Measurement of gluconeogenesis and pyruvate recycling in the rat liver: a simple analysis of glucose and glutamate isotopomers during metabolism of [1,2,3-$^{13}C_3$]propionate

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Abstract Simple equations that relate glucose and glutamate ^{13}C-NMR multiplet areas to gluconeogenesis and pyruvate recycling during metabolism of [1,2,3-$^{13}C_3$]propionate are presented. In isolated rat livers, gluconeogenic flux was 1.2 times TCA cycle flux and about 40% of the oxaloacetate pool underwent recycling to pyruvate prior to formation of glucose. The ^{13}C spectra of glucose collected from rats after gastric versus intravenous administration of [1,2,3-$^{13}C_3$]propionate indicated that pyruvate recycling was slightly higher in vivo (49%) while glucose production was unchanged. This indicates that a direct measure of gluconeogenesis and pyruvate recycling may be obtained from a single ^{13}C-NMR spectrum of blood collected after oral administration of enriched propionate.

Key words: ^{13}C isotopomer analysis; Gluconeogenesis; Pyruvate recycling

1. Introduction

There is widespread interest in measurement of flux through gluconeogenesis in animals and humans with the goal of a better understanding of dietary and hormonal regulation of fluxes through all associated pathways. Numerous techniques have been used for such measurements, including GC-mass spectrometry [1–3] NMR [4–6], and radiotracers [7,8]. One key control point that connects these two pathways is the interconversion of PEP and pyruvate, catalyzed in the forward direction by pyruvate kinase and in the reverse direction by the combined action of pyruvate carboxylase and phosphoenolpyruvate carboxykinase. Many early studies of gluconeogenesis detected excess cycling of three carbon units through this metabolic intersection. This process, often referred to as pyruvate recycling or an excess substrate cycle, has been detected in studies of isolated hepatocytes [9,10], perfused livers [11], and in rats and humans [12,13].

Most early metabolic models have relied on measurements of isotope enrichment in two or more sites of various product molecules. In prior metabolic studies of pathways associated with the Krebs citric acid cycle, we have emphasized the detection of ^{13}C–^{13}C spin–spin NMR couplings in product molecules. This approach, which we refer to as ^{13}C isotopomer analysis, can in some cases provide more information about isotope labeling patterns than other tracer methods. Recently, we reported the analysis of propionate metabolism in the isolated heart under conditions where the pathway succinyl-CoA → pyruvate → acetyl-CoA was significant [14]. In contrast to entry of labeled acetyl-CoA and scrambling of this label in cycle reactions, this metabolic condition yields a series of non-linear equations, which in general are difficult to solve analytically. Although the Newton-Raphson procedure is well known [15], the complexity of the equations in the comprehensive model obscures some simple and helpful relations between the ^{13}C-NMR spectrum and metabolic state. One such condition occurs during hepatic metabolism of [1,2,3-$^{13}C_3$]propionate.

In the present study, a simple interpretation of the ^{13}C-NMR spectrum of glucose and glutamate is presented in terms of gluconeogenesis and pyruvate recycling. The method is demonstrated in isolated perfused rat livers and in samples collected from rats after intragastric versus intravenous administration of propionate. The data demonstrate that gluconeogenesis and pyruvate recycling can be measured in a single ^{13}C-NMR spectrum of blood after oral administration of [1,2,3-$^{13}C_3$]propionate.

2. Materials and methods

2.1. Perfused livers

Male Sprague-Dawley rats (175–275 g, Sasco, Houston, TX) were fasted overnight before their livers were isolated and perfused via cannulation of the vena porta as described previously [16]. The livers were flushed in situ for 10 min using non-recirculating Krebs-Henseleit bicarbonate buffer, pH 7.4, at 37°C containing 2 mM NH_4Cl, 15 mM lactate, 1.5 mM pyruvate, 1.5 mM acetate, and 2 mM propionate at a flow rate of 50 ml/min. Oxygenation of the perfusion fluid was maintained by flow over an oxygenating net in a humidified atmosphere of air/CO_2 (95:5, v/v). The perfusion fluid was then switched to a fresh 300 ml portion of the modified Krebs-Henseleit buffer prepared with 2 mM sodium [1,2,3-$^{13}C_3$]propionate and recirculating perfusion was continued for 60 min to achieve steady-state conditions. The tissue was then freeze-clamped, extracted, and freeze-dried. Glutamate was isolated by ion-exchange chromatography as described previously [1], freeze-dried and redissolved in D_2O for NMR analyses. The glucose content in deproteinized perfusion fluid samples and perchloric acid extracts of the freeze-clamped livers was determined using the hexokinase/glucose-6-phosphate dehydrogenase coupled assay [17]. Glucose was quantitatively oxidized to gluconate by dissolving the lyophilized perfusion fluid sample in 10 ml of 1 mM sodium phosphate buffer, pH 5.5 containing 100 U glucose oxidase (Type VII-S, Sigma) and incubating the mixture for 3–12 h at 25°C with gentle

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bubbling (≈10–20 ml/min) of air [18]. The reaction was terminated by adding 5 ml of 8% perchloric acid. Sodium [1,2,3-$^{13}C_3$]propionate and sodium [^{13}C]bicarbonate (99% enriched) were obtained from Cambridge Isotope Labs (Cambridge, MA). Other materials were obtained from Sigma (St. Louis, MO).

2.2. In vivo studies

Male Sprague-Dawley rats weighing 280–350 g were anaesthetized (ketamine/xylazine) and intubated after an overnight fast. An esophagal tube was inserted for gastric feeding, and a catheter inserted into the jugular vein. An aqueous solution of [1,2,3-$^{13}C_3$]propionate was administered either into the stomach (at 5 min intervals for 15 min) or via the jugular vein (4 ml/h for 30 min) at 400 mg propionate/kg body weight. When propionate was provided orally, saline was infused i.v., and vice versa. After 30 min, the liver was freeze-clamped and a sample of blood taken. These were extracted with perchloric acid, adjusted to pH 7.4 with KOH, freeze-dried, and dissolved in 600 μl of D_2O for analysis by ^{13}C-NMR.

2.3. NMR spectroscopy

Proton decoupled ^{13}C-NMR spectra were obtained using a 5 mm probe on a 9.4 T General Electric Omega spectrometer operating at 100.61 MHz. Free-induction decays were digitized into 32 K points and were routinely multiplied by a 1 Hz exponential prior to Fourier transform. ^{13}C-NMR spectra were analyzed with the NUTS curve-fitting program (Acorn NMR Inc., Fremont, CA). Individual multiplet areas of C1, C2 and C3 carbons of glutamate and gluconate (C1D, C2D12, C2D23, C2Q and C3D) are defined as a fraction of total resonance area, as described [18,19]. ^{1}H-NMR spectra were obtained with and without ^{13}C single-frequency decoupling of glutamate C2 at 55.2 ppm.

2.4. Mathematical model

The metabolic pathways shown in Fig. 1 were modeled using input–output methods as described elsewhere [20] with the following assumptions: (1) metabolic and isotopic steady-state, (2) acetyl-CoA was derived from unenriched sources, (3) OAA was fully randomized in the symmetric four carbon pools, and (4) $^{13}CO_2$ did not contribute significantly to the bicarbonate pool involved in pyruvate carboxylation. Each flux variable was defined relative to flux through citrate synthase: y_{pc}, flux through pyruvate carboxylation; y_s, flux into succinyl-CoA via propionyl-CoA carboxylase or an equivalent pathway; y, total anaplerosis $= y_{pc}+y_s$; PK, flux from phosphoenolpyruvate to pyruvate; and g, glucose production $= (y-\text{PK})/2$ (division by 2 is necessary to convert the rate of PEP production to the rate of glucose production). This analysis, like other isotopic studies of pyruvate recycling and gluconeogenesis, does not distinguish flux through the malic enzyme (ME) from the combined action of phosphoenolpyruvate carboxykinase (PEPCK) and pyruvate kinase (PK). Therefore in the equations presented below, PK+ME indicates the combined flux through the malic enzyme and pyruvate kinase. Although a complete ^{13}C isotopomer analysis of all gluconate (glucose) and glutamate multiplet areas provides a complete description of the metabolic pathways of Fig. 1, the constraints imposed by the assumptions stated above resulted in a few simple relationships:

$$\text{glutamate C2 (55.2 ppm)} \quad y = (\text{C2D23}-\text{C2D12})/\text{C2D12} \tag{1}$$

$$\text{PK}+\text{ME} = (\text{C2D23}-\text{C2Q})/\text{C2D12} \tag{2}$$

$$g = (\text{C2Q}-\text{C2D12})/(2\cdot\text{C2D12}) \tag{3}$$

$$\text{glutamate C3 (27.8 ppm)} \quad g = (\text{C3D}-0.5)/(2-2\cdot\text{C3D}) \tag{4}$$

$$\text{gluconate C1 (180 ppm)} \quad g = (\text{C1D}-0.5)/(2-2\cdot\text{C1D}) \tag{5}$$

$$\text{gluconate C2 (75.9 ppm)} \quad y = (\text{C2D12}-\text{C2D23})/\text{C2D23} \tag{6}$$

$$\text{PK}+\text{ME} = (\text{C2D12}-\text{C2Q})/\text{C2D23} \tag{7}$$

$$g = (\text{C2Q}-\text{C2D23})/(2\cdot\text{C2D23}) \tag{8}$$

These equations show that the ^{13}C-NMR spectrum of either glutamate C2 or gluconate C2 yield a direct measure of anaplerosis (y), glucose production (g) and pyruvate kinase (actually PK+ME) flux while glutamate C3 and gluconate C1 provide a direct measure of g. Note that in the absence of pyruvate recycling (PK+ME = 0), C2D23 would equal C2Q in glutamate, C2D12 would equal C2Q in gluconate, and the equations simplify to $y = 2g$.

3. Results

^{13}C enrichment in carbons 1, 2 and 3 of glutamate and gluconate were easily detected in extracts of isolated livers. The C2 resonances shown in Fig. 2 display extensive ^{13}C–^{13}C coupling indicative of multiply-enriched isotopomers. The fractional enrichment in glutamate C2 was 0.174 ± 0.047 (measured by ^{1}H-NMR) while ^{13}C enrichment of glutamate C4 was less than 2%, verifying the model assumption that labeling of acetyl-CoA was insignificant. The relative multiplets areas of glutamate C2 and gluconate C2 were identical (Table 1; note the inverse relationship between C2D12 and C2D23 of glutamate and gluconate). This indicates that (1) glutamate and glucose were derived from the same pool of OAA, and (2) OAA was fully randomized by exchange with symmetric TCA cycle intermediates. Analysis of these data using Eq. 1–3 and 6–8 indicated that total anaplerotic flux, y, was about 4 times citrate synthase flux (see Table 1) while glucose production was about 1.2 times citrate synthase flux. There was no significant difference between the metabolic results derived from either spectrum. It is important to note that the equations for glutamate C2 and gluconate C2 do not contain a contribution from C2S and hence are insensitive to any natural abundance contributions to the spectra. The equations for glutamate C3 and gluconate C1 (Eq. 4 and 5) however would be sensitive to natural abundance contributions. After correcting for a small (–5%) natural abundance contribution to gluconate C1 and glutamate C3 as determined by ^{1}H-NMR, values of g estimated using Eq. 4 and 5 were not significantly different from the values estimated from the C2 multiplet areas (Table 1).

Glucose production in these isolated livers was 0.37 ± 0.15 μmol/gww/min, compared a value of about 1 μmol/gww/min in the absence of propionate [21]. Blair et al. [22] also reported a significant drop in glucose production by perfused rat livers to 0.42 ± 0.06 μmol/gww/min when propionate (10 mM) was

Table 1
Gluconeogenesis and pyruvate recycling calculated from the multiplets in the ^{13}C-NMR spectrum of glutamate or gluconate from the isolated liver

	Glutamate	Gluconate
Multiplets		
C2S	0.10 ± 0.02	0.10 ± 0.02
C2D23	0.48 ± 0.01	0.10 ± 0.01
C2D12	0.10 ± 0.01	0.48 ± 0.01
C2Q	0.32 ± 0.03	0.33 ± 0.01
Relative fluxes (without $^{13}CO_2$)		
y, total anaplerosis	3.91 ± 0.61	4.13 ± 0.32
PK+ME	1.63 ± 0.51	1.65 ± 0.20
g, gluconeogenesis	1.15 ± 0.19	1.24 ± 0.09
(PK+ME)/y, pyruvate recycling	42 ± 8%	40 ± 2%
Relative fluxes (with $^{13}CO_2$)		
g, gluconeogenesis	1.14 ± 0.42	1.21 ± 0.17

Relative fluxes were calculated from the multiplets in the carbon 2 resonance. When [^{13}C]bicarbonate was available, gluconeogenesis (or glucose production) was calculated from the multiplets in carbon 1 or carbon 3 of gluconate or glutamate, respectively. All results are $n = 4$ or $n = 5$, mean ± SD.

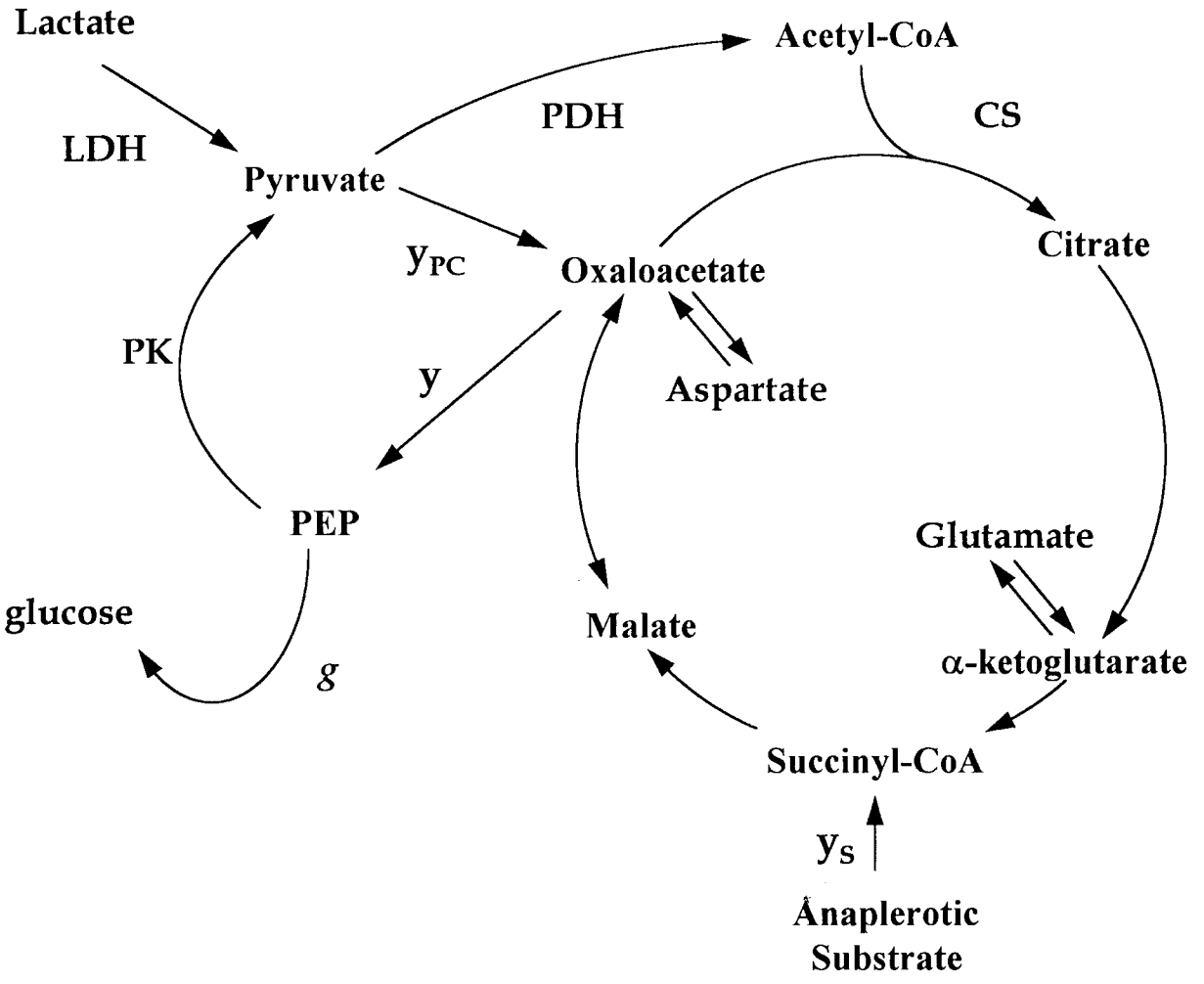

Fig. 1. Metabolic model. All fluxes are relative to flux through citrate synthase (CS). PDH, pyruvate dehydrogenase complex; LDH, lactate dehydrogenase; PK, pyruvate kinase; *g*, gluconeogenesis; y_S, flux through the combined anaplerotic pathways feeding succinyl-CoA, including propionate carboxylation; y_{PC}, flux through pyruvate carboxylase.

added to a mixture of L-lactate (10 mM), and pyruvate (1 mM). Since the rate of gluconeogenesis relative to citrate synthase was about 1.15 (*g* in Table 1), then flux through citrate synthase in these livers can be estimated at 0.37/1.15 = 0.32 μmol/gww/min. This is comparable to values of 0.13 to 0.25 μmol/gww/min for citrate synthase reported by DiDonato and colleagues [23]. This of course assumes that no glucose was derived from liver glycogen in these starved animals.

3.1. Measurement of gluconeogenesis when CO_2 is enriched

The input-output analysis did not yield simple equations for carbon 2 of glutamate or glucose when $^{13}CO_2$ entered at the level of pyruvate carboxylase. Thus, Eq. 1–3 and 6–8 do not apply if CO_2 is significantly enriched. However, the glucose C1 multiplets and the glutamate C3 multiplets are not sensitive to $^{13}CO_2$ enrichment because ^{13}C entering the oxaloacetate pool via CO_2 can only enrich carbons 1 or 4 of OAA. Therefore, Eq. 4 and 5 are valid regardless of whether $^{13}CO_2$ contains enriched carbon or not. Fig. 2 compares representative glutamate and gluconate C2 multiplets derived from livers perfused with [1,2,3-$^{13}C_3$]propionate ± $^{13}CO_2$. Note that the C2 multiplets were quite sensitive to the presence of $^{13}CO_2$ (C2Q now dominates both C2 resonances) while the multiplets in glutamate C3 and gluconate C1 did not change (not shown). Glucose production in livers perfused with $^{13}CO_2$ was 1.14 ± 0.42 as reported by the glutamate C3 multiplet areas (Eq. 4) and 1.21 ± 0.17 as reported by the gluconate C1 multiplet areas (Eq. 5), identical to the values determined in the absence of CO_2 enrichment (Table 1).

3.2. Intragastric and intravenous administration of enriched propionate in vivo

Although high-quality spectra were obtained from the isolated perfused liver, we also wanted to test whether this analysis could be applied in vivo where numerous other substrates are also available for gluconeogenesis and whether the analysis could be done on blood glucose without prior oxidation to gluconate. ^{13}C-NMR spectra of glucose was obtained from both blood and freeze clamped liver after either oral ($n = 3$) or intravenous ($n = 3$) administration of [1,2,3-$^{13}C_3$]-propionate. The β-C2 resonance of glucose was sufficiently resolved from all other resonances to allow an analysis of multiplet areas (Fig. 3). All four spectra confirm a high level of ^{13}C enrichment with complex isotopomer mixtures, and there were no significant differences between the β-glucose C2 multiplets from blood versus liver tissue. Some of the multiplet areas from the glucose C2β resonance have to be calculated algebraically because not all the components were

resolved in the ^{13}C-NMR spectrum. These include the right hand component of the D23 doublet (which co-resonates with the right hand C2D12 component) and one of the quartet lines of C2Q (which co-resonates with the C2 singlet). Furthermore, both the doublet and quartet components show asymmetry due to strong coupling, hence correction factors are needed to relate the area of the resolvable component to that of its hidden companion. These were calculated from the known coupling constants (J_{12} = 47.0 Hz; J_{23} = 38.5 Hz), field strength, and chemical shifts of carbons 1, 2 and 3 [24]. The glucose C2D12 was 0.50 ± 0.04, 0.49 ± 0.02, 0.46 ± 0.06, and 0.44 ± 0.06 in the four groups (i.v. administration of propionate and blood sampled; i.v. propionate and liver sampled; oral propionate and blood sampled; oral propionate and liver

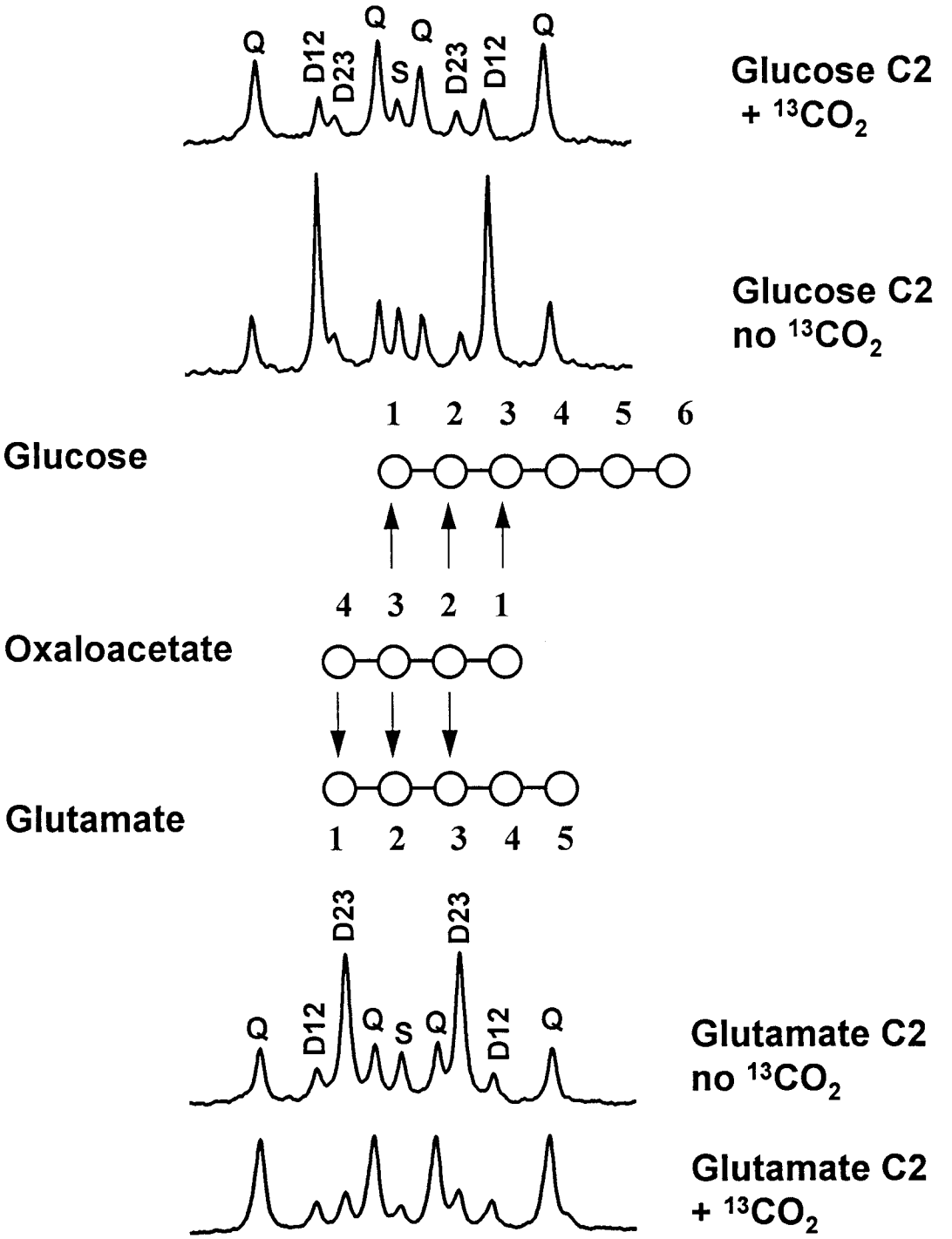

Fig. 2. Relation of ^{13}C enrichment in oxaloacetate to glutamate and glucose, and the effects of $^{13}CO_2$ on the proton decoupled ^{13}C-NMR spectra of carbon 2 from gluconate and glutamate. Multiplets are defined in the text. The spectra labeled 'no $^{13}CO_2$' were obtained from a liver supplied with [1,2,3-$^{13}C_3$]propionate plus unlabeled substrates as described in the text. The spectra labeled '+$^{13}CO_2$' were obtained under the same conditions except that $^{13}CO_2$ was present in high concentration. Since $^{13}CO_2$ would increase ^{13}C enrichment in OAA carbon 1 or carbon 4, the quartet (Q) increased in the C2 resonance of both glucose and gluconate.

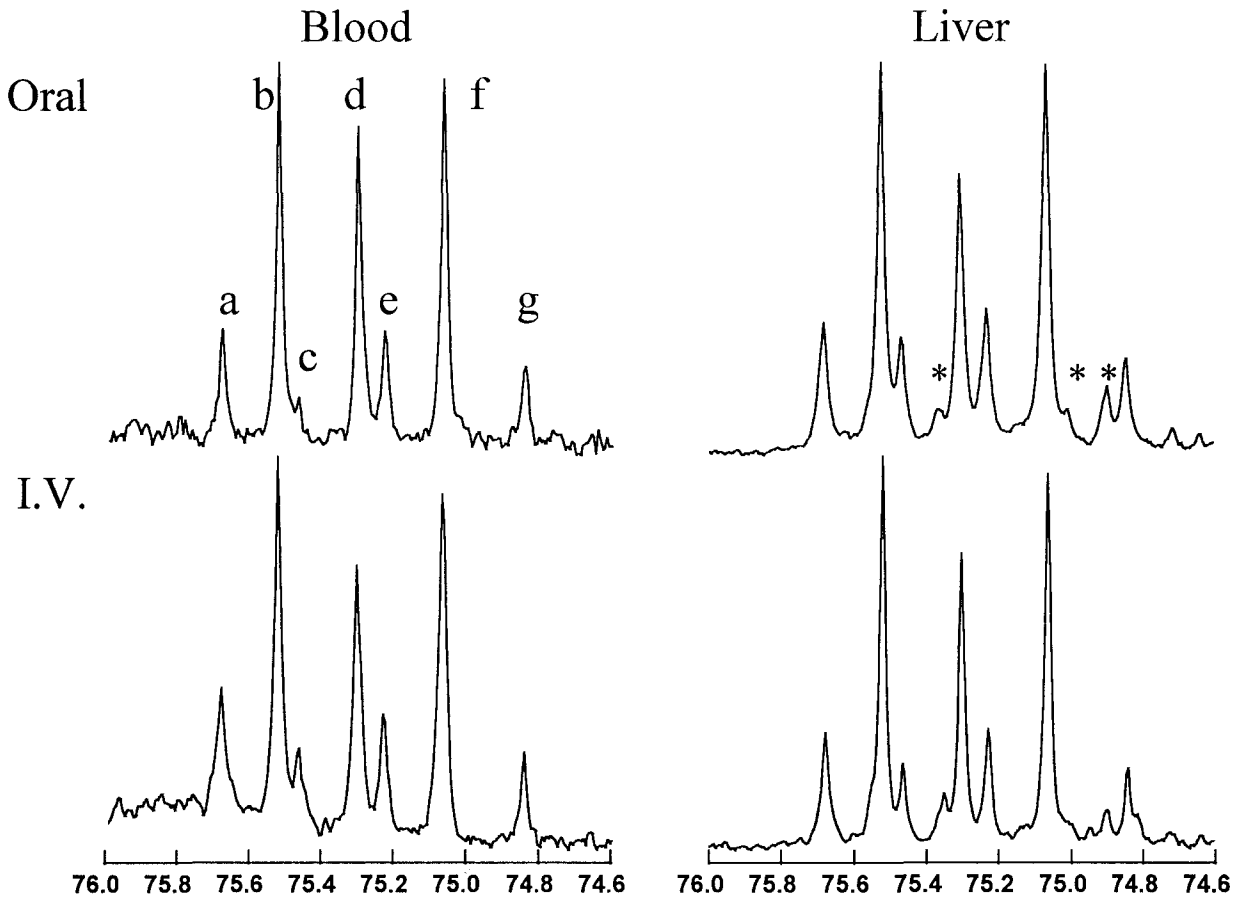

Fig. 3. 13C-NMR spectra of carbon 2 of the β-anomer of glucose from blood or liver after intravenous or intragastric administration of [1,2,3-$^{13}C_3$]propionate. Resonances **a**, **e** and **g** are the three resolvable components of the quartet (C2Q); resonance **b** is the resolvable component of the C2D12 doublet and resonance **c** is the resolvable component of the C2D23 doublet. Resonance **d** represents the sum of the C2S plus the unresolved C2Q component, while resonance **f** represents the sum of the unresolved C2D12 and C2D23 components.

sampled, respectively) which was not significantly different from the results obtained in the isolated liver (Table 1). The glucose C2D23 was 0.10 ± 0.01, 0.09 ± 0.01, 0.05 ± 0.02, and 0.09 ± 0.01 in the four groups, and the glucose C2Q was 0.29 ± 0.03, 0.28 ± 0.02, 0.25 ± 0.05, and 0.28 ± 0.03, respectively. These multiplet areas, when substituted into Eq. 6–8, gave the following results: $y = 4.57 \pm 1.15$, PK+ME = 2.26 ± 0.75 (pyruvate recycling = 49%), and $g = 1.16 \pm 0.25$. Anaplerosis and PK+ME were slightly higher in vivo compared to the isolated liver (Table 1) while gluconeogenesis was identical.

4. Discussion

We have derived simple relations between the ^{13}C multiplets of glucose and glutamate that allow a direct measure of relative gluconeogenesis and pyruvate recycling from a single ^{13}C-NMR spectrum of the liver. Glucose and glutamate analyses were chosen for analysis for several reasons. Glutamate (assumed equivalent to glutamine) can be sampled non-invasively in humans by chemical biopsy [12] while glucose of course is easily sampled from blood. Also, these two metabolites share a common metabolic intermediate in OAA so questions about compartmentation of OAA may be addressed. [1,2,3-$^{13}C_3$]propionate was chosen as the source of the ^{13}C label for several reasons. Unlike other gluconeogenic substrates such as lactate, pyruvate and alanine, propionate enters the citric acid cycle by a single and irreversible anaplerotic pathway [25]. Propionate is also efficiently extracted from the circulation [26] and is avidly utilized as an anaplerotic substrate by the liver [18,21]. Uniformly enriched propionate was chosen over a tracer enriched in a single carbon position so that all metabolic products would contain multiplets rather than singlets. This becomes an important factor whenever ^{13}C enrichment of metabolic products is low and one is uncertain whether the natural abundance ^{13}C signal contributes significantly to a spectrum [27]. Since the glutamate C2 and gluconate (glucose) C2 multiplet analyses (Eq. 1–3 and 6–8) do not contain a C2S term, the method should provide a reliable measure of gluconeogenesis and pyruvate recycling regardless of ^{13}C enrichment levels. The only limitation is that the enrichment must be sufficient so that the C2 doublet and quartet components are not overwhelmed by a large singlet component due to natural abundance ^{13}C. Note, however, the same is not true for the glutamate C3 and gluconate C1 analysis (a correction may be necessary for natural abundance ^{13}C in Eq. 4 and 5). A second advantage of [1,2,3-$^{13}C_3$]propionate is illustrated by the work of Landau et al. [8] who were unable to obtain a measure of pyruvate cycling using either [2-^{14}C]- or [3-^{14}C]propionate. They reported that gluconeogenic flux was two or more times Krebs cycle flux and that less than one-twenty-fifth of any pyruvate derived from propionate re-

entered the citric acid cycle as acetyl-CoA. These results are consistent with our values measured in both the perfused and in vivo rat liver.

The results reported in Table 1 are consistent with some but not all earlier reports. For example, Cohen et al. reported that PK flux relative to gluconeogenesis was 0.74–1.0 in isolated livers [5], while Grunnet and Katz [28] reported a range of PK/g from 0.18 to 0.72, and Friedman et al. found 0.86 in isolated livers [11]. In comparison, our value of (PK+ME)/g was 1.3 (Table 1). Recently, Petersen et al. reported (PK+ME)/y_{PC} of 0.26–0.40 in normal rats [13], in reasonable agreement with our finding of (PK+ME)/y=0.49 (where y in our experiment=y_S+y_{PC}). On the other hand, results in Table 1 are in the low end of the range reported in humans by Magnusson et al. [12] who found the following fluxes relative to citrate synthase: PK, 1.5–3.7; gluconeogenesis, 1.4–3.5; and total anaplerosis, 2.8–7. This wide range of reported values for variables related to gluconeogenesis is likely due to variations in nutritional state, available substrates, and species differences. We also note that it is not clear in some instances if 'gluconeogenesis' indicates flux from phosphoenolpyruvate to glucose or the rate of glucose production.

We have previously observed conditions where the gluconate C2D12 was significantly larger than the gluconate C2Q in livers supplied with [1,2,3-$^{13}C_3$]propionate. This observation, confirmed here, was interpreted as evidence for incomplete randomization of symmetric 4 carbon citric acid cycle intermediates [21]. Subsequently, we pointed out that this multiplet pattern could also be observed whenever pyruvate recycling was significant [29]. In these earlier reports the conclusions were solely based on the enrichment patterns detected in gluconate C2, aspartate C2 or lactate C2 (each reflecting the C2 carbon of OAA) and this did not provide sufficient information to distinguish between pyruvate recycling or orientation conserved transfer. In this study, glutamate was also examined because it was present in sufficient concentration for analysis, and comparison with gluconate allows us to distinguish between these two metabolic possibilities. If orientation conserved transfer is the mechanism for gluconate C2D12 > C2Q, then the glutamate C2Q should have been significantly larger than either doublet. This was not observed. The alternative hypothesis, that gluconate C2D12 > C2Q because of recycling, then glutamate C2D23 > C2Q, exactly as observed. We conclude that under these conditions the ^{13}C spectra of glutamate and gluconate are consistent with significant pyruvate recycling but not consistent with orientation conserved transfer of symmetric intermediates. Furthermore, our observation that glutamate C2D23 = gluconate C2D12 and glutamate C2D12=gluconate C2D23 shows that there was complete randomization of symmetric citric acid cycle intermediates and that both metabolites originated from a common OAA. This conclusion is consistent with most metabolic models of the liver, but it should be noted that labeling studies with [1,2,3-$^{13}C_3$]lactate have shown significant differences in the labeling patterns of these metabolites [1,30]. Our finding that OAA was fully randomized is also consistent the conclusion by Landau and colleagues [12] that backwards scrambling of OAA into the symmetric 4-carbon pool is rapid relative to citric acid cycle flux in humans.

Under conditions where backwards scrambling of OAA is substantially incomplete (i.e. < 80%), equations derived for the C2 multiplets of glucose and glutamate are no longer valid, while the equations derived for the glucose C1 and glutamate C3 multiplets still apply. Incomplete OAA-fumarate equilibration in the presence of pyruvate recycling causes asymmetric labeling of OAA carbons 1 and 4. This will produce different glucose and glutamate C2 isotopomer distributions, hence the C2 multiplet analyses of glucose and glutamate will yield incorrect and inconsistent values for y, PK+ME and g. However, the glucose C1 and glutamate C3 multiplets are insensitive to the labeling status of OAA carbons 1 and 4, hence after correction for natural abundance contributions, both multiplets will report a consistent and correct value for g under these conditions.

In conclusion, the ^{13}C isotopomer method presented here should be easily adapted to human studies since it uses a non-radioactive tracer that can be given orally. Blood glucose (or, alternatively, gluconate) can be obtained easily from humans for ^{13}C-NMR analysis. From preliminary studies where 20 ml of blood was drawn from normal human subjects following ingestion of ~1.5 g [1,2,3-$^{13}C_3$]propionate, we obtained glucose spectra of similar quality to those shown in this report (unpublished data). Analysis of blood glucose rather than the liver itself may be also preferable in animal studies because the ^{13}C-NMR spectra of blood samples were less contaminated by labeled metabolites (note the absence of unknowns labeled '*' in Fig. 3). This method may prove to be a useful alternative to other reported techniques for measuring gluconeogenesis and pyruvate recycling.

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