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^{13}C enrichment of carbons 2 and 8 of purine by folate-dependent reactions after [^{13}C]formate and [2-^{13}C]glycine dosing in adult humans

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Abstract

The 10-formyl moiety of 10-formyltetrahydrofolate is the source of carbons at the positions 8 (C_8) and 2 (C_2) of the purine ring, originating from formate and a few amino acids. Uric acid is the final catabolic product of purines. In adult humans, we independently measured the ^{13}C enrichment of the C_2 and C_8 positions of urinary uric acid after an oral dose of [^{13}C]sodium formate and that of the C_2 and C_8 plus C_5 positions after [2-^{13}C]glycine. A liquid chromatography-mass spectrometric method was used to measure the ^{13}C enrichment of uric acid in urine, which was collected for 3 to 4 days. Purine catabolism to uric acid does not alter the positions of carbons in the ring. After the formate dose, the ^{13}C enrichment at C_2 was greater than at C_8, and a circadian rhythm was observed in the enrichment at C_2. After the glycine dose, the C_8 plus C_5 positions were enriched, whereas no significant enrichment at C_2 was found. These ^{13}C enrichment patterns are not consistent with previous accepted metabolism. To our knowledge, this is the first study to investigate ^{13}C enrichment from formate and glycine independently into the C_2 and C_8 positions of purine in the same subjects. Possible mechanisms explaining our findings are discussed. Oral [^{13}C]formate or [2-^{13}C]glycine dosing and urine collection can be used to study purine biosynthesis in humans.

1. Introduction

Purine de novo nucleotide biosynthesis is a fundamental process producing building blocks of DNA and RNA. Glycinamide ribotide (GAR) and aminoimidazolecarboxamide ribotide (AICAR) transformylases utilize folate coenzymes to introduce carbons 8 (C_8 in red, Fig. 1) and 2 (C_2 in blue, Fig. 1) into the purine ring, respectively [1].

Formate is one source of the formyl carbon (green, Fig. 1) of 10-formyltetrahydrofolate (10-HCO-H_4folate), which is a substrate for these 2 enzymes. 10-HCO-H_4folate synthetase forms this substrate from tetrahydrofolate (H_4folate), formate, and adenosine triphosphate (ATP) [2]. Under normal conditions, human plasma formate concentrations range 20 to 250 μmol/L, which is about 50% of those in erythrocytes, and formate is derived from many sources [3,4]. In animals, ^{14}C-labeled formate given in vivo is predominantly found at C_2 and C_8 of purines, whereas only a small amount appears in the other positions [5-10], and the $^{14}C_2/^{14}C_8$ ratio was reported to be about 1.0 [6-8]. However, this ratio in humans has never been documented.

The second carbon of glycine (green, Fig. 1) is also potentially incorporated into C_2 and C_8 of purines by folate-dependent reactions and into C_5 by folate-independent metabolism. Glycine in the presence of H_4folate is metabolized to 5,10-methylenetetrahydrofolate (5,10-CH_2-H_4folate), CO_2, and NH_3 by the glycine cleavage system (GCS) [11]. The second carbon of glycine (green, Fig. 1), now the methylene carbon of 5,10-CH_2-H_4folate, can be converted to 10-HCO-H_4folate by 5,10-CH_2-H_4folate dehydrogenase and 5,10-methenyltetrahydrofolate (5,10-CH-H_4folate) cyclohydrolase [2].

Uric acid is the final catabolite of purines in humans, and the catabolic process does not alter the carbon positions of purines [1]. In mammals, C_2 and C_8 of purines are not more labile to simple isotope exchange in vivo than the other carbons of purines [12,13]. The peak in labeled urinary uric acid occurs 1 to 3 days after a dose of labeled formate or glycine in humans [14-19], representing catabolism of newly synthesized purines [14]. We measured the ^{13}C enrichment independently at C_2 and C_8 of uric acid by

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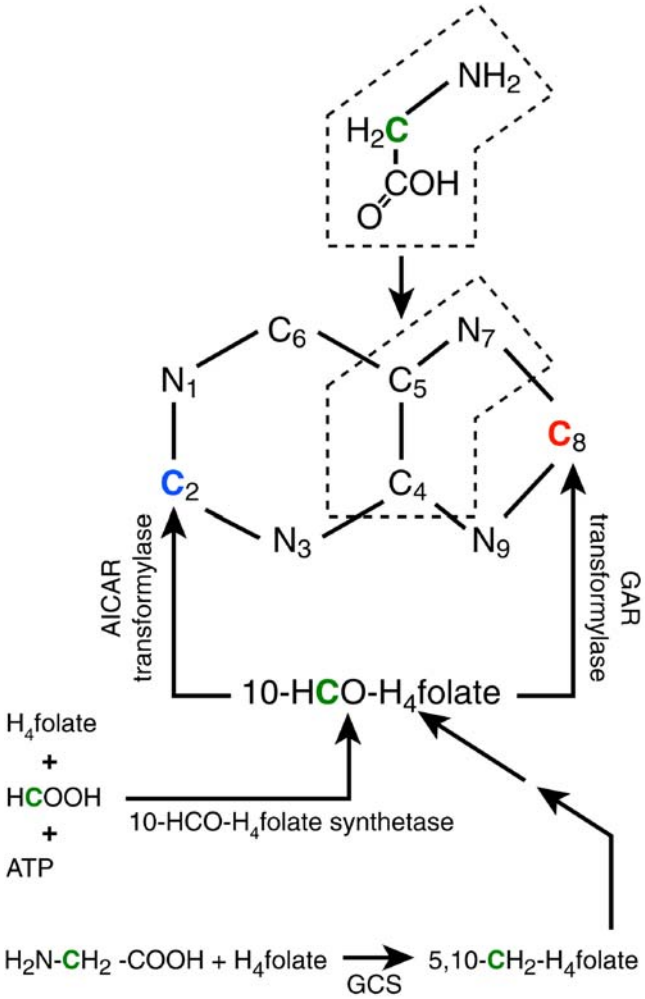

Fig. 1. The origin of C_2, C_4 C_5, C_8, and N_7 atoms of the purine ring from formate and glycine. The carbon of formate may be incorporated into C_2 and C_8 via the 10-HCO-H_4folate synthetase, and GAR and AICAR transformylases. The first and second carbon and nitrogen of glycine are incorporated directly to C_4, C_5, and N_7, respectively (in broken lines). The second carbon of glycine may be incorporated into C_2 and C_8 via GCS and GAR and AICAR transformylases. All carbons potentially incorporated into C_2 and C_8 are in green, and C_2 and C_8 are in blue and red, respectively, which are matched with those of bars in Fig. 2.

expanding the in vivo formate pool with an oral [^{13}C]formate dose and by using a liquid chromatography-mass spectrometry (LC/MS/MS) method [20]. We also measured the ^{13}C enrichment at C_2 and C_8 plus C_5 after expanding the glycine pool with [2-^{13}C]glycine.

2. Methods

2.1. Human study

The study was approved by the Institutional Review Board for Human Use at the University of Alabama at Birmingham. Three healthy adult men collected urine at each void for 72 to 96 hours after an oral dose of 14.5 mmol [^{13}C]sodium formate (1.0 g, ^{13}C 99%, Cambridge Isotope Laboratories, Andover, MA) with 100 mL of water at about noon. The volume of each void was measured and urine samples were stored at −80°C until analysis. In addition, as baseline experiments, similar urine collections for 72 hours were performed without a [^{13}C]sodium formate dose (once in subject B and twice in subjects A and C). With a minimum of a 12-month interval, the identical procedure was repeated in 2 subjects using 41.7 mmol of [2-^{13}C]glycine (2.5 g, ^{13}C 99%, Cambridge Isotope Laboratories). Subjects were asked to maintain a regular lifestyle including their diet and physical activity during the study.

2.2. Measurement of ^{13}C enrichment

Independent ^{13}C enrichment from [^{13}C]formate into the C_2 and C_8 positions of uric acid was measured by the LC/MS/MS method [20] followed by the calculation as described below. The area under the curve (AUC) of the extracted ion chromatograms (XICs) for m/z 168 → 124 and 168 → 125 and 169 → 125 represents the amount of ^{13}C at C_2, C_8, and both C_2 and C_8, respectively. The AUC of XIC for m/z 167 → 124 represents uric acid containing only ^{12}C, ^{1}H, ^{14}N, and ^{16}O. The percentage of ^{13}C at C_2 (% $^{13}C_2$) and C_8 (% $^{13}C_8$) in total uric acid was estimated by using the following formulae: % $^{13}C_2$ = (AUC for XIC m/z 168 → 124) ÷ (AUC for XIC m/z 167 → 124 + AUC for XIC m/z 169 → 125) × 100 and % $^{13}C_8$ = (AUC for XIC m/z 168 → 125) ÷ (AUC for XIC m/z 167 → 124 + AUC for XIC m/z 169 → 125) × 100.

Similar calculations were made to estimate the % ^{13}C after a [2-^{13}C]glycine dose. Although the LS/MS/MS method allows one to measure % ^{13}C at C_2 cleanly, % ^{13}C at C_8 also includes that at C_5 [20]. The amount of ^{13}C at both C_2 and C_8 is included in the denominator because it is unlikely that both positions would be simultaneously enriched by a [^{13}C]formate or [2-^{13}C]glycine dose. The ^{13}C enrichment at C_2 and C_8 (plus C_5) following [^{13}C]formate or [2-^{13}C]glycine dosing was calculated for each void by subtracting baseline % $^{13}C_2$ and % $^{13}C_8$ values that were paired for the subject and time of day when void was collected from the values obtained after the dose. The values of % $^{13}C_2$ and % $^{13}C_8$ from baseline experiments were subtracted from each other in 2 subjects (A and C) to measure the variability of baseline values. It is important to note that the measurements of baseline values are essential for such a study, as lifestyle or dietary habit can affect the ^{13}C enrichment of human samples [21,22].

2.3. Statistical analysis

The Wilcoxon paired-sample test was used to detect a significant difference from 0 in mean ^{13}C enrichment from [^{13}C]formate for each day, where more than 8 voids were obtained. For subject C with fewer than 8 voids per day, the data from 3 days were combined [23]. The same test was used to detect the ^{13}C enrichment from [^{13}C]glycine where data of 3 days were combined. The runs above and below the median test ("runs test") was performed to detect rhythmicity in subjects with greater than 25 voids. Significantly fewer than

expected runs above and below the median indicate a nonrandom temporal distribution of the data, suggesting a rhythmic pattern. This runs test avoids having to force-fit the data to a cosine function. The details of the principle of runs test are presented by Sokal and Rohlf [23].

3. Results

3.1. [^{13}C]Formate dose

The % ^{13}C enrichment after a [^{13}C]formate dose did not significantly correlate with the amount of uric acid excreted in each void, uric acid concentration, or urine volume. The ^{13}C enrichment at C_2 (blue columns) and C_8 (red columns) from subjects A and B is shown in Fig. 2. The peak % ^{13}C enrichment from [^{13}C]formate at C_2 was 0.74% to 5.7% and that at C_8 was 0.08% to 0.24%, and mean % ^{13}C enrichment at C_2 was significantly greater than 0 in all 3 subjects (Table 1, Fig. 2). Mean % ^{13}C enrichment at C_8 was significant greater than 0 in subjects B and C. Lower mean % ^{13}C enrichments at C_8 were generally found compared with C_2. Thus, contrary to our expectation, the ^{13}C enrichment ratio of C_2/C_8 was far from 1.0, and median C_2/C_8 ratios were 6.6, 6.5, and 3.0 for subjects A and B (obtained from the data presented in Fig. 2) and C (data not presented), respectively. Only positive ratios were used to determine the median. We observed that only 11 of 66 voids, where the C_2/C_8 ratios were positive, fell in the range 0.5 to 2.0, which could be generously considered close to 1.0.

To verify that our methods did not yield spurious positive enrichments, 2 paired-baseline values were subtracted from each other in subjects A and C (Table 1). In theory, this subtraction should have yielded 0% enrichment for all voids; however, this subtraction yielded nonsignificant

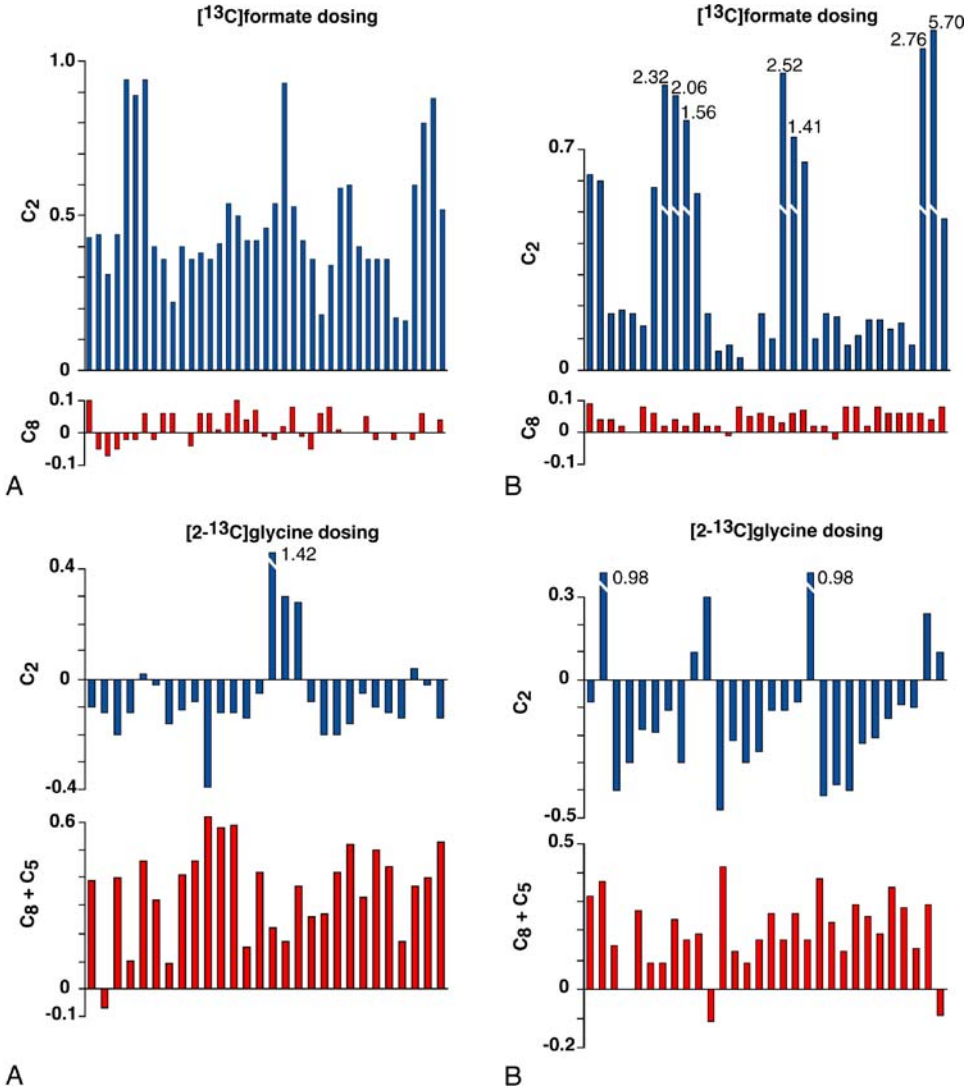

Fig. 2. The % ^{13}C enrichment at C_2 and C_8 or C_8 plus C_5 of uric acid in each void after [^{13}C]formate or [2-^{13}C]glycine dosing. Bars represent ^{13}C enrichment at C_2 (blue) and C_8 or C_8 plus C_5 (red) in voids collected for 72 to 96 hours after [^{13}C] formate or [2-^{13}C]glycine dosing in subjects A and B. The x-axis is time after dosing; however, the intervals between voids were different. Therefore, we did not show specify time of the void. The numbers next to the bars are percent enrichments that were out of range on the y-axis.

Table 1
Mean (±SD) % ^{13}C enrichment at the C_2 and C_8 positions of uric acid after [^{13}C]formate dose and at the C_2 and C_8 plus C_5 positions of uric acid after a [2-^{13}C]glycine dose

Subject	Dose (mmol)	% Enrichment at C_2 (no. of voids analyzed)			% Enrichment at C_8 (no. of voids analyzed)		
		Day 1	Day 2	Day 3	Day 1	Day 2	Day 3
[^{13}C]Formate							
A	14.5	0.58 ± 0.25[a] (9)	0.42 ± 0.09[a] (12)	0.47 ± 0.19[a] (10)	0.00 ± 0.06 (9)	0.03 ± 0.04 (12)	0.02 ± 0.04 (10)
B	14.5	0.84 ± 0.78[a] (10)	0.51 ± 0.78[a] (10)	0.40 ± 0.73[a] (12)	0.04 ± 0.03[a] (10)	0.04 ± 0.03[a] (10)	0.05 ± 0.03[a] (12)
A	0	−0.06 ± 0.05 (8)	0.02 ± 0.04 (9)	0.03 ± 0.06 (9)	−0.01 ± 0.09 (8)	0.04 ± 0.08 (9)	0.04 ± 0.08 (9)
C	14.5		0.43 ± 0.18[a,b] (16)			0.16 ± 0.04[a,b] (16)	
C	0		0.03 ± 0.16[b] (16)			0.01 ± 0.09[b] (16)	
[2-^{13}C]Glycine							
A	41.7		−0.03 ± 0.31[b] (28)			0.39 ± 0.17[a,b] (28)	
B	41.7		−0.06 ± 0.35[b] (28)			0.19 ± 0.13[a,b] (28)	

[a] Significantly greater than 0 by the Wilcoxon paired-sample test ($P < .05$).
[b] Data from these 3 rows were combined.

mean percent enrichments or mean percent enrichments below 0 at C_2 and C_8 because of unavoidable errors in the LC/MS/MS method and calculations [20]. The % ^{13}C enrichments at C_8 in subject A was similar to the values that were found when 2 paired baseline values were subtracted from each other, indicating no enrichment at C_8.

To test rhythmicity (circadian rhythm), the run test was applied to the data of subjects A and B [23]. Using data in Fig. 2, % ^{13}C enrichment at C_2 had much fewer runs (ie, 8) than the predicted number of 25 and 23 for these subjects, indicating rhythmicity in the data ($P < .01$). In contrast, % ^{13}C enrichment at C_8 had 19 and 17 runs for these 2 subjects, which is consistent with a random pattern, indicating no rhythmicity ($P > .05$) [23]. Subject C did not have a sufficient number of voids to perform the test for rhythmicity.

3.2. [2-^{13}C]glycine dose

There was low ^{13}C enrichment at C_2 after the [2-^{13}C]glycine dose (Table 1, Fig. 2). Of 28 voids collected for each subject, more than 80% was 0 or negative, indicating that the ratio of C_2/C_8 (plus C_5) after a [2-^{13}C]glycine dose was low. In both subjects, significant ^{13}C enrichments at C_2 after [2-^{13}C]glycine were detected in only several voids that correspond to the timing of high C_2 enrichments from [^{13}C]formate (Fig. 2). [2-^{13}C]Glycine enriched the C_8 plus C_5 (red column) positions, and peak % ^{13}C enrichments at C_8 plus C_5 were 0.62% and 0.42% in subjects A and B, respectively (Fig. 2, Table 1). Unlike the formate dose, no rhythmicity was found in the ^{13}C enrichment at C_8 plus C_5.

4. Discussion

We found that [^{13}C]formate predominantly enriched the C_2 position of purines, whereas significantly greater than zero ^{13}C enrichment was only found at the combination of the C_8 and C_5 positions after a [2-^{13}C]glycine dose (Table 1, Fig. 2). We shall describe our hypotheses to explain these findings below; however, we realize that these hypotheses (see Fig. 3) may require reevaluation or modification in the future, or they may be incorrect.

4.1. Mechanistic explanation of the enrichment at the C_2 position by [^{13}C]formate

Our finding differs from the previously reported C_2/C_8 ratio of about 1.0 in animals [6-8]. We postulate that the liver stops purine biosynthesis de novo at AICAR because isolated mammalian hepatocytes do not metabolize AICAR to inosine monophosphate (IMP) or to any other purines [24-28]. This is consistent with the low capacity of the mammalian hepatocytes or liver slices to synthesize purines in vitro from radioactive formate or serine [29-31]. The liver cannot enrich C_2 from [^{13}C]formate because AICAR cannot be metabolized to IMP (Fig. 3). Therefore, to explain our data, we tried to identify cells that (*a*) predominantly utilize formate as a source of one-carbons, (*b*) have AICAR transformylase and 10-HCO-H_4folate synthetase, and (*c*) have an external supply of AICAR. We identified erythrocytes as being a prime candidate that fulfills all these requirements because erythrocytes (*a*) contain double the amount of formate compared to plasma [3], (*b*) possess the above 2 key enzymes but neither GAR transformylase, GCS, nor serine hydroxymethyltransferase (SHMT) [11,32-34], and (*c*) are exposed to AICA riboside or its base in the circulation, because AICA is a normal constituent of human urine [35]. In fact, AICAR accumulates in erythrocytes in Lesch-Nyhan syndrome, some forms of gout, and a genetic defect of AICAR transformylase [36,37]. Therefore, we hypothesized that the formation of IMP from AICAR with formate and H_4folate occurs in erythrocytes. The idea of human erythrocytes participating in purine biosynthesis in such a way is not novel. Bertino et al [32] suggested this more than 40 years ago based on the findings of abundant activities of 10-HCO-H_4folate synthetase and AICAR transformylase. In fact, Lowy et al [33] and Wagner and Levitch [34] reported that the in vitro incubation of intact erythrocytes with [^{14}C]formate and AICA riboside lead to the formation of IMP, and that this metabolism required H_4folate, ATP, and the formation of AICAR from AICA riboside. The mass of human erythrocytes, almost equal to that of the liver, should not be underestimated in its ability to metabolize purines [38]. In human erythrocytes, however,

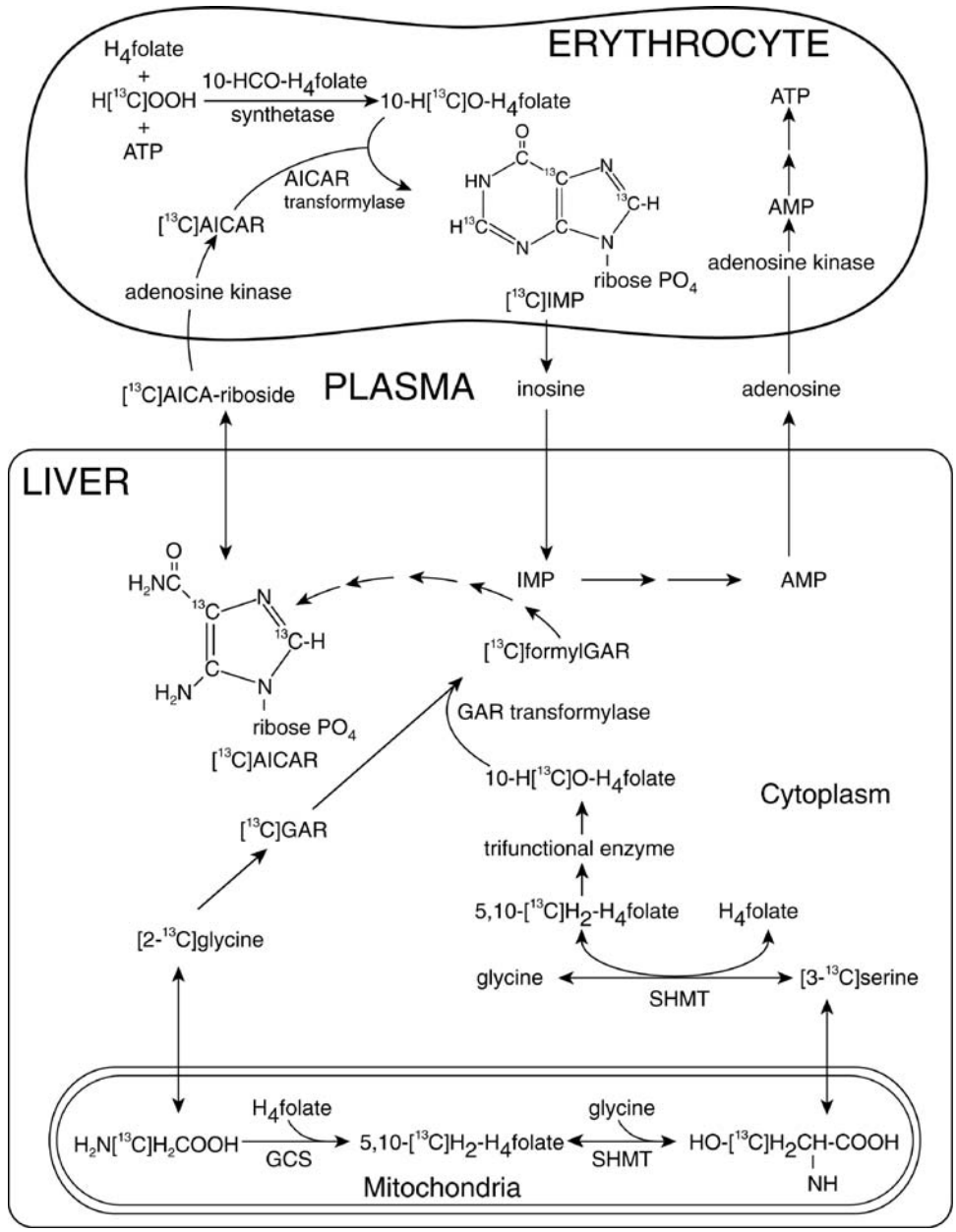

Fig. 3. Proposed metabolic pathways explaining ^{13}C enrichment of purine by [^{13}C]formate and [2-^{13}C]glycine. Using liver mitochondrial GCS and SHMT, ^{13}C of [2-^{13}C]glycine produces [3-^{13}C]serine, which is transported to cytoplasm. Cytoplasmic SHMT and the trifunctional enzyme produce 5,10-H[^{13}C]O-H_4folate, which is incorporated into the formyl moiety of formyl-GAR by GAR transformylase. [2-^{13}C]Glycine is also incorporated into GAR as glycine. Formyl-GAR is converted to AICAR, which is exported to plasma as its riboside and taken up by erythrocytes. This is converted back to AICAR by adenosine kinase in erythrocytes. Erythrocytes utilize [^{13}C]formate and H_4folate by means of 10-HCO-H_4folate synthetase and AICAR transformylase to synthesize IMP. Thus, C_2 of purine is labeled by [^{13}C]formate and C_8 plus C_5 are labeled by [2-^{13}C]glycine (see Fig. 1). The liver converts inosine that is exported from erythrocytes to AMP. This AMP is then returned to erythrocytes as shown in order to maintain ATP concentrations. The coordinated IMP and AMP metabolism in the liver and erythrocytes has been previously established [38,39].

the lack of GAR transformylase and most of the other enzymes participating in purine biosynthesis precludes the ^{13}C enrichment at C_8 [33].

Using Fig. 3, let us explain our thoughts on how erythrocytes and the liver metabolize and shuttle purines. First, where does AICAR in erythrocytes come from? We propose that the liver synthesizes AICAR de novo and exports into the circulation as AICA riboside, which is then phosphorylated to AICAR by erythrocyte adenosine kinase. It has been shown that isolated rat hepatocytes cycle AICAR to AICA riboside and the latter can be released from these cells to the medium [28]. Second, what is the fate of erythrocyte IMP, a minor component of its purine pool? Other researchers have established that IMP cannot be converted to adenosine monophosphate (AMP) in erythrocytes [33,39]; therefore, it must be

exported to the circulation as inosine or hypoxanthine [40]. It has been shown that human erythrocytes loaded with [8-^{4}C]IMP ex vivo rapidly release radioactivity in vivo ($t_{1/2}$ = 1 hour) when reinjected back to their donor [40]. Presumably, IMP is released as inosine or hypoxanthine, which is present in blood at 0.1 to 0.5 μmol/L [41]. It is known that AMP is again exported from the liver to the circulation as adenosine that is incorporated back to erythrocytes and metabolized back to AMP by adenosine kinase to maintain adequate erythrocyte ATP concentration [38,39] (Fig. 3). Plasma adenosine concentrations range from 0.1 to 0.5 μmol/L [41].

We now present possible reasons why [^{13}C]formate failed to enrich C_8. This may be due to the dilution of 10-H[^{13}C]O-H_4folate by channeling of 10-H[^{12}C]O-H_4folate to hepatic GAR transformylase (Fig. 3). Avian hepatic GAR transformylase forms a complex with the trifunctional enzyme and SHMT [42,43]. Thus, 5,10-CH_2-H_4folate or 5,10-CH-H_4folate, formed from glycine, serine, and histidine, could be channeled to GAR transformylase as 10-H[^{12}C]O-H_4folate resulting in the dilution of the ^{13}C enrichment at C_8. Although this is a possible explanation, the existence of such an enzyme complex, however, has not been proven in human liver. In addition, formate is readily oxidized such that about 25% of a tracer dose of [^{14}C]formate is lost as $^{14}CO_2$ in 2 hours, whereas only 1% is incorporated into uric acid in 11 days in humans [19]. Some of this oxidation likely takes place in hepatic peroxisomes [44]. Thus, formate may be preferentially metabolized to CO_2 rather than participating in purine biosynthesis in human liver.

We observed the circadian rhythm in the ^{13}C enrichment at C_2. Erythrocyte adenosine kinase activity parallels blood inosine and hypoxanthine concentrations with a similar circadian rhythm [41]. Therefore, it is possible that the circadian rhythm in adenosine kinase activity could account for rhythmicity in the ^{13}C enrichment at C_2 (Fig. 3).

4.2. Mechanistic explanation of the enrichment of the C_2, C_8 and C_5 positions by [2-^{13}C]glycine

We postulate that the formation of GAR and formyl-GAR from [2-^{13}C]glycine occurs in the liver [45] and enriches both C_8 and C_5 (Figs. 1 and 3). We are forced to report in this way, because our method does not distinguish independent enrichment at C_8 and C_5 [20]. Based on the findings by Pimstone et al [46], this may not impose problems in interpreting our ^{13}C enrichment data at C_8. They found that 20% of [2-^{14}C]glycine is incorporated into the C_2 and C_8 positions through folate-dependent reactions and the remaining 80% into the C_4 and C_5 positions through the folate-independent pathway [46]. They used a method involving degradation of uric acid that does not distinguish between the ^{14}C incorporation at C_2 and C_8. Although their data cannot be directly compared with ours, we hypothesize that most of this 20% of the ^{13}C enrichment found in our study is at C_8. It is unlikely that our method was not sensitive enough to detect ^{13}C enrichment at the C_2 positions. Our data further agree with those by Heinrich and Wilson [7] who found that there was no labeling at C_2 of guanine in rat carcass after [1,2-^{14}C]glycine administration, whereas C_8 was labeled.

The substantial ^{13}C enrichments at C_2 after [2-^{13}C]glycine in subject B corresponded to the timing of high enrichments at C_2 by [^{13}C]formate (Fig. 2). This finding suggests that some [^{13}C]formate was produced from [2-^{13}C]glycine or 10-H[^{13}C]O-H_4folate and enriched C_2. The [2-^{13}C]glycine dose used by us might have made a small but detectable contribution to the [^{13}C]formate pool. However, considering many metabolic pathways involving glycine and our larger dose of glycine than formate on molar basis, its pathway to formate may be minor. Various substrates contribute to the formate pool without involving folate coenzymes, including methylthioadenosine, tryptophan, choline, acetate, and others [47-49].

Relatively low ^{13}C enrichment at C_2 with [2-^{13}C]glycine supports our interpretation of the [^{13}C]formate data because erythrocytes with the absence of mitochondria have no GCS activity, whereas the liver has high GCS activity [11]. A possible metabolic pathway for [2-^{13}C]glycine includes the following. In hepatic mitochondria, GCS with H_4folate cleaves [2-^{12}C]glycine to 5,10-[^{13}C]H_2-H_4folate, CO_2, and NH_3. Mitochondrial SHMT in the presence of glycine converts 5,10-[^{13}C]H_2-H_4folate to [3-^{13}C]serine, which is then transported to the cytoplasm [2,11], as shown in Fig. 3. Cytoplasmic 5,10-[^{13}C]H_2-H_4folate is formed from H_4folate and [3-^{13}C]serine catalyzed by cytoplasmic SHMT and further metabolized to 10-H[^{13}C]O-H_4folate by the trifunctional enzyme (Fig. 3).

The % C_{13} enrichment at C_8 of 0.19 to 0.39 from [2-^{12}C]glycine is less than that at C_2 of 0.40 to 0.84 from [^{13}C]formate in subjects A and B, although the glycine dose was greater (Table 1). This may be due to channeling of ^{12}C and dilution of ^{13}C by ^{12}C in the GAR transformylase, trifunctional enzyme, and SHMT complex as discussed above [42,43].

Using [2-^{13}C]glycine, we unavoidably tested the ^{13}C enrichment as if we used [3-^{13}C]serine. As we discussed previously, [2-^{12}C]glycine and [2-^{13}C]glycine can form [3-^{13}C]serine in the presence of GCS and SHMT in hepatic mitochondria [11], and [3-^{13}C]serine can be transported to the cytoplasm (Fig. 3) [2].

4.3. Conclusion

The ^{13}C enrichment at C_2 of uric acid was greater than at C_8 after a [^{13}C]formate dose, and a circadian rhythm was seen in the ^{13}C enrichment at C_2 in humans. After a [2-^{13}C]glycine dose, however, no significant ^{13}C enrichment at C_2 was found. To our knowledge, this is the first study to measure ^{13}C enrichment from ^{13}C-labeled formate and glycine independently into the C_2 and C_8 positions of purine in the same subjects. Although the number of subjects was small, the specificity and consistency of our data are compelling. However, it is necessary to stress that this research is in its

infancy, and further investigations are required to confirm our findings and to prove our hypotheses.

Contrary to popular belief, our data suggest that ^{13}C from these sources behave differently, and the incorporation of ^{13}C of formate and the second ^{13}C of glycine into purines may require coordination, which could be made not only on the molecular level but also on the organ level (erythrocytes and the liver). We take it for granted that purine metabolism presented in textbooks obtained mostly by using uricotelic animals and microorganisms can be extrapolated to humans; however, our data suggest how fragile and precarious such an assumption is. Our noninvasive method of oral [^{13}C]formate or [2-^{13}C]glycine dosing and urine collection, although expensive, could be used to better understand human purine biosynthesis.

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