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Metabolism of Fructose to Oxalate and Glycolate

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Abstract

Much attention has been recently directed at fructose consumption because of its association with obesity and subsequent development of chronic diseases. It was recently reported that an increased fructose intake increases the risk of forming kidney stones. It was postulated that fructose consumption may increase urinary oxalate, a risk factor for calcium oxalate kidney stone disease. However, conflicting results have been obtained in human studies examining the relationship between fructose metabolism and oxalate synthesis. To test whether fructose intake influences urinary excretions impacting kidney stone risk, healthy subjects consumed diets controlled in their contents of fructose, oxalate, calcium, and other nutrients. Subjects consumed diets containing 4, 13, and 21% of calories as fructose in a randomized order. No changes in the excretions of oxalate, calcium, and uric acid were observed. In vitro investigations with cultured liver cells incubated with ¹³C-labeled sugars indicated that neither fructose nor glucose was converted to oxalate by these cells. Fructose metabolism accounted for $12.4 \pm 1.6\%$ of the glycolate detected in the culture medium and glucose $6.4 \pm 0.9\%$. Our results suggest that mechanisms for stone risk associated with fructose intake may lie in factors other than those affecting the major stone risk parameters in urine.

Keywords

nutrition; urolithiasis; HepG2 cells; glyoxal; glucose

Introduction

Dietary factors have a strong impact on calcium oxalate kidney stone formation [1–3]. Low intakes of calcium, magnesium, and potassium and high intakes of sodium and oxalate may contribute to stone formation. Fructose consumption was recently shown to be a risk factor for kidney stone formation [4]. Since fructose consumption has increased substantially over the past 3 decades, an understanding of how it might increase stone risk is warranted. The increased risk was identified in 3 large cohorts consisting of the Nurses' Health Study I (females of median age 46 years), the Nurses' Health Study II (females of median age 36 years), and the Health Professionals Follow-up Study (males of median age 55 years). The majority of stones formed in these cohorts consisted predominantly of calcium oxalate [4]. The multivariate-adjusted relative risk for stones ranged from 1.27–1.37 for the quintiles consuming the highest amount of total fructose in the diet providing a median intake of 13.8–15.2% of dietary calories compared with the quintiles consuming the lowest amount of total fructose providing a median intake of 5.6–5.7% of dietary calories. It was argued that

fructose consumption could increase stone risk by altering urine composition. There is evidence that it could increase the excretion of calcium [5], oxalate [6], and uric acid [7] and, if it causes insulin resistance, it could possibly decrease urinary pH [8]. Increasing urinary calcium and oxalate could increase the risk of calcium oxalate stone formation [9], whereas increasing urinary uric acid excretion and decreasing urinary pH could increase the risk of uric acid stones [10].

Research studies on the relationship of fructose metabolism with oxalate synthesis are equivocal. An oral load of glucose (75 g), but not fructose (75 g) induced a 62% increase in urinary oxalate excretion [11]. In contrast, an intravenous infusion of fructose but not glucose increased oxalate excretion by 60% [6]. However, the plasma fructose level was 10-fold higher with the infusion compared to the oral load. Fructose handling and metabolism in humans is distinguished from glucose metabolism in several ways. Fructose is absorbed from the small intestine at half of the rate of glucose, but is cleared from the circulation at twice the rate of glucose [12]. The majority of fructose metabolism occurs in the liver and is depicted in Fig. 1. Of note is the conversion of fructose and glucose to different trioses, with glucose getting converted to glyceraldehyde-3-P (GAP) and dihydroxyacetone-P (DHAP), and fructose to DHAP and glyceraldehyde (GA). Glyceraldehyde can be subsequently converted to GAP, glycerol or glycerate. There is no known pathway that would result in a significant conversion of any of the trioses produced to oxalate. The rapid phosphorylation of fructose in the liver results in ATP depletion, the accumulation of AMP, and acceleration of its breakdown to uric acid [12], thus providing a pathway whereby fructose consumption may stimulate uric acid synthesis.

To determine what effects fructose consumption has on urinary excretions in healthy subjects, we conducted a comprehensive nutritional study with subjects consuming diets that were controlled in their contents of nutrients known to affect the excretions of stone risk factors. The oxalate content was low, 51 mg/day, to diminish dietary oxalate contributions to urinary oxalate excretion and to amplify contributions from potential changes in endogenous oxalate synthesis. 3 levels of fructose were consumed in random order contributing 4, 13, and 21 % of calories, which covered the range of estimated fructose consumptions in the cohorts studied by Taylor and Curhan [4]. In addition, the metabolism of fructose and glucose was investigated in a hepatic cell line to determine if metabolism of sugars in liver cells can contribute to the generation of oxalate.

Subjects and Methods

Chemicals

Reagent grade chemicals were primarily obtained from either Sigma-Aldrich Chemicals (St Louis, MO, USA) or Fisher Scientific (Pittsburg, PA, USA). ¹³C-isotopes were obtained from Cambridge Isotopes (Andover, MA, USA).

Subjects

7 healthy, non-kidney stone forming adults (4 females and 3 males) with a mean age of 30 ± 5 years and a mean BMI of 25 ± 4 kg/m² were recruited for this study, which was approved by the Institutional Review Board. All subjects were initially evaluated with a serum comprehensive metabolic profile and collected two 24-h urine specimens to assess stone risk parameters while consuming self-selected diets.

Dietary protocol

Subjects consumed the controlled diets shown in Table 1 in a randomized order for 1 week each and obtained 24-h urine collections on the last 4 days of each diet. Caloric levels of

diets were adjusted to 2 000 or 2 500 kcal, whichever matched most closely their calculated caloric needs estimated using the Harris–Benedict equation with an additional activity factor. There was a 1 week washout period between each diet. The fructose content of the diet was calculated as the sum of the fructose level and half of the sucrose level as reported in USDA National Nutrient Database for Standard Reference-Release 19. Fructose was provided primarily as a fructose-supplemented berry punch on the medium and high fructose diets. All diets were developed using ProNutra (Viocare Technologies, Princeton, NJ, USA) menu development software (v. 3.3) and contained 16% protein, 30% fat, and 54% carbohydrate.

Analytical Methods

Urinary creatinine, calcium, magnesium, sodium, potassium, phosphorus, urea-N, uric acid, oxalate, and citrate were measured on a Beckman CSE Analyzer. Sodium and potassium were measured by ion specific electrodes, oxalate using a kit provided by Trinity Biotech (St Louis, MO, USA), citrate using a kit provided by Boehringer Mannheim (Darmstadt, Germany), and other analytes using kits provided by the manufacturer. The pH of urine was determined using a Beckman pH meter.

Cell culture oxalate analysis

Total oxalate was determined in cell culture media by ion chromatography (Dionex Corp., Sunnyvale, CA, USA) with suppressed conductivity detection (ASRS300) using a AS22 2 × 250 mm ion exchange column, and 2.5 mM sodium carbonate/1.7 mM sodium bicarbonate at 0.4 ml min⁻¹. Cell culture media was diluted 2-fold in 0.8 M boric acid prior to storage at -70°C to prevent any oxalogenesis. Prior to analysis, samples were filtered on boric acid-washed centrifugal filters with a 10 000 nominal molecular weight cutoff limit (NMWC).

Glycolate and ¹³C₂-isotope analysis by RFIC-MS

Reagent-FreeTM ion chromatography coupled with negative electrospray mass spectrometry (RFIC-MS) (Dionex Corp.) was used to quantitate total glycolate, ¹³C₂-glycolate, and ¹³C₂-oxalate in cell culture media. The IC portion of the IC-MS consists of an AS11-HC 2 × 250 mm anion exchange column with guard, potassium hydroxide eluent generator, and ASRS300 suppressor. The MS is a Thermo-Finnigan MSQTM ELMO single quadrupole mass spectrometer that is specifically designed for the analysis of small molecular weight ions. Enrichment standard curves containing mixtures of ¹²C₂-glycolate, ¹³C₂-glycolate, ¹²C₂-oxalate, and ¹³C₂-oxalate were used to quantitate total glycolate and ¹³C₂-isotopes in samples. Media samples were stored at -70°C and filtered prior to analysis.

Cell culture

HepG2 cells were obtained from the American Type Culture Collection (Rockville, MD, USA) and were used only until passage 30. They were routinely grown at 37°C in Dulbecco's modified Eagle's medium (DMEM) containing 10 % fetal bovine serum (FBS), 2 mM glutamine, 1 mM sodium pyruvate, and 25 mM glucose (Invitrogen, Carlsbad, CA, USA) in a humidified atmosphere containing 5% CO₂. For precursor and ¹³C-sugar experiments, tissue culture treated polystyrene 35 mm dishes (Corning Inc, Lowell, MA, USA) were seeded with 2 × 10⁶ cells and grown to confluency in DMEM.

Cell culture incubations

DMEM media (1 ml) containing 10% FBS, 2 mM glutamine, 1 mM sodium pyruvate, 25 mM glucose, and either 1 mM or 10 mM precursor was added to the confluent cells and the media harvested 48 h later for the measurement of oxalate and glycolate. Glycolaldehyde, (+)-D-glyceraldehyde, and glyoxal were obtained from Sigma. Glyoxylic acid and sodium

glycolate were obtained from Fisher. Each precursor stock preparation and the DMEM without FBS were analyzed for glycolate and oxalate content before experiments and did not contain glycolate or oxalate. The 10 % FBS added to media contained oxalate (2 μ M) and glycolate (4 μ M), and these blanks were subtracted from experimental results.

Cell culture ^{13}C -sugar incubations

$^{13}\text{C}_6$ -Glucose or $^{13}\text{C}_6$ -fructose (1 or 25 mM), was added to DMEM basic base powder (Sigma-Aldrich), which contains no sugar, and reconstituted in water along with glutamine, sodium pyruvate, and sodium bicarbonate, and sterile filtered. 1 ml of media formulation was added to confluent HepG2 cells and after 48 h the media was harvested for measurement of total oxalate, total glycolate and $^{13}\text{C}_2$ -glycolate, and $^{13}\text{C}_2$ -oxalate. $^{13}\text{C}_6$ -Fructose experiments do not contain any added glucose.

Protein analysis

The protein content of HepG2 cell monolayers was measured using a Coomassie Plus assay kit (Pierce, Rockford, IL, USA), with bovine serum albumin as the standard, after dissolution of the cells with 0.1 M NaOH.

Statistical analyses

All analyses were performed using SAS v9.2 (SAS Institute Inc., Cary, NC, USA). The effects of dietary fructose on urinary excretions were determined by repeated measures ANOVA. The tests of significance used in the model were adjusted for gender, age, BMI, and within-individual correlation. For cell culture experiments, differences in oxalate and glycolate synthesis were compared using a *t*-test. Data are presented as the raw mean $\pm$ s.e.m, unless otherwise stated. *p*-Values less than 0.05 were considered significant.

Results

Varying the fructose content of the diet from 4–21 % did not significantly alter any stone risk parameters, including the urinary excretions of calcium, oxalate, and uric acid, and urinary pH (Table 2). Urinary glycolate excretion was also unaffected, not supporting a substantial flux to glycolate, or to glycolaldehyde and glyoxylate, which are potential precursors of glycolate and oxalate during fructose metabolism [13]. Potassium excretion increased by 19% as fructose intake increased (*p* = 0.004).

The metabolism of $^{13}\text{C}_6$ -glucose and $^{13}\text{C}_6$ -fructose was examined in HepG2 cells. These cells established from a human hepatoma retain many hepatocyte-specific functions, and have been shown to synthesize oxalate and glycolate in culture [14, 15]. Fig. 2a shows that these cells metabolized only small amounts of these sugars to $^{13}\text{C}_2$ -oxalate, representing < 1.5% of the total oxalate produced with either 1 or 25 mM concentrations of fructose or glucose in the growth medium. Following incubation with 25 mM fructose, 28% more oxalate was produced compared to 25 mM glucose. However, this was not significant. This indicates that neither glucose nor fructose is a significant source of the oxalate synthesized by these cells. In contrast, $^{13}\text{C}_2$ -glycolate was formed from both $^{13}\text{C}_6$ -glucose ($6.4 \pm 0.9\%$ of the total glycolate pool was enriched with $^{13}\text{C}_2$ -glycolate) and $^{13}\text{C}_6$ -fructose ($12.5 \pm 1.6\%$) with 25 mM sugar in the medium, but <1.5% with a 1 mM concentration of the sugars (Fig. 2b). When the sugar concentration was 1 mM in the growth medium, all the sugar was completely metabolized within 12 h. An increased synthesis of total oxalate was observed in cells incubated with 1 mM sugars in comparison to the incubations with 25 mM sugars, suggesting that sugar deprivation promoted oxalate synthesis in these cells. The existence of pathways that may result in the synthesis of glycolate and oxalate from sugar metabolites was investigated by incubating HepG2 cells with several potential 2- and 3-carbon

precursors (Fig. 3). Glycolaldehyde, glyoxal, and glyoxylate were all effective precursors of glycolate. Only small amounts of glycolate were synthesized from ethylene glycol, but it was twice as much as in control cells (13.3 ± 1.5 nmol/mg protein vs. 5.5 ± 1.6 nmol/mg protein). No glycolate was formed from glyceraldehyde. Glyoxylate was the best source of oxalate and significant amounts were also synthesized from glyoxal. Glycolate at 10 mM was a moderate source of oxalate, but no oxalate was formed from this concentration of glycolaldehyde, glyceraldehyde or ethylene glycol.

Discussion

The positive association of incident kidney stones with fructose intake in 3 different cohorts suggested that the amount of fructose consumed in the diet is a risk factor for stone formation [4]. Taylor and Curhan speculated that increasing fructose consumption may increase urinary calcium, oxalate and/or uric acid excretion, and/or reduce urinary pH. Several studies support the existence of a pathway for the formation of oxalate in the metabolism of fructose [5, 13]. Rofe et al. observed the conversion of small but significant amounts (~0.1%) of ^{14}C -labelled fructose to oxalate in incubations with isolated rat hepatocytes [16]. These results have not as yet been confirmed in other laboratories. Conflicting results have been obtained in human studies. No increase in urinary oxalate was observed in one study with an oral load of 75 g of fructose [11] where serum fructose reached 0.67 mM 60–90 min after the load. However, a short intravenous infusion of ~35 g of fructose did increase oxalate excretion [6]. The serum concentration of fructose reached at 15 min with this infusion was high, 5.5 mM, suggesting that high concentrations of plasma fructose may be required to produce an increase in oxalate synthesis.

Our study was designed to test whether fructose consumption over a 4.9-fold range would affect urinary stone risk parameters. The upper limit, 21 % of calories, would be equivalent to consuming nearly 6 cans of a sweetened soda, if the soda provided all of the fructose. Our results clearly showed that oxalate excretion was not affected by fructose consumption in the individuals tested. Glycolate excretion is potentially a sensitive marker of oxalate synthesis as both oxalate and glycolate have glyoxylate as a common precursor [17] as shown in Fig. 4. Because glyoxylate reductase has a higher affinity for glyoxylate than lactate dehydrogenase, in most conditions where significant glyoxylate is formed, such as when hydroxyproline is metabolized, the production of glycolate is 5–10 times higher than that of oxalate [14, 18]. Glycolate excretion did not change on these diets further suggesting that a pathway associated with glyoxylate production is not stimulated by fructose consumption. We also found that fructose consumption did not impact any of the other urinary parameters measured except potassium, which went up with increasing fructose consumption. The reason for the increased potassium excretion is unclear. It is possible that it is related to phosphate excretion, which also increased in this study ($p = 0.086$). Phosphate excretion has been reported to increase with the intravenous infusion of fructose [6] and with increased amounts of fructose in the diet [5].

We cannot entirely exclude an effect of fructose consumption on the absorption of dietary oxalate. A low oxalate diet (51 mg/day) was used in the studies reported here to limit the contribution of dietary oxalate to urinary oxalate excretion and to accentuate effects on endogenous oxalate synthesis. Fructose consumption has been shown to increase the balance of several ions, including calcium, magnesium, zinc, copper and iron and to decrease phosphate balance [5, 19]. The mechanisms associated with these changes have not been elucidated and whether oxalate absorption may be affected remains unknown.

Our studies with cultured HepG2 cells, showed that prolonged exposure of these cells to high levels of fructose produced more oxalate and glycolate compared to prolonged

incubation with glucose. However, the amounts produced were only minor compared to the total levels of oxalate and glycolate produced by these cells, and the amount of oxalate produced was not significantly different from that produced by equivalent glucose levels. $^{13}\text{C}_6$ -Fructose incubations did produce significantly more $^{13}\text{C}_2$ -glycolate than $^{13}\text{C}_6$ -glucose. This glycolate is potentially formed from glycolaldehyde (Fig. 4), as glycolaldehyde is formed by an aldolase-catalyzed hydrolysis of xylulose-1-phosphate [17, 20]. However, there is no known report of any difference in fructose and glucose metabolism that affects the synthesis or metabolism of xylulose-1-phosphate.

Incubation of glyceraldehyde and a range of potential 2 carbon precursors with HepG2 cells showed that neither glyceraldehyde nor glycolaldehyde could be metabolized to oxalate. In contrast, glyoxylate, glyoxal, and glycolate were converted to oxalate with glyoxylate > glyoxal >> glycolate. Although glycolaldehyde is converted to glycolate, it appears that the intracellular concentration reached was not sufficient to stimulate oxalate synthesis. Pathways describing how these 2 and 3 carbon aldehydes and acids are converted to oxalate are shown in Fig. 4. The exact role of aldehyde dehydrogenase (ALDH) and which of the isoforms may be active in reducing glycolaldehyde to glycolate and glyoxal to glyoxylate are unclear. There is evidence that ALDH isolated from human liver homogenates can reduce both glyoxal and glycolaldehyde to glyoxylate and glycolate, respectively [21]. It is of interest that glyoxal could be converted to oxalate in HepG2 cells. This dialdehyde could be expected to be converted to glycolate by the glyoxalase system after reacting with glutathione [22]. Our results suggest that some glyoxal may escape glutathionylation and instead be converted to glyoxylate by ALDH. The formation of glyoxal, a product of cellular peroxidation [23], may be accelerated in these cells when they are deprived of glucose as increased peroxidation is observed under these conditions [24]. The increase in oxalate synthesis in cells incubated with 1 mM sugar may in part be due to increased glyoxal formation. The role of peroxidation and glyoxal formation in endogenous oxalate synthesis warrants further investigation.

Fructose infusions and oral fructose loads are associated with increased uric acid synthesis [7,25,26]. An increased uric acid excretion is a risk factor for uric acid stones [10]. This increased uric acid production is believed to be due to an accelerated adenosine diphosphate (ADP) breakdown during fructose metabolism [27]. We observed no effect of an elevated fructose consumption for 1 week on uric acid excretion. This response is similar to that reported by Narins et al. [28], who supplemented the diet of 8 subjects with 100 g of fructose per day for 4 days and observed no change in urinary uric acid excretion. A longer period of fructose consumption, the consumption of higher levels of fructose, or a less healthy study population may have produced different results.

The other risk factors potentially influenced by a high fructose intake are an increased calcium excretion and a lowered urinary pH. We found that both calcium excretion and urinary pH were unaffected by the amount of fructose consumed. It remains possible that dietary imbalances occur in diets where fructose or sucrose sweetened items that are energy-rich and nutrient-poor are excessively consumed. Milne and Nielsen [5] observed a decrease in calcium balance with a high fructose, low magnesium diet, but no change in urinary calcium excretion in normal subjects.

If the risk for stone disease from fructose consumption is not associated with the urinary excretions of oxalate, calcium or uric acid, how might fructose exert its effect? One possibility is that fructose affects early lithogenic events such as the formation of Randall's plaques [29]. Although a magnesium deficiency has been ruled out [4], it is possible that fructose effects manifest only in an imbalanced diet. In rats, an imbalance of either dietary phosphorus or magnesium leads to nephrocalcinosis and a lower urinary pH in animals on a

fructose-enriched but not on a glucose-enriched diet [30]. Urinary calcium excretion was unaffected.

This study has limitations in relating the results obtained with the observations of Taylor and Curhan that increased fructose consumption increases stone risk [4]. The populations they studied were older. The sample size in our study was small because of the comprehensive controlled diets that were utilized. The possibility remains that a minor segment of the population metabolizes fructose differently from the bulk of normal individuals. Hyperoxaluric stone formers may be enriched in this group as they may not have the appropriate compensatory pathways/mechanisms to limit oxalate synthesis from fructose metabolism. A study comparing the responses of normal and stone-forming populations would be required to address this issue. The high fructose diet was consumed for only 1 week, whereas individuals in the cohorts studied by Taylor and Curhan reported on their food consumption over the preceding 12 months. Our studies with cultured HepG2 cells did show a minor increase in glycolate and oxalate synthesis, suggesting that prolonged exposure of hepatocytes to high levels of fructose may lead to increased oxalate synthesis. Furthermore, it remains possible that elevated fructose metabolism within the liver or other tissues over a long period of time produces changes that predispose individuals to the risk of incident stone formation. The mechanisms by which this occurs or whether it interacts with other factors are not understood at this time. In our study, subjects consumed a low oxalate diet to accentuate the urinary oxalate excretion associated with endogenous oxalate synthesis. It remains possible that dietary fructose modifies intestinal oxalate absorption. Lastly, the in vitro experiments with cultured hepatoma cells may not reflect the metabolism occurring in human liver tissue or other tissues of the body that contribute to endogenous oxalate production.

Conclusions

Our study demonstrates that in a small group of normal, healthy individuals on a low oxalate diet, urinary excretions of oxalate, calcium, urate and hydrogen ions are not affected by fructose intake. In vitro investigations revealed that HepG2 cells do not metabolize fructose or glucose to oxalate, but convert a minor portion of these sugars to glycolate. Glyoxylate and glyoxal appear to be important precursors of oxalate.

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References

1. Assimos DG, Holmes RP. Role of diet in the therapy of urolithiasis. *Urol Clin North Amer.* 2000; 27:255–268. [PubMed: 10778468]
2. Taylor EN, Curhan GC. Diet and fluid prescription in stone disease. *Kidney Int.* 2006; 70:835–839. [PubMed: 16837923]
3. Taylor EN, Fung TT, Curhan GC. DASH-style diet associates with reduced risk for kidney stones. *J Am Soc Nephrol.* 2009; 20:2253–2259. [PubMed: 19679672]
4. Taylor EN, Curhan GC. Fructose consumption and the risk of kidney stones. *Kidney Int.* 2008; 73:207–212. [PubMed: 17928824]
5. Milne DB, Nielsen FH. The interaction between dietary fructose and magnesium adversely affects macromineral homeostasis in men. *J Am Coll Nutr.* 2000; 19:31–37. [PubMed: 10682873]
6. Nguyen NU, Dumoulin G, Henriot M-T, Regnard J. Increase in urinary calcium and oxalate after fructose infusion. *Horm Metab Res.* 1995; 27:155–158. [PubMed: 7607607]

7. Fox IH, Kelley WN. Studies on the mechanism of fructose-induced hyperuricemia in man. *Metabolism*. 1972; 21:713–721. [PubMed: 5047915]
8. Abate N, Chandalia M, Cabo-Chan AV Jr, Moe OW, Sakhaee K. The metabolic syndrome and uric acid nephrolithiasis: novel features of renal manifestation of insulin resistance. *Kidney Int*. 2004; 65:386–392. [PubMed: 14717908]
9. Curhan GC, Taylor EN. 24-h uric acid excretion and the risk of kidney stones. *Kidney Int*. 2008; 73:489–496. [PubMed: 18059457]
10. Sakhaee K. Recent advances in the pathophysiology of nephrolithiasis. *Kidney Int*. 2009; 75:585–595. [PubMed: 19078968]
11. Nguyen NU, Dumoulin G, Wolf JP, Berthelay S. Urinary calcium and oxalate excretion during oral fructose or glucose load in man. *Horm Metab Res*. 1989; 21:96–99. [PubMed: 2722135]
12. Steinmann, B.; Gitzelmann, R.; Berghe, GVD. Disorders of fructose metabolism. In: Scriver, CR.; Beaudet, AL.; Sly, WS.; Valle, D., editors. *The Metabolic and Molecular Bases of Inherited Disease*. 8. New York: McGraw-Hill; 2001. p. 1489-1520.
13. Conyers RAJ, Bais R, Rofo AM. The relation of clinical catastrophes, endogenous oxalate production, and urolithiasis. *Clin Chem*. 1990; 36:1717–1730. [PubMed: 2208646]
14. Baker PR, Cramer SD, Kennedy M, Assimios DG, Holmes RP. Glycolate and glyoxylate metabolism in HepG2 cells. *Amer J Physiol*. 2004; 287:C1359–C1365.
15. Javitt NB. Hep G2 cells as a resource for metabolic studies: lipoprotein, cholesterol, and bile acids. *FASEB J*. 1990; 4:161–168. [PubMed: 2153592]
16. Rofo AM, James HM, Bais R, Conyers RAJ. The production of [14C] oxalate during the metabolism of [14C] carbohydrates in isolated rat hepatocytes. *Austral J Exp Biol Med Sci*. 1980; 58:103–116.
17. Holmes RP, Assimios DG. Glyoxylate synthesis, and its modulation and its influence on oxalate synthesis. *J Urol*. 1998; 160:1617–1624. [PubMed: 9783918]
18. Knight J, Jiang J, Assimios DG, Holmes RP. Hydroxyproline ingestion and urinary oxalate and glycolate excretion. *Kidney Int*. 2006; 70:1929–1934. [PubMed: 17021603]
19. Holbrook JT, Smith JC Jr, Reiser S. Dietary fructose or starch: effects on copper, zinc, iron, manganese, calcium, and magnesium balances in humans. *Am J Clin Nutr*. 1989; 49:1290–1294. [PubMed: 2729168]
20. Barngrover DA, Stevens HC, Dills WL. D-Xylulose-1-phosphate: enzymatic assay and production in isolated rat hepatocytes. *Biochem Biophys Res Commun*. 1981; 102:75–80. [PubMed: 6458298]
21. Kraemer RJ, Deitrich RA. Isolation and characterization of human liver aldehyde dehydrogenase. *J Biol Chem*. 1968; 243:6402–6408. [PubMed: 4387261]
22. Thornalley PJ. Glyoxalase I – structure, function and a critical role in the enzymatic defence against glycation. *Biochem Soc Trans*. 2003; 31:1343–1348. [PubMed: 14641060]
23. Shangari N, Bruce WR, Poon R, O'Brien PJ. Toxicity of glyoxals – role of oxidative stress, metabolic detoxification and thiamine deficiency. *Biochem Soc Trans*. 2003; 31:1390–1393. [PubMed: 14641070]
24. Chang SH, Garcia J, Melendez JA, Kilberg MS, Agarwal A. Haem oxygenase 1 gene induction by glucose deprivation is mediated by reactive oxygen species via the mitochondrial electron-transport chain. *Biochem J*. 2003; 371:877–885. [PubMed: 12585963]
25. Emmerson BT. Effect of oral fructose on urate production. *Ann Rheum Dis*. 1974; 33:276–280. [PubMed: 4843132]
26. Stirpe F, Della Corte E, Bonetti E, Abbondanza A, Abbati A, De Stefano F. Fructose-induced hyperuricaemia. *Lancet*. 1970; 2:1310–1311. [PubMed: 4098798]
27. Hallfrisch J. Metabolic effects of dietary fructose. *FASEB J*. 1990; 4:2652–2660. [PubMed: 2189777]
28. Narins RG, Weisberg JS, Myers AR. Effects of carbohydrates on uric acid metabolism. *Metabolism*. 1974; 23:455–465. [PubMed: 4825302]

29. Evan AP, Lingeman JE, Coe FL, Parks JH, Bledsoe SB, Shao Y, Sommer AJ, Paterson RF, Kuo RL, Grynepas M. Randall's plaque of patients with nephrolithiasis begins in basement membranes of thin loops of Henle. *J Clin Invest.* 2003; 111:607–616. [PubMed: 12618515]
30. Bergstra AE, Lemmens AG, Beynen AC. Dietary fructose vs. glucose stimulates nephrocalcinogenesis in female rats. *J Nutr.* 1993; 123:1320–1327. [PubMed: 8320569]

Fig. 1. Pathways for the metabolism of glucose and fructose. (1) = glucokinase or hexokinase; (2) = glucose 6-phosphatase; (3) = phosphohexose isomerase; (4) = fructose 1,6-bisphosphatase; (5) = fructose 6-phosphate-1-kinase; (6) = aldolase; and (7) = fructokinase.

Fig. 2. Cell culture media levels of oxalate (Panel **a**) and glycolate (Panel **b**) after 48-h incubation of HepG2 cells with either $^{13}\text{C}_6$ -glucose (open) or $^{13}\text{C}_6$ -fructose (hatched). Black filled in areas represent level of $^{13}\text{C}_2$ -isotope. # represents a significant difference ($p = 0.0049$) in $^{13}\text{C}_2$ -glycolate levels between fructose and glucose incubations. Data are expressed as mean $\pm$ SD of 3 replicates. Similar results were obtained in 2 identical experiments.

Fig. 3. Cell culture media levels of oxalate (Panel **a**) and glycolate (Panel **b**) after 48-h incubation of HepG2 cells with a variety of possible precursors. Incubations were with 10 mM concentrations except for glyoxylate and glyoxal, which were added at 1 mM because of evidence of toxicity at 10 mM. EG: ethylene glycol. Data expressed as mean $\pm$ SD of 3 replicates. *represents significant difference ($p < 0.01$) between control (no added precursor) and experiment.

Fig. 4.

Pathways for the conversion of 2 carbon precursors to glycolate and oxalate. (1)=alcohol dehydrogenase; (2) = aldehyde dehydrogenase; (3) = glycolate oxidase; (4) = glyoxylate reductase; (5) = lactate dehydrogenase; and (6) = glyoxalase.

Table 1

Composition of low, medium, and high fructose diets

	Low Fructose (4.3 % of calories)	Medium Fructose (12.7 % of calories)	High Fructose (21.1 % of calories)
Energy (kcal)	2512	2512	2511
Oxalate (mg)	51	51	51
Calcium (mg)	1026	1019	1020
Magnesium (mg)	230	250	242
Phosphorus (mg)	1422	1421	1403
Sodium (mg)	4244	3956	4006
Vitamin C (mg)	140	135	117
Potassium (mg)	2367	2422	2391
Sucrose (g)	22	35	37
Fructose (g)	15	58	107
Total Fructose (g)*	26	76	126

* Total fructose was calculated as the fructose content of diets plus half of the sucrose. The total carbohydrate content of each diet was 54 % of calories, fat 30 %, and protein 16 %

Table 2

The effect of the amount of fructose in the diet on 24-h urinary excretions

	Dietary Fructose Levels			
	Low	Medium	High	p-Value* Self-selected
Volume (ml)	1491 (144)	1693 (156)	1747 (131)	0.144 1259 (182)
pH	5.93 (0.10)	5.82 (0.06)	5.96 (0.13)	0.230 6.15 (0.14)
Creatinine (mg)	1507 (114)	1514 (127)	1592 (138)	0.217 1569 (197)
Oxalate (mg/g cr)	16.1 (0.92)	16.1 (1.25)	15.8 (1.05)	0.886 17.6 (2.80)
Glycolate (mg/g cr)	14.0 (1.16)	13.3 (0.98)	13.90 (1.32)	0.617 14.9 (1.15)
Sodium (mmol/g cr)	91.4 (5.7)	90.3 (4.6)	93.4 (5.9)	0.545 101.3 (9.5)
Potassium (mmol/g cr)	29.7 (1.6)	32.7 (1.9)	34.5 (2.2)	0.004 42.2 (3.2)
Calcium (mg/g cr)	109.6 (14.6)	103.3 (10.7)	101.2 (11.8)	0.667 116.7 (13.5)
Magnesium (mg/g cr)	48.3 (3.2)	51.4 (3.4)	47.4 (3.3)	0.281 59.1 (5.3)
Citrate (mg/g cr)	305.0 (25.2)	309.3 (30.9)	345.5 (36.6)	0.072 333.4 (35.5)
Phosphorus (mg/g cr)	593.3 (36.6)	608.9 (42.3)	654.5 (45.1)	0.086 573.1 (30.7)
Urate (mg/g cr)	386.8 (20.8)	381.7 (26.8)	386.9 (29.2)	0.849 366.4 (15.4)
Urea nitrogen (mg/g cr)	6.64 (0.47)	6.90 (0.50)	7.20 (0.48)	0.264 5.90 (0.48)

* Test for difference in response to fructose level where the type 1 error rate is 0.05. Excretions on the self-selected diets were not included in the model and are presented for comparative purposes only. The model was adjusted for BMI, gender, age, diet order, and within-individual correlation. Probabilities of less than 0.05 are considered statistically significant