Two-Carbon Oxalogenesis Compared in Recurrent Calcium Oxalate Stone Formers and Normal Subjects

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We used a xylitol load to test the two-carbon pathway to oxalate production in humans. Use of this pentose sugar caused a fourfold increase in glycolate excretion, indicating its suitability as a dynamic function test of two-carbon metabolism. However, despite this increase in glycolate excretion in 10 recurrent stone formers and six normal subjects, there was no concomitant increase in oxalate excretion in either group. By comparison, a sucrose load produced no increase in excretion of either glycolate or oxalate. In addition, when we studied four recurrent calcium stone formers on successive diets with various fat content, we found no correlation between high fat intake and increased glycolate or oxalate excretion. In summary, there was no evidence of abnormal fluxes through the two-carbon pathway to oxalate in recurrent stone formers, nor of hyperoxaluria as related to increased intake of sucrose or fat.

Additional Keyphrases: lack of effect of sucrose, fat in diet · xylitol loading · origins of hyperoxaluria

Increased urinary excretion of oxalate is considered to be a major factor predisposing to recurrent calcium nephrolithiasis (1, 2). Oxalate may be derived essentially from three major sources: dietary oxalate, ascorbate, and two-carbon metabolites from glycolaldehyde (3) (Figure 1). Normally, dietary oxalate contributes up to 20% of the urinary oxalate excretion (4, 5); 30–50% is contributed by asarobate (2, 6) and 40–50% by two-carbon metabolites (2, 7). The main precursors of the two-carbon pathway are glycine and ethanolamine (2, 7, 8), although recent evidence indicates that some carbohydrates, particularly xylitol, may contribute directly to this pathway (9). The increase in calcium stone disease in westernized societies also has been shown to correlate with affluence (2, 10), presumably because of an increased intake of animal protein and refined carbohydrates (11, 12). However, careful studies of dietary intakes have not indicated significant differences between the diets of normal healthy controls and recurrent stone formers (13).

We recently concluded that an impairment in intestinal absorption of ascorbate by some recurrent calcium oxalate stone formers led to a significantly increased oxalogenesis from this vitamin (14). However, aberrant two-carbon metabolism or increased intestinal uptake of oxalate (4, 5) may be important factors in other oxalate stone formers. Investigating the possibility that an impaired two-carbon metabolism might cause hyperoxaluria in oxalate-stone formers, we used oral xylitol loading to measure the activity of this pathway in humans. In a second related study, we measured the effect of variations in dietary fat intake on the excretion of oxalate by recurrent calcium oxalate stone formers, excessive intraluminal fat being a major contributor to the excessive absorption and excretion of oxalate seen in pa-

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Fig. 1. Major pathways of oxalate metabolism in humans

The two-carbon pathway from glycolaldehyde to oxalate is emphasized by the dashed border. The vitamin B₆ transaminase and B₆ α-ketoglutarate:glyoxylate carboxylase pathways, which metabolize glyoxylate, are also indicated.

 Patients: The patients were three women and nine men, ages 22 to 63 years, all of whom had formed more than one mixed oxalate/phosphate stone in the past three to 32 years. They were carefully instructed to avoid foods with high oxalate or calcium content and to maintain a high fluid intake. During the study all were healthy, with normal renal function as evidenced by their concentrations of creatinine and urea in plasma. None of the patients had hyperparathyroidism, enteric hyperoxaluria, vitamin D intoxication, or renal tubular acidosis. Included in the dietary fat studies were three additional patients with enteric hyperoxaluria and recurrent calcium-stone episodes resulting from jejuno-ileal resections; these patients have been described already (21). Calculi obtained from all patients and analyzed by a wet chemical quantitative method (22) indicated the presence of mixed calcium phosphate/oxalate calculi.

Normal healthy volunteers were laboratory personnel, five men and three women, ages 20 to 48 years.

Xylitol and sucrose loads: In this study 10 patients and six normal volunteers fasted overnight. An overnight urine was voided and discarded at 0600 hours and a baseline urine was collected between 0600 and 0800 hours. At 0900 hours the subjects drank 16 g (105 mmol) of xylitol in 300 mL of water and we collected three further 2-h urine specimens. During this period all subjects were allowed only water ad libitum. Similarly, we tested five stone formers and four normal volunteers with an oral load of 50 g (146 mmol) of sucrose.
We measured the concentrations of glycolate, glyoxalate, and oxalate in all samples collected.

**Fat studies:** We assessed the effect of dietary fat on four recurrent calcium stone formers in hospital. The fat in the diets was 10 to 30% vegetable fat, 70 to 90% animal fat. The patients were maintained on a medium-fat diet (100 g of fat daily) for three days, followed by a low-fat diet (50 g/day) for two days. The medium-fat diet was then resumed for a further two days, followed by a high-fat diet (140 g/day) for two days. Each day we measured the urinary excretions of glycolate and oxalate from each subject.

**Statistics:** In comparing urinary excrections of the normal subjects and the stone formers, we used the Wilcoxon nonparametric two-sample statistic (23).

**Results**

Results of oral xylitol loading on urinary glycolate excretion are shown in Figure 2A. On average, the glycolate excretion rate increased fourfold ($P < 0.005$) during the first 2 h after the xylitol load, with no significant difference between the normal subjects and the stone formers. To calculate the percentage of the xylitol load excreted as glycolate, we made a linear extrapolation between the minus 2- to 0-h and the 4- to 6-h urine collections and subtracted this baseline from the glycolate excretion rate at 0 to 2 h and 2 to 4 h. For six normal persons the proportion of the xylitol dose excreted as glycolate ranged from 0.04 to 0.27% (mean 0.18%), not significantly different from results for the 10 recurrent calcium stone formers (0.08 to 0.28%; mean 0.18%). Figure 2B shows the oxalate excretion rate after the xylitol load. The rate was similar in both stone formers and normal subjects, with no obvious peak of excretion. Like oxalate, there was no peak or significant difference in the glyoxylic acid excretion for both groups of subjects. The mean ($\pm$SD) glyoxalate excretion rate for controls and stone formers were respectively 4.3 (3.5) and 2.9 (1.4) $\mu$mol/h before the xylitol load and 4.2 (2.2), 3.4 (1.8), and 2.3 (1.6) vs 3.9 (1.3), 2.8 (1.6), and 4.6 (3.1) $\mu$mol/h for each subsequent 2-h sample.

Glycolate and oxalate excretion rates after sucrose loads are shown in Figure 2, C and D. There was no observable peak in glycolate excretion, unlike the case after the xylitol load. The oxalate excretion rate was also constant, which was similar to that seen after the xylitol load.

We found no significant difference between patients and controls in vitamin B6 status (data not shown).

Figure 3 illustrates the profile for daily urinary oxalate excretion by the four recurrent stone formers on hospital diets with low, medium, and high fat content. Oxalate excretion in these patients remained normal and was unaffected by the fat content of the diet. Daily glycolate excretion was also normal (24)—493 (SD 175) $\mu$mol ($n = 16$) and was unaffected by the fat content of the diets, the mean ($\pm$SD) excretions after low-, medium-, and high-fat diets being 700 (118), 675 (99), and 671 (100) $\mu$mol/d, respectively.
Discussion

To determine whether the two-carbon pathway for metabolizing proteins and carbohydrates to oxalate is hyperactive in stone formers, we used the xylitol loading test. Xylitol is converted by action of D-xylulose reductase (EC 1.1.1.9) and fructokinase (EC 2.7.1.14) to xylulose 1-phosphate. Aldolase (EC 4.1.2.13) converts xylulose 1-phosphate to dihydroxyacetone phosphate and glyceraldehyde, the latter being a substrate for the two-carbon pathway (25).

In our study, the vitamin B-mediated salvage pathways were well able to cope with the moderate stress on the two-carbon pathway by the xylitol load. Urinary oxalate excretion after the load was unaffected despite the fourfold increase in glycolate excretion (Figure 2A). However, even at a greater oral load, sucrose had a poor capability to generate two-carbon metabolites and oxalate (see Figure 2C), confirming findings of others (9, 28).

That increased flux down the two-carbon pathway to oxalate is not the cause of hyperoxaluria in recurrent stone formers is also supported by our previous study (24). There we showed that 24-h urinary glycolate and glyoxylate excretions were similar for stone formers and normal subjects, despite the fact that stone formers had significantly increased 24-h excretions of oxalate.

Controlled diets with low-, medium-, and high-fat intakes were given to test the hypothesis that excess intraluminal fat might increase calcium binding and thereby make more soluble oxalate salts free for absorption, as is the case of patients with fat malabsorption and enteric hyperoxaluria (15). However, we found no correlation between high fat intake and increased oxalate excretion. Subclinical degrees of fat malabsorption resulting in excess intraluminal fat therefore seem unlikely to be a cause of hyperoxaluria. The absence of increased glycolate excretion by these patients on the high-fat diets indicates only a small metabolic flux of fat metabolites through the two-carbon pathway.

Further, the oxalate content of the diets given these patients apparently was adequate: three patients with enteric hyperoxaluria and receiving the medium-fat diet had urinary oxalate excretion rates of 0.6 to 1.23 mmol/d (reference range <0.5 mmol/d; 17, 18). It is therefore improbable that the oxalate content of the hospital diet was so low as to obscure any fat-enhanced absorption of oxalate in the stone-forming patients we studied.

In summary, the procedure we described (the 16-g oral xylitol loading test) was effective in stressing the two-carbon pathway for oxalate synthesis, increasing the excretion of glycolate, but it did not increase oxalate excretion significantly in either stone formers or normal subjects. This suggests that the hyperoxaluria reported for patients with calcium oxalate stone disease is unlikely to result from a hyperactive two-carbon pathway and supports our finding that hyperoxaluria in such patients is due to malabsorption of ascorbate and other hydroxycarboxylic acids (14, 24) or to inadvertent intake of foods rich in oxalate. Our future studies will be aimed at further elucidating the hyperoxaluria seen in recurrent calcium stone formers.

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References