Glutamate, a Window on Liver Intermediary Metabolism$^{1,2}$

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ABSTRACT In isotopic experiments, the labeling pattern of glutamate opens a window on hepatic metabolism, particularly the citric acid cycle, gluconeogenesis and fatty acid oxidation. This is because glutamate is in isotopic equilibrium with $\alpha$-ketoglutarate, whose labeling pattern is influenced by the following: 1) the contributions of glucose and fatty acids to acetyl-CoA, 2) the relative contributions of pyruvate carboxylase and pyruvate dehydrogenase to the entry of pyruvate carbon into the citric acid cycle, and 3) the rate of gluconeogenesis in relation to citric acid cycle activity. In humans and primates, hepatic glutamate can be sampled noninvasively via urinary phenylacetylglutamine, which is formed in liver from phenylacetate (a side product of phenylalanine catabolism) and glutamine (which equilibrates with liver glutamate and $\alpha$-ketoglutarate). The $^{14}\text{C}$- or $^{13}\text{C}$-labeling pattern of the glutamate moiety of phenylacetylglutamine can be measured by sequential degradations to $^{14}\text{CO}_2$, gas chromatography-mass spectrometry or nuclear magnetic resonance (NMR). When phenylacetylglutamine is labeled from singly labeled $^{13}\text{C}$- or $^{13}\text{C}$substrates, relative metabolic rates can be computed from the labeling pattern using Landau’s model. In diabetic patients infused with $[3\text{-}^{13}\text{C}]$pyruvate, the noninvasive sampling of hepatic glutamate via phenylacetylglutamine allows one to test the degree of liver insulinization via the (pyruvate carboxylase)/(pyruvate dehydrogenase) activity ratio. This ratio regulates gluconeogenesis in part. Its measurement may allow the identification of patients who might benefit from the intraperitoneal administration of insulin, or from recently developed antidiabetic drugs. J. Nutr. 130: 991S–994S, 2000.

KEY WORDS: • phenylacetylglutamine • gluconeogenesis • tricarboxylic acid cycle • isotopic methods • diabetes

Liver glutamate and gluconeogenesis

In the postabsorptive state, blood glucose concentration is kept constant by a combination of glycogenolysis and gluconeogenesis. During prolonged fasting, when hepatic glycogen is exhausted, gluconeogenesis becomes the only source of plasma glucose. Gluconeogenesis (the production of new glucose molecules) occurs mainly in liver and, to a small extent, in kidneys. It is fueled $\sim90\%$ by the gluconeogenic amino acids, released by proteolysis, and $\sim10\%$ by glycerol, released by lipolysis. Lactate, which is an intermediate of the Cori cycle (i.e., glucose $\rightarrow$ lactate $\rightarrow$ glucose), and alanine, derived from proteolysis, are converted to glucose via pyruvate by the same reactions.

A number of isotopic techniques have been developed to study gluconeogenesis in vivo and ex vivo. This review concentrates on isotopic techniques developed to assess the regulation of hepatic gluconeogenesis and its relationship to other pathways of intermediary metabolism, particularly the citric acid cycle and fatty acid oxidation. Such investigations are complicated by the fact that oxaloacetate is an intermediate common to gluconeogenesis and the citric acid cycle (Landau, 1993). This results in numerous isotopic exchanges between gluconeogenic and citric acid cycle intermediates. These isotopic exchanges result in variable losses of carbon label between gluconeogenic precursors (pyruvate, lactate, alanine) and glucose. In addition, the substrate cycle pyruvate $\rightarrow$ oxaloacetate $\rightarrow$ phosphoenolpyruvate $\rightarrow$ pyruvate results in additional loss of label in phosphoenolpyruvate before it is converted to glucose. Last, pyruvate enters the citric acid cycle via two reactions, one catalyzed by pyruvate carboxylase to form oxaloacetate, and the other by pyruvate dehydrogenase to form acetyl-CoA. The two routes of entry of pyruvate carbon into the citric acid cycle result in a complex labeling pattern of cycle intermediates. In addition to affecting the labeling pattern of citric acid cycle intermediates, the (pyruvate carboxylase)/(pyruvate dehydrogenase) activity ratio is a key regulator of gluconeogenesis. It is increased in starvation and diabetes, and decreased by insulin treatment. Because of the importance of this activity ratio, much effort has been devoted to setting up techniques for its estimation, using both invasive and noninvasive procedures. Some of these techniques take advantage of the rapid interconversion that occurs in liver mitochondria between $\alpha$-ketoglutarate and glutamate (Fig. 1).
This interconversion is catalyzed by glutamate dehydrogenase, glutamate-oxaloacetate aminotransferase and glutamate-pyruvate aminotransferase. Thus the labeling pattern of liver glutamate, equivalent to that of \( \alpha \)-ketoglutarate, yields useful information on the regulation of hepatic gluconeogenesis.

Modeling of the \( ^{14} \)C- or \( ^{13} \)C-labeling pattern of glutamate

Landau and co-workers (Magnusson et al. 1991) developed a mathematical model of the \( ^{14} \)C-labeling pattern of glutamate that is applicable to experiments in which glutamate is labeled from lactate, or pyruvate labeled with \( ^{14} \)C on carbon 3. The data introduced into the model are the specific activities of the five glutamate carbons. Computation yields rates of citric acid cycle reactions and of gluconeogenesis expressed relative to the net flux through the citric acid cycle (setting the rate of the citrate synthase reaction to 1.0). This model is also applicable to experiments conducted with \([3-^{13} \)C]lactate or \([3-^{13} \)C]pyruvate, in which the computation uses the \( ^{13} \)C-enrichments of the five glutamate carbons (Yang et al. 1996). Magnusson et al. (1991) and Di Donato et al. (1993) have discussed some limitations of the model, particularly the sensitivity of some parameters to errors in measurements of the small amount of label on carbon 5 of glutamate.

Determination of the \( ^{14} \)C- or \( ^{13} \)C-labeling pattern of glutamate

The \( ^{14} \)C-specific activity of each of the five carbons of glutamate can be determined by isolating glutamate in pure form, degrading it sequentially to five molecules of CO\(_2\) and measuring the label in the five CO\(_2\) samples (Magnusson et al. 1991). The \( ^{13} \)C-enrichment of the glutamate carbons can be determined by gas chromatography-mass spectrometry of various glutamate derivatives. Only \(~50\) nmol of glutamate is required for the analyses. When the total \( ^{13} \)C-labeling of glutamate is sufficiently low so that, on average, most molecules contain no more than one \( ^{13} \)C atom, analysis of a single derivative of glutamate yields the complete labeling pattern (Beylot et al. 1993). When glutamate is more heavily labeled, the analysis requires running up to five glutamate derivatives on the mass spectrometer (Di Donato et al. 1993). Unfortunately, no present mass spectrometric technique allows the direct measurement of the \( ^{13} \)C-enrichment of carbon 5 of glutamate. This enrichment is calculated as the difference between the enrichments of two fragments of the glutamate molecule. When the enrichment of carbon 5 is low compared with that of carbon 4, the value for carbon 5 enrichment is imprecise.

The problem of the low \( ^{13} \)C-enrichment of carbon 5 of glutamate can be solved, at a price, by assaying the labeling pattern of glutamate by high-power \( ^{13} \)C or \( ^{1} \)H nuclear magnetic resonance (NMR). However, to be able to assay enrichments on carbon 5 of the order of 0.1–0.5\%, \(~50\) mg of semipurified phenylacetylglutamine is required. Such amounts cannot be obtained without administering phenylacetate or phenylbutyrate to the subjects (typically 2.5 g) (Cline et al. 1994, Jones et al. 1998). An advantage of the NMR assay is that it can identify adjacent \( ^{13} \)C atoms in the glutamate molecule (Jones et al. 1998).

Noninvasive sampling of liver glutamate via urinary phenylacetylglutamine

It has been known for a long time that the liver of humans and primates contains an enzyme that conjugates glutamine with phenylacetate (a side-product of phenylalanine catabolism) to form phenylacetylglutamine (Fig. 2), which is excreted in urine (James et al. 1972). This process involves the activation of phenylacetate to phenylacetyl-CoA, followed by conjugation with glutamine catalyzed by an acyl-CoA : glutamine N-acetyltransferase (Webster et al. 1976). Exogenous phenylacetate can be ingested in gram amounts without toxicity. The main fate of this ingested phenylacetate is the formation and excretion of phenylacetylglutamine.

The formation of phenylacetylglutamine is used in the treatment of a number of inborn errors of the urea cycle enzymes that lead to the accumulation of toxic concentrations
of ammonia in body fluids. In these children treated with phenylacetate, nitrogen derived from protein catabolism is excreted in the form of phenylacetylglutamine rather than urea (Brusilow 1991). Because of the objectionable smell of phenylacetate, it is often administered as a less volatile precursor, phenylbutyrate. In the liver, phenylbutyrate undergoes β-oxidation to phenylacetyl-CoA, the immediate precursor of phenylacetylglutamine (Piscitelli et al. 1995). Note that prolonged administration of phenylbutyrate can lead to glutamine depletion (Darmaun et al. 1998).

Magnusson et al. (1991) were the first to use the labeling pattern of urinary phenylacetylglutamine as a noninvasive probe of the liver citric acid cycle intermediates and of gluconeogenesis. This was the first of a number of studies, some of which are ongoing, that have capitalized on this elegant technique. Some of these studies used 14C-substrates (Esenmo et al. 1992, Landau et al. 1995, Schumann et al. 1991); other studies used 13C-substrates, assaying the concentration and/or labeling pattern of phenylacetylglutamine by gas chromatography-mass spectrometry (Diraie et al. 1998, Yang et al. 1993 and 1996) or NMR (Cline et al. 1994, Dugelay et al. 1994, Jones et al. 1998).

New knowledge derived from the labeling pattern of glutamate

Glutamate assayed as such in tissue extracts. Katz et al. (1993) infused food-deprived rats with [U-13C3]lactate or [2,3-13C]acetate and assayed the mass isotopomer distribution of liver glutamate. From the mass isotopomer distribution of glutamate and glucose, they deduced the absolute rates of gluconeogenesis, recycling of phosphoenolpyruvate, Cori cycling and citric acid cycle flux.

Beylot et al. (1995) determined the 13C-labeling pattern of rat liver glutamate (Beylot et al. 1993) under two sets of conditions as follows: 1) in isolated livers perfused with [3-13C]lactate, [2,13C]acetate or [3,3-13C]isocaproate, and 2) in liver taken from rats that had been infused with the same tracers. With [3-13C]lactate, the labeling pattern of liver glutamate was identical in the livers perfused with the tracer and in livers removed after infusion of the tracer in vivo. In contrast, with [2-13C]acetate and α-keto[3-13C]isocaproate, the labeling pattern of glutamate was very different in the livers perfused with the tracer and in livers removed after infusion of the tracer in vivo. This discrepancy results from the intense metabolism of [2,13C]acetate and α-keto[3-13C]isocaproate in muscle, which releases 13CO2 and [13C]glutamine; these are carried by blood to the liver where they modify the labeling pattern of glutamate made locally. With the latter tracers, the labeling patterns of muscle and liver glutamine are very different, as shown by direct analysis. This study confirmed the conclusion of Schumann et al. (1991) that the bulk of the metabolism of [2-13C]acetate is extrahepatic (see below). Also, Large et al. (1997) applied the model of Magnusson et al. (1991) to studies of gluconeogenesis in perfused rat livers with an emphasis on the effect of modulators of pyruvate metabolism.

Glutamate isolated from urinary phenylacetylglutamine. Landau and co-workers used the labeling pattern of phenylacetylglutamine to investigate the suitability of various 14C-tracers to gain insight into the regulation of gluconeogenesis and the citric acid cycle in human liver. One of the issues to be resolved was whether, with some tracers, the labeling pattern of liver glutamate could be altered by circulating labeled products of the tracer’s metabolism in extrahepatic tissues, particularly muscle. They compared the labeling patterns of phenylacetylglutamine and of plasma glucose. They developed a technique to correct the labeling pattern of phenylacetylglutamine and glucose for reincorporation of 14CO2, which labels carbon 1 of the glutamate moiety of phenylacetylglutamine, as well as carbons 3 and 4 of glucose. This was achieved by using the specific activity of urea measured in the experiments with various tracers as well as in control experiments in which the production of 14CO2 was simulated by an infusion of NaH13CO3. These studies showed that [13C]acetate, but not [14C]acetate, is suitable for tracing gluconeogenesis in liver (Magnusson et al. 1991, Schumann et al. 1991). They stimulated Beylot et al. (1995) to compare the labeling patterns of liver glutamate in perfused rat livers and in livers from rats infused with various 13C-substrates (see above).

To determine directly whether the labeling pattern of urinary phenylacetylglutamine reflects that of liver glutamate and α-ketoglutarate, Yang et al. (1996) infused anesthetized monkeys with phenylacetate and various 13C-substrates, which label the citric acid cycle intermediates either via pyruvate carboxylase and pyruvate dehydrogenase ([3-13C]lactate, [U-13C]acetate) or via other reactions ([1,2-13C2]acetate, α-keto[3,3-13C]isocaproate). Figure 3 shows the comparison of the labeled mass isotopomer distributions of the glutamate moiety of phenylacetylglutamine in monkeys infused either with [U-13C3]lactate (upper panel) or with [1,2-13C2]acetate (lower panel). Mass isotopomers are molecules containing one to four 13C atoms (M1, M2, M3, M4). In the presence of [U-13C3]lactate, the mass isotopomer distributions of liver glutamate and urinary phenylacetylglutamine are practically identical. The isotopomer distributions of muscle and kidney glutamate do not match those of liver glutamate and phenylacetylglutamine. In

![Figure 3](https://example.com/figure3.png)

**FIGURE 3** Comparison between the labeling patterns of urinary phenylacetylglutamine and glutamate in the liver, muscle and kidneys of monkeys infused with [U-13C3]lactate (upper panel) or [1,2-13C2]acetate (lower panel). Labeling patterns are presented as percentage distributions of labeled mass isotopomers, whose molecules contain 1, 2, 3 or 4 13C atoms (M1, M2, M3, M4).
the presence of [1,2-13C]acetate, the mass isomoposer distribution of phenylacetylglutamine does not match that of liver glutamate, but is closer to those of muscle and kidney glutamate. This study demonstrated that the labeling pattern of urinary phenylacetylglutamine is identical to that of liver α-ketoglutarate and glutamate only when carbon label is administered as lactate or pyruvate. For a review on the applications of mass isomoposer analysis to nutrition research, see Brunengraber et al. (1997).

Cline et al. (1994) assayed the labeling pattern of phenylacetylglutamine in control and type II diabetic patients infused with [1-13C]glucose. They concluded that patients with poorly controlled insulin-dependent diabetes mellitus have augmented hepatic gluconeogenesis and relatively decreased rates of hepatic pyruvate oxidation. Diraion et al. (1998) infused [3-13C]lactate in control and stable type II diabetic patients and, using the labeling pattern of phenylacetylglutamine, did not find evidence of increased gluconeogenesis. Landau et al. (1995) studied in a healthy subject infused with [3,4-13C]lactate and NaH13CO3 after 60 h of food deprivation. From the labeling patterns of glucose and phenylacetylglutamine, they calculated rates of pyruvate cycling and contributions of gluconeogenesis to total glucose production (80 and 45% in control and diabetic subjects, respectively).

Recently, Jones et al. (1998) reported the labeling pattern of phenylacetylglutamine in humans who had ingested [U-13C]propionate. This pattern, determined by NMR, was used with a model of the citric acid cycle in which label enters via an anaplerotic process. Note that computation of the labeling pattern of phenylacetylglutamine or of glutamate does not yield absolute rates of gluconeogenesis, but rather relative rates of cellular reactions that participate in the control of gluconeogenesis. The information, gathered from the labeling pattern of phenylacetylglutamine, is thus complementary to techniques used to measure carbon flux through gluconeogenesis and glycolgenolysis.

The precision of the measurements of the distribution of 13C between the glutamate and phenylacetylglutamine carboxyls depends very much on the following: 1) the nature of the tracer, 2) whether the tracer is singly or uniformly labeled, 3) the dose or administration rate of (expensive) tracers, 4) the sensitivity of the analytical equipment, and 5) the analytical skills of the investigators. Readers are referred to the original references for further evaluation of the technologies.

Future directions

There is now good evidence that the labeling pattern of urinary phenylacetylglutamine yields useful information on the regulation of gluconeogenesis via determination of the (pyruvate carboxylase)/(pyruvate dehydrogenase) activity ratio in humans who had ingested [3-13C]pyruvate or [3-13C]lactate. Also, the mass isomoposer distribution of plasma glucose, labeled during the same experiment, yields the contribution of gluconeogenesis to total glucose production. This technique can now be applied to study perturbations of gluconeogenesis that occur in type I and type II diabetes. In type I diabetics, the (pyruvate carboxylase)/ (pyruvate dehydrogenase) ratio yields an index of the degree of insulinization of the liver. Physiologically, insulin reaches the liver by the portal vein. In contrast, in treated type I diabetics who receive insulin by subcutaneous injection, insulinization of the liver may be suboptimal. Sufficient insulinization of the liver may require hyperinsulinization of peripheral tissues. Probing the parameters of gluconeogenesis by phenylacetylglutamine may identify a subset of type I diabetics who might benefit from the intraperitoneal administration of insulin. In type II diabetics, the labeling pattern of phenylacetylglutamine may prove useful in assessing the efficacy of nutritional and/or pharmacologic therapy.

LITERATURE CITED


