

Origin of Urinary Oxalate

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Abstract. Urinary oxalate is mostly derived from the absorption of ingested oxalate and endogenous synthesis. The breakdown of vitamin C may also contribute small amounts to the urinary oxalate pool. The amount of oxalate absorbed is influenced by the oxalate content of the diet, the concentrations of divalent cations in the gut, the presence of oxalate-degrading organisms, transport characteristics of the intestinal epithelium, and other factors associated with the intestinal environment. Knowledge of pathways associated with endogenous oxalate synthesis is limited. Urinary oxalate excretion can be modified using strategies that limit dietary oxalate absorption and the ingestion of oxalogenic substrates such as hydroxyproline.

Keywords: dietary oxalate, oxalate synthesis, intestinal absorption, ascorbate.

PACS: 82.39.-k

INTRODUCTION

Urinary oxalate is derived from 3 sources: the diet, endogenous synthesis, and the breakdown of ascorbic acid. In the absence of any disease that enhances oxalate excretion (primary hyperoxaluria, enteric diseases, bariatric or intestinal surgery), the amount of oxalate excreted in the urine may vary from 5 to 60 mg per day, and the concentration from 0.04 to 0.75 mM. There may be slight differences in excretion due to gender [1], while the impact of age and race are not well defined. Oxalate is not metabolized in the body and is removed from the body principally by urinary excretion. Under certain conditions related to the gradient of oxalate across the intestinal epithelium, secretion of oxalate into the intestine may occur [2].

ASCORBIC ACID BREAKDOWN

Ascorbic acid can breakdown non-enzymatically to produce oxalate. This process is accelerated at alkaline pH. Initial experiments suggested that this breakdown was a significant source of oxalate, but a close scrutiny of the procedures indicated that the breakdown occurred due to processing urine at a high pH [3]. Two studies have examined the effects of 2 g of supplemental ascorbic acid in normal and stone forming subjects who consumed diets controlled in oxalate and other nutrients [4,5]. They showed increases in urinary oxalate excretion of 7.5 to 21.5% in normal subjects and 16.4 to 32.6% in stone forming subjects. An analysis of the diets of 1473 men who formed stones also indicated that the ingestion of more than 1000 mg of ascorbate per day was a risk factor for stone disease [6]. These results suggest that to avoid

CP900, *Renal Stone Disease, 1st Annual International Urolithiasis Research Symposium*,

edited by A. P. Evan, J. E. Lingeman, and J. C. Williams, Jr.

© 2007 American Institute of Physics 978-0-7354-0406-9/07/\$23.00

unnecessary contributions to urinary oxalate excretion, stone formers should be advised to ingest no more than 400 mg of ascorbic acid per day [7].

DIETARY OXALATE

The contribution of dietary oxalate to urinary oxalate excretion varies considerably when individuals consume a self-selected diet. A number of factors most likely contribute to this variability due to their influence on oxalate absorption. They include the oxalate content of the diet, the bioavailability of the oxalate, divalent cations in the intestinal stream, oxalate degradation in the gut, intestinal transit times, intestinal physiology, and other intestinal substances or organisms that can influence any one of these parameters. In a small number of individuals ($n = 5$), we observed that the mean oxalate intake was 150 mg/day. Several studies indicate that normally 1 to 15% of the ingested oxalate is absorbed [8-10]. Hesse and colleagues have suggested that absorptions above 15% of a soluble oxalate load represent a hyperabsorption of oxalate [10]. High absorptions of oxalate will lead to transitory oxalate loads on the kidney and will increase the supersaturation of urine with calcium oxalate. Thus, oxalate hyperabsorption could be a risk factor for stone disease and Voss et al have confirmed this in a case-control study using soluble $^{13}\text{C}_2$ -oxalate to monitor absorption [10].

The amount of calcium ingested appears to have a strong influence on intestinal oxalate absorption. A prospective study in male health professionals showed that there was an inverse dose effect of dietary calcium on stone formation [11]. In another prospective study, Borghi et al showed that calcium intake was partially responsible for limiting stone formation in a cohort of stone formers [12]. An experimental group with a high calcium intake had a diminished oxalate excretion, as well as a reduced stone recurrence in comparison to a group with a low calcium intake. Both of these prospective studies suggest that the effect of a high calcium intake is to bind to oxalate in the gut and limit its absorption. This effect of dietary calcium on oxalate absorption and urinary oxalate excretion was confirmed by von Unruh et al [13].

Of the other factors that may influence oxalate absorption, there is current interest in the role of the oxalate-degrading organism, *Oxalobacter formigenes* (OxF), in decreasing the amount of intestinal oxalate available for absorption and of the oxalate transporter SLC26A6, which is present in much of the human intestine. Preliminary data suggest that stone formers have a lower colonization rate with OxF than non-stone formers and that this may increase their urinary oxalate excretion [14]. Experiments with control of dietary oxalate, calcium and other nutrients are required to confirm and quantify the effects of OxF on oxalate absorption. The role of SLC26A6 in oxalate transport became apparent after studies in mice in which this gene had been knocked out [15,16]. These mice were hyperoxaluric. Surprisingly, flux experiments on isolated epithelium indicated that intestinal oxalate secretion was defective, suggesting that significant cycling of oxalate occurs across the luminal membrane in this tissue. These results further suggest that another process (transporter?) is responsible for oxalate influx into enterocytes. It is possible that either

the absorptive or secretory components of this cycling process are altered in individuals who hyperabsorb oxalate. A more complete analysis of the transport processes underlying oxalate absorption and an accurate phenotyping of the absorptive characteristics of individuals is required.

ENDOGENOUS OXALATE SYNTHESIS

All tissues can apparently synthesize oxalate as measurable levels of glycolate, glyoxylate, oxalate, glyoxylate reductase and lactate dehydrogenase are found throughout the body. These metabolites and enzymes are centrally involved in the terminal steps of oxalate biosynthesis [17]. The liver, the major metabolic organ in the body, is the main site of oxalate production. The carbon sources utilized for oxalate synthesis are only partially identified [17]. Such sources include the amino acids, hydroxyproline, glycine, phenylalanine and tyrosine. However, their relative contributions have not been accurately quantified and some may be of only minor significance. Carbohydrates can also be converted to oxalate via pathways that result in xylulose-5-phosphate formation. Aldolase can catalyze the breakdown of xylulose-5-phosphate to glycolaldehyde and eventually to oxalate. Carbohydrates known to be converted to oxalate include glucuronate, xylitol and xylulose. The pathway associated with the metabolism of these sugars was demonstrated when patients in Australia and Germany developed oxalosis and organ dysfunction after being infused with large doses of xylitol [18].

To identify pathways that lead to oxalate synthesis we have utilized HepG2 cells, a hepatoma cell line that retains many hepatocyte functions including the synthesis of oxalate, glycolate and glyoxylate [19]. The metabolism of glycine and hydroxyproline by HepG2 cells to glycolate and oxalate is shown in Fig. 1.

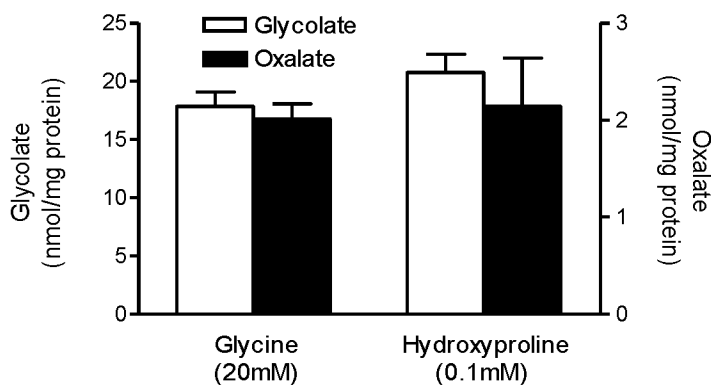


FIGURE 1. Increased glycolate and oxalate synthesis from supplemental glycine and hydroxyproline after 48 hrs incubation in low glucose medium. $^{13}\text{C}_2$ -glycine was used to trace the metabolism of glycine to $^{13}\text{C}_2$ -glycolate and $^{13}\text{C}_2$ -oxalate. Basal amounts of glycolate and oxalate synthesis were 6.1 ± 1.1 nmoles and 1.9 ± 1.0 nmoles/mg protein, respectively.

These results show that HepG2 cells possess pathways to metabolize both hydroxyproline and glycine to glycolate and oxalate, but much more is produced from hydroxyproline as the concentration used was 1/200 that of glycine. The daily turnover of glycine in the body, however, may be 200 times that of hydroxyproline. There is a single catabolic pathway for hydroxyproline degradation where one mole of the amino acid results in the formation of one mole of glyoxylate. This glyoxylate can subsequently be converted to glycolate, oxalate and glycine. In contrast, glycine has numerous metabolic fates, including a decarboxylation, a conversion to serine and utilization in protein synthesis as well as oxidation to glyoxylate. The formation of $^{13}\text{C}_2$ -oxalate and $^{13}\text{C}_2$ -glycolate, but not any $^{13}\text{C}_1$ -oxalate or $^{13}\text{C}_1$ -glycolate from glycine suggests that $^{13}\text{C}_2$ -glyoxylate is an intermediate and is formed through the activity of the peroxisomal enzyme, D-amino acid oxidase. A 10-fold differential in the amounts of glycolate and oxalate formed from hydroxyproline and glycine apparently occurs regardless of the subcellular compartmentation of the site of glyoxylate formation (mitochondria for hydroxyproline and peroxisome for glycine). A limited conversion of glycine to glyoxylate and oxalate was observed in isolated peroxisomes, consistent with the limited conversion by HepG2 cells of glycine to glycolate (~0.01%) and oxalate (~0.001%) [20]. A more extensive conversion of glycine to oxalate has been reported *in vivo* (0.05%) [21], suggesting that more extensive metabolism occurs *in vivo* in comparison to that observed *in vitro*. Alternatively, the methodology used in the 1960 publication may not have been accurate. To clarify these issues, the metabolism of glycine to oxalate *in vivo* should be re-evaluated.

The metabolism of hydroxyproline can potentially make an important contribution to oxalate synthesis as collagen turnover leads to the metabolism of 240 to 420 mg of hydroxyproline each day. Hydroxyproline may be also ingested either as collagen in meats and meat products or as gelatin. We have recently shown that the ingestion of gelatin leads to significant increases in urinary oxalate and glycolate excretion [22]. Quantitative estimates of the contribution of hydroxyproline breakdown to endogenous oxalate synthesis are yet to be determined.

The data previously discussed illustrate that the metabolic pathways that lead to oxalate synthesis are still poorly defined. Such information is clinically relevant as it could lead to possible enzymatic targets for drug therapy or dietary strategies that could limit oxalate synthesis. Decreasing endogenous oxalate synthesis could be important in the treatment of the primary hyperoxalurias and calcium oxalate stone disease [23]. The development of knockout mouse models for the primary hyperoxalurias should provide important insights into the biochemical pathways that result in oxalate synthesis, and lead to better strategies for limiting the conversion of glyoxylate to oxalate.

OXALATE-FREE DIETS

The use of oxalate-free diets is one tool we have used to quantify endogenous oxalate synthesis. These studies have also provided an insight into inter- and intra-

individual variability in the parameter. A limitation of the approach is that the diet could potentially influence the amount of endogenous synthesis. The composition of the diet mirrors that of the commercially available product, Ensure®, and was made from the base ingredients to eliminate all sources of oxalate [24]. The response of 14 individuals to this diet is shown below in Fig. 2.

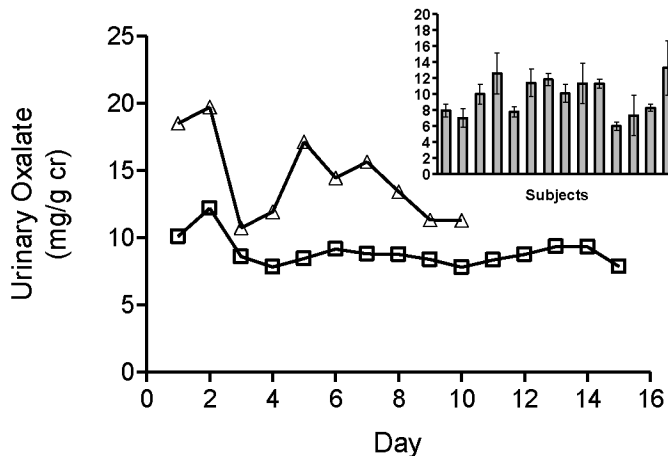


FIGURE 2. Urinary oxalate excretion of two normal subjects on an oxalate-free formula diet. Data in the inset show the mean (\pm SD) oxalate excretion of 14 subjects on days 3 to 5 of the diet.

Urinary oxalate excretion on an oxalate-free diet is substantially reduced from that observed on controlled oxalate diets [25]. The pattern of oxalate excretion over a 10 to 15 day period is shown for 2 individuals in Fig. 2. The mean 24 hr excretions of 14 other individuals on days 3 to 5 of the diet are shown in the inset. The results show that there is substantial inter- and intra-subject variability in endogenous oxalate synthesis. The mean intra-individual coefficient of variation (CV) was 15.8% and the mean inter-individual CV was 23.5%. Factors that may underlie this variability are unknown. We have shown that glucagon modifies oxalate synthesis in guinea pigs [26], indicating that variations in the levels of metabolic hormones may have an important role.

MODIFICATION OF URINARY OXALATE

Whereas the amount of calcium excreted in urine can be reduced with thiazide drugs, there is no such medical therapy available for reducing urinary oxalate excretion. A subset of individuals with primary hyperoxaluria type 1 respond to pyridoxine therapy with a decreased urinary oxalate excretion [27], but other individuals do not appear to respond to pyridoxine [28]. Dietary therapy is, however, one available option and there are several steps that can be taken to modify the diet

without much difficulty, thus enhancing compliance. Reducing oxalate intake is the first step that should be taken. It should be possible with a judicious choice of foods to decrease oxalate intake to less than 75 mg/day. The second step is to ensure that calcium intake is at least 1200 mg/day. Hesse et al illustrated the importance of calcium intake by showing that increasing calcium intake from 1211 to 3858 mg offsets the hyperoxaluria associated with a diet containing 2200 mg oxalate/day [29]. Urinary calcium did increase with the high calcium diet by 20%, but there was no increase in calcium oxalate crystallization indices. The response of individuals with hypercalciuria to calcium intakes above 1200 mg/day remains to be investigated. The third step is to balance calcium and oxalate intakes throughout the day to maintain a molar excess of calcium over oxalate with each meal or snack.

Modifying endogenous oxalate synthesis requires a more complete understanding of the biosynthetic pathways involved. Clearly, excessive ingestion of collagen or gelatin should be avoided to limit hydroxyproline metabolism to oxalate. Some individuals take large doses of these proteins as can be ascertained by an internet search for collagen or gelatin supplements and their uses. Other dietary components or supplements that contribute to oxalate synthesis also warrant consideration.

ACKNOWLEDGMENTS

Our research on dietary oxalate and the endogenous production of oxalate has been supported by NIH grants DK50466, DK62284, DK73732 and MO1 RR07122.

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