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Metabolism of primed, constant infusions of [1,2-$^{13}C_2$] glycine and [1-$^{13}C_1$] phenylalanine to urinary oxalate

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ABSTRACT

Experiments in humans and rodents using oral doses of glycine and phenylalanine have suggested that the metabolism of these amino acids contributes to urinary oxalate excretion. To better define this contribution, we have examined the primed, constant infusion of [1-$^{13}C_1$] phenylalanine and [1,2-$^{13}C_2$] glycine in the postabsorptive state in healthy adults. Subjects were infused for 5 hours, hourly urines were collected, and blood was drawn every 30 minutes. Ion chromatography/mass spectrometry was used to measure [^{13}C] enrichment in urinary oxalate, glycolate, and hippurate; and the enrichment of ^{13}C-amino acids in plasma samples was measured by gas chromatography/mass spectrometry. Following infusion with either 6 μmol/(kg h) [1-$^{13}C_1$] phenylalanine or 6 μmol/(kg h) [1,2-$^{13}C_2$] glycine, no isotopic glycolate or oxalate was detected in urine. Based on the limits of detection of our ion chromatography/mass spectroscopy method, these data indicate that less than 0.7% of the urinary oxalate could be derived from phenylalanine catabolism and less than 5% from glycine catabolism. Infusions with high levels of [1,2-$^{13}C_2$] glycine, 60 μmol/(kg h), increased mean plasma glycine by 29% and the whole-body flux of glycine by 72%. Under these conditions, glycine contributed 16.0% ± 1.6% and 16.6% ± 3.2% to urinary oxalate and glycolate excretion, respectively. Experiments using cultured hepatoma cells demonstrated that only at supraphysiological levels (>1 mmol/L) did glycine and phenylalanine metabolism increase oxalate synthesis. These data suggest that glycine and phenylalanine metabolism make only minor contributions to oxalate synthesis and urinary oxalate excretion.

1. Introduction

Small amounts of oxalate, 15 to 25 mg, are normally synthesized in the body each day as a by-product of metabolism [1,2]. This oxalate is not further metabolized and is excreted predominantly in urine. The amount excreted in urine is of pathological significance because it is a critical determinant in idiopathic calcium oxalate kidney stone formation and the deposition of calcium oxalate in the kidneys of individuals with primary hyperoxaluria [3,4]. Amino acid metabolism is thought to be a major contributor to endogenous oxalate synthesis [1]. Experiments in both humans and animal models have shown that glycine and the aromatic amino acids phenylalanine, tyrosine, and

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tryptophan can be metabolized to oxalate [5-9]. A pathway for the metabolism of glycine to oxalate was proposed soon after glycine oxidase activity was detected in liver and kidney tissue and glyoxylate was determined to be the product of the oxidation [10]. Further experiments established that the oxidation of glycine was catalyzed by D-amino acid oxidase (DAO) [11]. The glyoxylate (CHO.COOH) formed by this oxidation is an immediate precursor of oxalate and the only one clearly identified to date [1]. The studies of Atkins and colleagues [5] and by Elder and Wyngaarden suggested that glycine catabolism produced 18% to 50% of urinary oxalate. Crawhall et al [7] also showed that $^{13}C_1$-oxalate could be detected in urine after $^{13}C_1$-glycine was ingested. However, a limitation of these studies is that they were based on oral consumption of labeled glycine, which results in a complex labeling pattern that changes over time in the various glycine compartments/pools within the body.

Phenylalanine metabolism is another potential source of oxalate. It has been reported to yield urinary oxalate following its intraperitoneal injection in rats [6,12]. The metabolism of phenylalanine in humans occurs primarily via its conversion to tyrosine, but other minor pathways exist where phenylalanine is first deaminated to form phenylpyruvate [13]. It is possible that phenylpyruvate and hydroxyphenylpyruvate formed from tyrosine are broken down to oxalate following the formation of unstable enols [14,15].

In a recent study, we found that increasing the protein content of the diet, which results in an increased catabolism of amino acids, did not alter urinary oxalate excretion [16]. These results suggested that amino acid catabolism is not a major source of endogenously produced oxalate. To readdress whether glycine or phenylalanine metabolism contributes to endogenous oxalate synthesis, we used a primed, constant infusion of ^{13}C-isotopes of these amino acids to trace their metabolism [17]. We have also examined the metabolism of these amino acids in vitro with HepG2 cells, a human hepatoma cell line that retains many aspects of hepatocyte metabolism including the synthesis of both oxalate and glycolate [18].

2. Methods

2.1. Chemicals

[1,2-$^{13}C_2$] glycine, [1-$^{13}C_1$] phenylalanine, [1,2-$^{13}C_2$] oxalate, and [1,2-$^{13}C_2$] glycolate were purchased from Cambridge Isotopes Laboratories (Andover, MA). Reagent-grade chemicals were obtained from Sigma-Aldrich Chemicals (St Louis, MO).

2.2. Study subjects

Healthy adults, as assessed by their medical history and a normal complete serum metabolic profile, participated in this study. Five (3 women and 2 men; mean age, 30.8 ± 3.3 years; mean body mass index [BMI], 22.5 ± 2.8 kg/m^2) participated in the low-glycine study, 6 (3 women and 3 men; mean age, 30.3 ± 6.1 years; mean BMI, 22.7 ± 4.1 kg/m^2) in the high-glycine study, and 5 in the phenylalanine study (3 women and 2 men; mean age, 33.0 ± 3.5 years; mean BMI, 22.7 ± 2.6 kg/m^2).

2.3. Study protocol

Before infusions, subjects consumed for 3 days controlled diets prepared in the metabolic kitchen of our institution's General Clinical Research Center (GCRC) that contained 16% protein, 30% fat, and 54% carbohydrate to normalize their metabolism. These diets contained 50 mg oxalate and 1000 mg calcium per day.

Subjects were studied in the postabsorptive state following a 12-hour overnight fast. The subjects arose at 6:00 AM, emptied their bladder, and drank 750 mL of water to ensure an adequate urine flow. Upon arrival at the GCRC at 7:00 AM, a catheter was inserted in an antecubital vein for infusion of the isotopic solutions; and another catheter was inserted into a superficial hand vein of the other arm for blood collection. Subjects drank 250 mL water per hour for the next 5 hours, collecting urine hourly. Infusions were initiated at 8:00 AM with a priming dose (5 μmol/kg) for both the low [1,2-$^{13}C_2$] glycine and [1-$^{13}C_1$] phenylalanine infusion study and 50 μmol/kg for the high [1,2-$^{13}C_2$] glycine infusion study, administered over a 5-minute period. The 4-hour constant infusion followed immediately after the priming dose and delivered 6 μmol/(kg h) for both the low [1,2-$^{13}C_2$] glycine and [1-$^{13}C_1$] phenylalanine studies and 60 μmol/(kg h) for the high [1,2-$^{13}C_2$] glycine study.

Blood samples (~3.5 mL) were drawn every hour, including 2 preinfusion specimens. Before blood collection, the targeted hand was placed in a warming box at 68°C for at least 10 minutes to achieve arterialization of the venous blood [19]. The patency of the sampling catheter was maintained with a slow infusion of 0.9% saline.

2.4. Analyses

Glycine and phenylalanine were measured in plasma by the AccQ Tag method (Waters, Milford, MA), as previously described [20]. The protein content of HepG2 cell monolayers was measured using a Coomassie Plus assay kit (Pierce, Rockford, IL), with bovine serum albumin as the standard, after dissolution of the cells with 0.1 mol/L NaOH. Total oxalate was determined in urine and cell culture media by ion chromatography (IC) with suppressed conductivity detection (Dionex, Sunnyvale, CA) using an AS22, 2 × 250 mm, ion exchange column and with 2.5 mmol/L sodium carbonate/1.7 mmol/L sodium bicarbonate as the mobile phase running at 0.3 mL/min. Reagent-free ion chromatography coupled with negative ion electrospray mass spectrometry (IC/MS) (Dionex) was used to measure total glycolate and total hippurate, and [^{13}C] enrichment in oxalate, glycolate, and hippurate. A Thermo-Finnigan MSQTM ELMO single quadrupole mass spectrometer (West Palm Beach, FL) that is specifically designed for the analysis of low–molecular weight ions was used for mass determinations. The IC portion of the IC/MS consisted of an ED50 conductivity detector, a GS50 gradient pump, an AS50 refrigerated autosampler, an EG50 potassium hydroxide gradient generator, and an AS50 thermal compartment containing an AS11-HC, 2 × 150 mm, anion exchange column at a controlled temperature of 30°C and an ASRS 300 2-mm suppressor. A gradient of KOH from 0.5 to 80 mmol/L over 60 minutes at a flow rate of 0.4 mL/min was used to separate anions in samples. The relative abundance of specific

anions was determined by selected-ion monitoring (SIM) at the following mass/charge ratios: glycolate (SIM75), [1-^{13}C] glycolate (SIM76), [1,2-^{13}C] glycolate (SIM77), oxalate (SIM89), [1-^{13}C] oxalate (SIM90), [1,2-^{13}C] oxalate (SIM91), hippurate (SIM178), and [1,2-^{13}C] hippurate (SIM180). Enrichment curves were prepared using known amounts of [1,2-$^{13}C_2$] glycolate and [1,2-$^{13}C_2$] oxalate in the range 0% to 3% enrichment, as previously described [21]. The IC/MS method has a *limit of detection* (LOD), defined as the mean mass signal ratio of an unenriched sample plus 3× SD, of 0.09% enrichment for [1,2-$^{13}C_2$] oxalate and 0.32% for [1,2-^{13}C] glycolate. [1,2-^{13}C] hippurate and [1-^{13}C] oxalate cannot be purchased commercially; and thus, appropriate enrichment curves to accurately quantitate these isotope levels in samples could not be determined. Values for [1,2-^{13}C] hippurate and [1-^{13}C] oxalate are thus only estimates and were calculated by firstly correcting the isotope mass signal of [1,2-^{13}C] hippurate and [1-^{13}C] oxalate for natural abundance. The level of isotope in samples was calculated using carbon 12 hippurate and carbon 12 oxalate standard curves. The enrichment of ^{13}C-amino acids in plasma samples was measured by gas chromatography/mass spectroscopy analysis by Metabolic Solutions (Nashua, NH).

2.5. Calculations of amino acid flux and contribution of amino acid to urinary oxalate and glycolate

The flux of phenylalanine was calculated in the same way as previously described [22], where it was assumed that phenylalanine, as an essential amino acid, is not synthesized in man and enters the plasma pool only from protein breakdown. It exits the pool only via protein synthesis or by hydroxylation to tyrosine, an irreversible step. The calculation of phenylalanine flux (Q_{Phe}) used the standard equation:

$$Q_{Phe} = i[(E_i / E_{Phe}) - 1],$$

where i is the tracer infusion rate in micromoles per kilogram per hour and E_i/E_{Phe} is the ratio of isotopic enrichment of the infusate (E_i) and plasma phenylalanine (E_{Phe}).

Glycine flux was calculated from plasma [1,2-$^{13}C_2$] glycine enrichment after correcting for the overestimation of the intracellular [1,2-$^{13}C_2$] glycine enrichment that occurs when plasma E_p of the glycine tracer is used. This prediction of intracellular [1,2-$^{13}C_2$] glycine enrichment ($E_p'_{Gly}$) was accomplished by multiplying the observed plasma [1,2-$^{13}C_2$] glycine enrichment by a correction factor of 0.4, derived from previous glycine tracer infusion studies in humans [23,24]. Glycine flux (Q_{Gly}) was calculated using the equation:

$$Q_{Gly} = i[(E_i / E_P' \text{ Gly}) - 1].$$

The percentage contribution (C) of amino acid metabolism to urinary oxalate and glycolate excretion used the equation:

$$C = (E_U / E_{Phe} \text{ or } E_P' \text{ Gly}) \times 100,$$

where E_U is urinary enrichment with [^{13}C] isotope.

2.6. Sample preparation and storage

For oxalate analysis, an aliquot of urine was diluted 5-fold in 2 mmol/L hydrochloric acid before –80°C storage to prevent any possible crystallization and oxalogenesis that may occur with storage and handling. For all other urine measures, whole urine was stored in aliquots at –70°C. For oxalate analysis, cell culture medium was diluted 2-fold in 0.8 mol/L boric acid before storage at –70°C to prevent any oxalogenesis. Before analysis, samples were filtered on acid-washed centrifugal filters with a 10 000 nominal molecular weight cutoff limit. For amino acid quantitation, plasma samples were extracted with trichloroacetic acid (10% final concentration) before analysis.

2.7. Cell culture

HepG2 cells were obtained from the American Type Culture Collection (Rockville, MD) and were used only until *passage* 30. They were routinely grown at 37°C in Dulbecco modified Eagle medium (DMEM) containing 10% fetal bovine serum, 2 mmol/L glutamine, 1 mmol/L sodium pyruvate, and 25 mmol/L glucose (Invitrogen, Carlsbad, CA) in a humidified atmosphere containing 5% CO_2.

2.8. Cell culture incubations with [1,2-$^{13}C_2$] glycine, and [1-$^{13}C_1$] phenylalanine

For experiments, 35-mm dishes were seeded with 2×10^6 cells and grown to confluency in DMEM before incubation with the isotope. The DMEM medium (1 mL) containing 10% fetal bovine serum, 2 mmol/L glutamine, 1 mmol/L sodium pyruvate, 25 mmol/L glucose, and varying concentrations of isotope was added to the confluent cells; and the medium was harvested 48 hours later for the measurement of oxalate and glycolate and [^{13}C] enrichment in oxalate and glycolate. Prepared medium was analyzed for total glycolate and total oxalate content before experiments and subtracted from experimental results.

2.9. Statistics

Comparisons between preinfusion and 4-hour postinfusion urine collections were performed using a paired Student t test. A probability (P) value < .05 was considered significant.

3. Results

3.1. Infusion with [1,2-$^{13}C_2$] glycine at 6 μmol/(kg h)

Five individuals were infused with tracer levels of [1,2-$^{13}C_2$] glycine to enrich plasma glycine with the isotope by 4% to 5% (Table 1A) and not significantly raise the plasma glycine concentration and alter glycine metabolism. Equilibration was reached in 1 hour, consistent with the results obtained in a previous study with glycine infusion [23]. With this level of [1,2-$^{13}C_2$] glycine enrichment, [1,2-$^{13}C_2$] oxalate and [1,2-$^{13}C_2$] glycolate were not detected in urine. Based on the LOD of this IC/MS assay, we would only be able to detect the conversion of glycine to oxalate and glycolate if glycine metabolism contributed greater than 5.0% and greater than 18.0% of the oxalate and glycolate, respectively, in urine (see "Methods" for calculations). These estimates rely on the assumption that the intracellular hepatic [1,2-$^{13}C_2$] glycine pool is diluted 40% from

Table 1A – Urinary and plasma parameters during an infusion of trace [1,2-$^{13}C_2$] glycine

Time (h)	E_p [1,2-$^{13}C_2$] glycine (%)	Urine oxalate (mg)	Urine glycolate (mg)	Urine hippurate (mg)	Estimated E_u [1,2-^{13}C] hippurate (%)
Preinfusion		0.82 ± 0.16	1.23 ± 0.58	8.3 ± 4.5	
3-4	4.11 ± 0.90	0.89 ± 0.18	1.63 ± 0.66	11.4 ± 7.7	1.67 ± 0.18

Data expressed as mean ± SD of samples from 5 individuals. Urinary measures pre- and postinfusion were not significantly different ($P > .05$). Plasma samples for enrichment analyses were obtained 4 hours postinfusion.

that in plasma, and that a similar dilution occurs in other tissues that may be able to synthesize oxalate and glycolate from glycine. These results suggest that previous reports of the conversion of glycine to oxalate were overestimated [5,9]. The urinary excretion of [1,2-$^{13}C_2$] hippuric acid derived from [1,2-$^{13}C_2$] glycine and benzoic acid was observed, illustrating that the intracellular glycine pool in the liver was effectively labeled by the [1,2-$^{13}C_2$] glycine infusion (Table 1A). Similar to previous reports, hippurate labeling did not reach equilibrium during the 4-hour infusion [23].

3.2. *Infusion with [1,2-$^{13}C_2$] glycine at 60 μmol/(kg h)*

To determine if glycine conversion to oxalate could be detected when the enrichment of plasma glycine was increased, 6 subjects were infused with 10 times the amount of glycine previously used. Enrichment increased to a mean of 23% ± 4% [1,2-$^{13}C_2$] glycine (Table 1B). Plasma glycine measurements were in keeping with this enrichment, increasing 29% from 268 ± 95 μmol/L preinfusion to 342 ± 113 μmol/L postinfusion ($P = .02$). The time course of labeling of plasma glycine and urinary hippurate (Fig. 1) was similar to that achieved with the lower glycine infusion rate. Enrichment of urinary oxalate and urinary glycolate was also detected, reaching 1.43% ± 0.31% and 1.53% ± 0.20%, respectively, after 4 hours of infusion (Fig. 1 and Table 1B). This level of enrichment could not be detected in plasma oxalate and glycolate because of their low circulating concentrations [25]. Based on these data, it was calculated that after 4 hours of infusion with this higher amount of glycine, 16.0% ± 1.6% of the urinary oxalate and 16.6% ± 3.2% of the urinary glycolate were derived from glycine metabolism. We did not detect the enrichment of either glycolate or oxalate with a single labeled carbon, indicating that the metabolism did not involve the splitting of the carbon-carbon bond. Whole-body flux values were calculated for each of the glycine infusion levels (Table 2) and were similar to those previously reported [23-27]. Our results suggest that glycine metabolism increased at the higher infusion rate when plasma glycine levels were increased, as the flux was 72% higher ($P = .02$).

3.3. *Infusion with [1-$^{13}C_1$] phenylalanine at 6 μmol/(kg h)*

Infusion of [1-$^{13}C_1$] phenylalanine did not significantly alter plasma phenylalanine levels that were 47.0 ± 15.4 μmol/L preinfusion and 52.0 ± 16.1 μmol/L postinfusion ($P = .71$). The infusion resulted in an E_p of 13.0% ± 0.5% (Table 1C). In keeping with previous studies [28], metabolism of phenylalanine to tyrosine produced a tyrosine to phenylalanine enrichment ratio of 13.7 ± 1.8. The calculated whole-body flux of phenylalanine was similar to that previously reported [29] (Table 2). No enrichment of urinary oxalate or glycolate was observed. Assuming a similar LOD by IC/MS for [1-^{13}C] oxalate enrichment, as determined for [1,2-$^{13}C_2$] oxalate enrichment, these data suggest that phenylalanine metabolism contributes less than 0.7% of the oxalate excreted in urine.

3.4. *Metabolism in HepG2 cells*

The synthesis of [^{13}C] glycolate and [^{13}C] oxalate following incubations of HepG2 cultured cells with [1,2-$^{13}C_2$] glycine and [1-$^{13}C_1$] phenylalanine is shown in Fig. 2. Incubation of HepG2 cells with either 10 mmol/L [1,2-$^{13}C_2$] glycine or 20 mmol/L [1-$^{13}C_1$] phenylalanine doubled media oxalate levels compared with cells not incubated with any isotope. There was a pronounced, concentration-dependent synthesis of [1,2-$^{13}C_2$] glycolate after incubation with [1,2-$^{13}C_2$] glycine; but none was detected at the highest tested phenylalanine concentration (20 mmol/L). These results show that these cells contain metabolic pathways that can metabolize glycine and phenylalanine to oxalate, but are only active when the concentrations of these amino acids are high.

4. Discussion

The oxidation of glyoxylate is the terminal step in endogenous oxalate synthesis in humans. Several sources of glyoxylate have been proposed, including sugars and carbohydrates [1]. We have previously estimated that the metabolism of hydroxyproline, which results in glyoxylate formation,

Table 1B – Urinary and plasma parameters during an infusion of high [1,2-$^{13}C_2$] glycine

Time (h)	E_p [1,2-$^{13}C_2$] glycine (%)	Urine oxalate (mg)	E_u [1,2-$^{13}C_2$] oxalate (%)	Urine glycolate (mg)	E_u [1,2-$^{13}C_2$] glycolate (%)	Urine hippurate (mg)	Estimated E_u [1,2-$^{13}C_2$] hippurate (%)
Preinfusion		0.80 ± 0.30		1.00 ± 0.40		16.4 ± 11.4	
3-4	22.8 ± 3.2	1.02 ± 0.30	1.43 ± 0.31	1.22 ± 0.18	1.53 ± 0.20	13.9 ± 8.0	14.2 ± 1.8

Data expressed as mean ± SD of samples from 6 individuals. Urinary measures pre- and postinfusion were not significantly different ($P > .05$). Plasma samples for enrichment analyses were obtained 4 hours postinfusion.

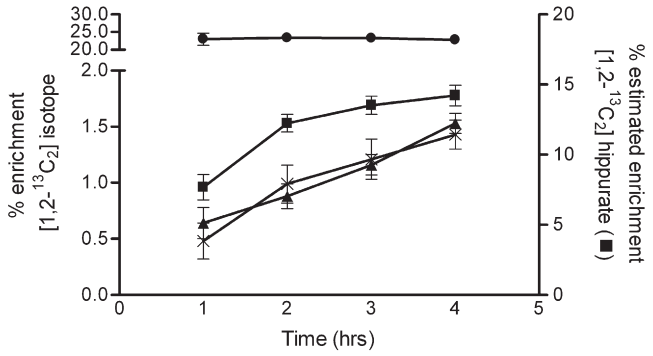

Fig. 1 – Plasma enrichment with [1,2-$^{13}C_2$] glycine (●) and urine enrichment with [1,2-$^{13}C_2$] oxalate (X), [1,2-$^{13}C_2$] glycolate (▲), and [1,2-$^{13}C_2$] hippurate (■) following a primed, constant infusion of 60 μmol of [1,2-$^{13}C_2$] glycine per kilogram per hour. The results are the mean ± SEM (n = 6).

Table 1C – Urinary and plasma parameters during an infusion of [1-$^{13}C_1$] phenylalanine

Time (h)	Urine oxalate (mg)	E_p [1-^{13}C] Phe (%)	E_p [1-^{13}C] Tyr (%)
Preinfusion	1.0 ± 0.39		
3-4	0.91 ± 0.59	12.7 ± 2.1	2.04 ± 0.46

Data expressed as mean ± SD of samples from 5 individuals. Urinary measures pre- and postinfusion were not significantly different ($P > .05$). Plasma samples for enrichment analyses were obtained 4 hours postinfusion.

contributes 5% to 20% of the endogenously produced oxalate excreted in urine [25]. In this investigation, we sought to determine whether glycine and phenylalanine are potential sources of endogenously produced oxalate.

Our results demonstrated that when tracer levels of glycine were infused, less than 5% of the urinary oxalate was derived from glycine metabolism. Conversion of glycine to oxalate increased when glycine infusion was increased 10-fold and reached a mean of 16% after 4 hours. This much higher rate of infusion increased the plasma concentration of glycine by a mean of 29% and increased the whole-body flux of glycine by 72%. This increase in plasma glycine is similar to the increase observed 2 hours after the ingestion of a protein-rich meal (300 g of roast beef) [30]. This contribution differs from that obtained in earlier studies using oral dosing with ^{13}C-glycine or ^{14}C-glycine. Elder and Wyngaarden [9] estimated that glycine metabolism accounted for the majority of the oxalate excreted in urine based on studies in 6 individuals following a single ingested dose of $^{14}C_1$-glycine. These investigators also determined that urinary oxalate was labeled considerably in excess of hippurate. This labeling of hippurate is in contrast to what we have observed with primed, constant infusions, where labeling of hippurate was 10 times greater than that of oxalate (Fig. 1 and Table 1B). Previous studies of individuals receiving oral doses of the stable isotope $^{13}C_1$-glycine every 6 hours for 4 days indicated that glycine metabolism accounted for 18% to 40% of the urinary oxalate excreted [5,7]. The inability of oral dosing to equilibrate endogenous glycine pools may have in part contributed to the high estimates of glycine oxidation to oxalate in these earlier studies in contrast to the results obtained in the current studies. There were also technical issues in these earlier studies. Urinary oxalate assays were not specific, relying on the isolation of calcium oxalate crystals precipitated from urine; and they may have promoted the conversion of glycine-derived products to oxalate. Glycine-derived products may also have contaminated crystals that were isolated.

The increased whole-body flux of glycine with the infusion of the higher glycine level suggests that there was an increased flux of glycine through several pathways associated with its metabolism. The metabolism of glycine by DAO is apparently one such pathway affected. D-Amino acid oxidase is expressed predominantly in kidney, liver, and brain tissue, with the highest activity in the kidney proximal tubule. Glycine is not a good substrate for this enzyme, as the K_m for glycine is high (60-180 mmol/L) and its activity is optimal at a pH of 10.3 [11,31]. This is consistent with our previous studies using isolated hepatic peroxisomes where only high, nonphysiological concentrations of glycine resulted in glyoxylate and oxalate synthesis [32]. The results with HepG2 cells in this study also suggest that high concentrations of extracellular glycine are required for it to be metabolized to glycolate and oxalate in liver cells. The formation of labeled glycolate suggests that some of the glyoxylate produced by the oxidation of glycine by DAO, which is localized in peroxisomes, moves into the cytoplasm where it is converted to glycolate by glyoxylate reductase and oxalate by lactate dehydrogenase. The amount of oxalate and glycolate produced from glycine metabolism is most likely influenced by the relative amounts oxidized in the liver and kidney. Hepatic peroxisomes contain alanine:glyoxylate transaminase that would result in the transamination

Table 2 – Whole-body fluxes (Q) and percentage contribution (%C) of the metabolism of amino acids to urinary glycolate and urinary oxalate 4 hours after initiating the infusion

Amino acid	Infusion rate (μmol/[kg h])	Amino acid E_p (%)	Q (μmol/[kg h])	%C glycolate	%C oxalate
[1,2-$^{13}C_2$] glycine	6.0	4.35 ± 0.63	341 ± 52	<18	<5.0
[1,2-$^{13}C_2$] glycine	60.0	23.4 ± 3.3	586 ± 95	16.6 ± 3.2	16.0 ± 1.6
[1-$^{13}C_1$] Phenylalanine	6.0	13.0 ± 0.50	39.6 ± 1.70	<2.5	<0.7

The percentage contribution for glycine infusions was corrected for intracellular dilution of plasma [1,2-$^{13}C_2$] glycine, as described in the "Methods."

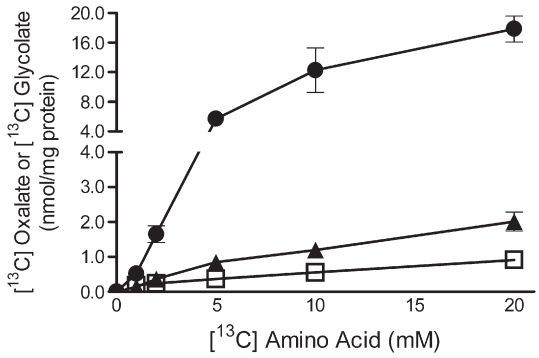

Fig. 2 – Metabolism of [1,2-$^{13}C_2$] glycine and [1-$^{13}C_1$] phenylalanine by HepG2 cells. The levels of [1,2-$^{13}C_2$] glycolate (•) and [1,2-$^{13}C_2$] oxalate (▲) produced after 48 hours of incubation with varying concentrations of [1,2-$^{13}C_2$] glycine, and [1-^{13}C] oxalate (□) after incubation with [1-$^{13}C_1$] phenylalanine are shown. No [1-$^{13}C_1$] glycolate was detected in cell culture medium after incubation with [1-$^{13}C_1$] phenylalanine. Data are expressed as mean ± SD from 3 replicates per [^{13}C] amino acid concentration tested.

of some of the glyoxylate produced in this tissue to glycine. As peroxisomes in proximal tubules lack alanine:glyoxylate transaminase and are enriched in DAO activity, the bulk of the oxalate synthesized with high glycine infusions may occur in the kidney.

We also investigated whether phenylalanine contributed to endogenous oxalate synthesis. With infusion of [1-$^{13}C_1$] phenylalanine, the conversion of phenylalanine to oxalate could not be detected, suggesting that it is a negligible source of endogenous oxalate production. The proposed pathway by which oxalate is formed from phenylalanine metabolism involves the nonenzymatic breakdown of phenylpyruvate, a minor metabolite of phenylalanine metabolism [14,15]. Phenyllactate is the normal end product of phenylpyruvate metabolism and is excreted in urine [33]. Measurements of [1-$^{13}C_1$] phenyllactate and [1-$^{13}C_1$] phenylpyruvate by IC/MS did not show significant increases over preinfusion urines, suggesting an insignificant flux of phenylalanine through this pathway (data not shown). As the plasma concentration of phenylalanine is approximately one fifth of that of glycine, it is unlikely that infusing increased amounts of this amino acid would produce a conversion to oxalate that was significant under normal conditions. There is one report that mentally retarded individuals with phenylketonuria and a presumed elevated plasma phenylalanine excreted urinary oxalate at levels twice normal [34]. This case report suggests that the flux of phenylalanine to oxalate may only occur when plasma phenylalanine is highly elevated (millimoles per liter levels). This is in keeping with our HepG2 cell culture experiments where a small flux of phenylalanine to oxalate was detected at very high concentrations of [1-$^{13}C_1$] phenylalanine. Furthermore, in cell culture experiments with [1-$^{13}C_1$] phenylalanine, no [1-^{13}C] glycolate was detected, supporting the hypothesis that synthesis of oxalate from phenylalanine occurs through the breakdown of phenylpyruvate and not glyoxylate.

The labeling of the metabolites, hippurate, glycolate, and oxalate in urine shows that they did not reach equilibrium in the 4 hours of infusion in contrast to the rapid equilibration reached with plasma glycine. Some of the delay in urine equilibration may be attributed to required equilibration in the kidney. Other metabolites or synthetic products of infused glycine, including plasma serine and apolipoprotein B, similarly did not reach equilibrium in 8-hour infusions [23,24]. This slow equilibration has been interpreted as resulting from the time taken for various intracellular glycine pools, such as in mitochondria, to reach a constant enrichment. With oxalate and glycolate synthesis, the glycine pool in peroxisomes, where DAO is localized, may take time to completely equilibrate. This slow equilibration becomes a limitation of this study. Fig. 2 shows that [1,2-$^{13}C_2$] oxalate and [1,2-$^{13}C_2$] glycolate synthesis continues to increase over the 4-hour infusion period, and it would not be surprising if it at least doubled with an extended infusion. Thus, our estimates of the contribution of the high dose of glycine to urinary oxalate synthesis may be underestimated. A longer infusion protocol would be required to establish the exact contribution glycine metabolism makes to its synthesis. Based on [1,2-$^{13}C_2$] glycine infusion studies examining the enrichment of urinary hippurate with [1,2-^{13}C] hippurate, an infusion of more than 8 hours may be needed before equilibration is reached [23].

In summary, the results of this study suggest that glycine and phenylalanine are not significant contributors to the endogenous generation of oxalate in healthy humans when fasted. However, it remains possible that this contribution becomes significant transiently following the ingestion of glycine-rich meals. Given the potential pathologic consequences of oxalate accumulation in the body, a continued search for the major sources of endogenously synthesized oxalate is warranted.

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