate from this diet may be far greater and this diet may be deficient in oxalate precursors, hence the restoration of urinary oxalate to near levels on the SS-diets does not provide evidence on endogenous synthesis.

Authors: We agree that we cannot conclude that endogenous synthesis is not altered by the formula diet and our remarks are offered as a suggestion only. As stated in the text, we agree with the possibility that the absorptive properties of the intestine are altered by the formula diet.

L.-C. Cao: Do the authors believe that there is a genetic defect (unrelated to other dietary factors) in the patients with mild idiopathic hyperoxaluria?

Authors: Patients with mild metabolic hyperoxaluria, we believe, are a mixture of individuals who are either homozygous or heterozygous for an allele promoting high levels of hepatic oxalate synthesis [18]. The homozygous individuals may require drug therapy (when one is developed) to reduce their synthesis unless an appropriate dietary regime is identified that lowers endogenous synthesis. The heterozygous individuals may have a diet-induced hyperoxaluria, as identified by Hatch [20], and should respond to dietary treatments (low oxalate and high Ca, Mg and fiber) that decrease intestinal oxalate absorption. The homozygous individuals would also benefit from such dietary treatments to minimize their oxalate absorption. We would stress that this gene cannot be regarded as abnormal or defective as it exists at least in a single dose in over half the population [18]. It helps create a susceptibility to stone disease but additional genetic and environmental risks are required for stone formation to occur.

L.-C. Cao: Can the authors speculate on the response to the oxalate-free formula diet in stone patients? Does the designed oxalate-free formula diet have a good tolerance for patients so as to be of any use in clinical practice?

Authors: Most calcium oxalate stone-formers would undoubtedly benefit by consuming the formula diet we have described as their only source of nutrition, due to the decrease in urinary oxalate excretion. The formula could also be adjusted to lower Ca and citrate excretion and increase urinary volume, other risk factors in stone disease. The formula diet may particularly benefit those individuals whose stone disease is refractory to other therapies. Compliance, we anticipate, would be propor-

tional to the severity and frequency of the stone episodes. Partial replacement of regular foods is a further option coupled with the intake of only low oxalate foods and the avoidance of most fruits and vegetables. The use of the formula diet may best serve stone patients by providing important nutritional information about normal individuals and by being used to nutritionally stabilize patients before a metabolic evaluation of their urinary excretions.

L.-C. Cao: What influence will the oxalate-free formula diet have on the renal handling of oxalate?

Authors: Without renal hemodynamic measurements, an effect of the formula diet on the renal handling of oxalate cannot be discounted. It is possible that a decreased renal secretion of oxalate, an increased re-absorption of oxalate, or a decreased glomerular filtration rate occurred and lowered urinary oxalate excretion. Enteral diets, however, are not known to influence the glomerular filtration rate or the renal handling of other anions. If such changes do affect oxalate excretion we believe they are small.

L.-C. Cao: Evidence from oxalate transport in cell culture models [54-56] and the Ussing chamber technique [22] has revealed a significant effect of the extracellular environment on oxalate transport (absorption and secretion). Do you think a diet can be designed for stone-formers manipulating these variables to lower oxalate excretion? Are these factors influencing your results with formula diets?

Authors: The question of modifying the diet to alter modifiers of discrete transcellular transport processes is an interesting one, but is not one that can be answered readily addressed until such modifiers are identified and ways to control them are established. Decreasing the free oxalate with Ca and Mg is an approach that should modify dietary oxalate absorption by transcellular and paracellular routes and has been discussed in the manuscript.

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