Mass and Positional Isotopomer Analysis of Glucose Metabolism in Periportal and Pericentral Hepatocytes*

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To determine whether the source of carbon for the indirect pathway of hepatic glycogen synthesis differs between the periportal and pericentral zones, we studied seven 24-h-fasted conscious rats given a constant 2-h intraduodenal infusion of glucose, 40% labeled with [U-13C]glucose (99% 13C enriched), to raise and maintain plasma glucose concentration at ~10 mm. Glycogen, glutamate, aspartate, and alanine were selectively sampled from the periportal and pericentral zones of the liver by the dual-digitonin pulse technique and analyzed by GC-MS for positional isotopomer distribution and by gas chromatography-mass spectrometry for mass isotopomer distribution. Plasma glucose mass isotopomer distribution was determined from gas chromatography-mass spectrometry. The isotopomer distribution indicates that there was no significant difference between the zones with respect to 1) percent direct flux of glucose into the glycogen (periportal, 34 ± 4; pericentral, 38 ± 4), 2) extent of oxaloacetate/fumarate equilibration (periportal, 0.54 ± 0.01; pericentral, 0.53 ± 0.01), 3) dilution of tracer in oxaloacetate (periportal, 0.64 ± 0.07; pericentral, 0.64 ± 0.07), or 4) influx of pyruvate versus tricarboxylic acid cycle flux (periportal, 0.70 ± 0.20; pericentral, 0.68 ± 0.16). Positional isotopomer populations, determined from the 13C-13C splitting in C3 and C4 of periportal and pericentral glycogen, were indistinguishable, indicating no significant differences in the source of the 3-carbon precursors for hepatic glycogen synthesis by the indirect pathway. In conclusion, glucose metabolism is the same in the periportal and pericentral zones with regard to 1) the relative flux of carbon via the direct/indirect pathways, 2) the source of the 3-carbon precursor used in the indirect pathway of glycogen synthesis, and 3) the flux of the 3-carbon precursors through the tricarboxylic acid cycle.

Liver parenchyma display a heterogenous spatial distribution of activity of those enzymes associated with gluconeogenesis and glycolysis, as determined by microdissection and microhistochemical assays (1). In vitro, liver perfusion studies showed that glycogen was deposited only in the periportal zone when gluconeogenic precursors were perfused and that glycogen was deposited only in the pericentral zone when glucose was perfused (2). Based on these observations, it has been proposed that hepatocytes in the periportal zone are gluconeogenic and that pericentral hepatocytes are glycolytic (3). If so, this has important implications for interpretation of data obtained from virtually all in vivo based studies of liver metabolism. In a recent study, we administered [1-13C]glucose to awake unstressed rats and selectively sampled periportal and pericentral glycogen by the dual-digitonin pulse technique (4, 5), and we found no difference between the periportal and pericentral zones in the relative percentages of the direct (Glc→Glc-6-P→Glc-1-P→UDP-glc→glycogen) and indirect (3-carbon units→→→glycogen) pathways of glycogen repletion (4).

However, it is still possible that the source of the 3-carbon precursors for liver glycogen may differ between the zones. Even though the relative contributions for the direct and indirect pathway were similar between the two populations of hepatocytes, it is still possible that the three carbon units for the indirect pathway of glycogen synthesis in one population of hepatocytes was derived by intracellular glycolysis while the other population of hepatocytes derive their three carbon units for glycogenesis from extrahepatic sources. This question carries additional weight in view of our recent study in awake triple-catheterized dogs, in which we found that during an intraduodenal glucose infusion net hepatic glucose uptake could account for all of the glycogen synthesized by the liver from both the direct and indirect pathways (6). These results imply that the liver is capable of undergoing substantial glycolytic and gluconeogenic flux simultaneously, raising the question as to whether or not, under these conditions, some hepatocytes are mostly glycolytic and others are mostly gluconeogenic or whether substrate cycling between glycolysis and gluconeogenesis is occurring to an equal extent within all hepatocytes.

The purpose of this study was to assess whether there are differences in the source of carbon for glycogen synthesized via the indirect pathway in the periportal and pericentral zones in vivo. Hepatic glycogen stores in awake, unstressed rats were depleted after a 24-h fast with a constant intraduodenal glucose infusion enriched with [U-13C]glucose. Periportal and pericentral cytosol were then selectively sampled by using the dual-digitonin-pulse technique, and the distribution of positional and mass isotopomers of glycogen, alanine, aspartate, and glutamate were ascertained by 13C-NMR and GC-MS3 spectrometry. Any difference in the source of carbon for the indirect pathway of glycogen repletion in the periportal and pericentral zones can be determined from differences in the isotopomer distribution of glycogen and of the amino acids that equilibrate with metabolites of the tricarboxylic acid cycle. From mass isotopomer analysis, the relative proportions of the direct to

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indirect flux of glucose in glycogen can be determined. In addition, the tricarboxylic acid cycle parameters of pyruvate carboxylase to citrate synthase flux and the magnitude of oxaloacetate-fumarate equilibration can be calculated. From analysis of the \(^{13}\)C-NMR spectra, the positional isotopomers of glycogen, and hence the relative flux of labeled to unlabeled 3-carbon precursors (i.e., glycerol and free fatty acids relative to pyruvate), can be evaluated. The data derived from the \(^{13}\)C-\(^{13}\)C coupling between the C3 and C4 carbons of glycogen is unique in that the \(^{13}\)C-\(^{13}\)C splitting pattern will be very different if the source of the three carbon units for the indirect pathway is derived from intracellular glycolysis versus extrahepatic sources since the C3 and C4 carbons reflect the contribution from two different pyruvate molecules. This type of positional isotopomer analysis is unique and the most comprehensive way of addressing this question since it reflects the \(^{13}\)C labeling in adjacent carbons within the same glucose molecule as opposed to examining the \(^{13}\)C labeling distribution within a population of glucose molecules. This type of analysis was not possible in our previous study since only \([1-{^{13}\text{C}}]\)glucose was used and does not lead to a comparable array of isotopomeric species, which provide a richer wealth of informational content.

**EXPERIMENTAL PROCEDURES**

**Materials**—Digitonin was purchased from ICN Biochemicals, Inc. (Costa Mesa, CA) and was rendered water soluble by recrystallization from methanol. Enzymes and chemicals were purchased from commercial sources (Sigma).

**Animals**—Male Sprague-Dawley rats (n = 7) weighing between 370 and 530 g were used. They were maintained on standard rat chow (Ralston-Purina Co.) and housed in an environmentally controlled room with a 12-h light/dark cycle. 1 week before the study, an internal jugular catheter, extending to the right atrium, and an intraduodenal catheter were implanted. The catheters were externalized through a skin incision through the back of the head. All rats were fasted 24 h before the study to deplete liver glycogen. On the day of the study, a prime (0.3 g/kg) constant (17 mg/kg/min) infusion of glucose (15 g/dl, enriched 40% with \([U-{^{13}\text{C}}]\)glucose, 99 atom % \(^{13}\text{C}\)) was begun via the duodenal catheter and continued throughout the study. Blood samples for plasma glucose concentration and \(^{13}\)C enrichment were taken at 15-min intervals. After 120 min, rats were anesthetized by intravenous administration of Nembutal (50 mg/kg), and the livers were perfused in situ as described below.

**Perfusion**—After anesthesia, the abdomens were incised, the portal vein cannulated, and the liver perfused in situ with glucose-free Krebs-Henseleit buffer (37 °C) at a rate of 25 ml/min. The superior vena cava was cannulated, and the inferior vena cava was ligated. The perfusion was switched to cold buffer (4 °C) at the same rate for 2 min. Perfusion was then switched to cold digitonin solution (5 mg/ml in glucose-free Krebs-Henseleit buffer) at a rate of 20 ml/min for 20 s, followed by a 5-min washout with digitonin-free buffer. Fractions were collected at intervals of 20 s. The direction of perfusion was then reversed and followed the same sequence: 2 min of buffer, followed by 20 s of digitonin, a washout, and sampling of 5 min. The initial direction, antegrade or retrograde, of the 2-min cold perfusion, digitonin pulse, and sampling varied between experiments to eliminate systematic errors of selective sampling of periporal or pericentral zones.

**Analysis**—Plasma glucose was determined using a commercial glucose analyzer (Beckman Instruments). Each fraction of effluent collected during the digitonin perfusion was assayed for lactate dehydrogenase activity (7) and glutamine synthetase activity (8). Glutamine synthetase activity is localized to the pericentral hepatocytes (9), whereas the fractions corresponding to maximum lactate dehydrogenase activity correspond to cytoplasmic contents from the periporal cells (10). The fractions corresponding to maximum enzyme activity were combined (periporal and pericentral fractions kept separate), and the glycogen was purified and digested to free glucose with amyloglucosidase for \(^{13}\)C NMR and GC-MS analysis as described previously (4).

Alanine, aspartate, and glutamate were purified from the supernatant from the initial pelleting of glycogen by means of a 0.9 × 150-cm Dowex-50 (200–400 mesh) column equilibrated with ammonium formate (0.1 M, pH 3.0), as described previously (11). The residual glycogen following digitonin treatment was extracted and purified by perchloric acid homogenization and cold ethanol precipitation, dialyzed extensively with water, and digested to free glucose with amyloglucosidase as described previously (4, 11, 16).

**NMR Methodology**—\(^{13}\)C NMR spectra were acquired at 125.76 MHz (AM 500, Bruker Instruments, Inc.) using a standard \(^{13}\)C/H probe (4). Briefly, spectra were acquired using a 30 °C pulse, quadrature detection, digital resolution of 2.7 Hz/point, and with a pulse program for inverse-gated heteronuclear WALTZ decoupling with a delay of 1 s between pulses.

**GC-MS Methodology**—GC-MS analysis was performed with a Hewlett-Packard 5890 gas chromatograph (HP-1 capillary column, 12 m × 0.2 mm × 0.33 μm film thickness) interfaced to a Hewlett-Packard 5971A mass selective detector operating in the positive chemical ionization mode with methane as reagent gas. Glucose was derivatized as the pentaacetate. Mass isotopomer distribution of \(m_1+1\) to \(m_1+6\) (C1 through C6) was determined from the ion intensities of m/z 331–339. Amino acids were derivatized and analyzed by GC-MS as the trifluoroacetyl-n-butyldiesteryl (12). Mass isotopomer distribution of alanine \(m_1+1\) to \(m_1+3\) was determined from the ion intensities of m/z 242–247, aspartame \(m_1+1\) to \(m_1+5\) from m/z 342–348, and glutamate \(m_1+1\) to \(m_1+5\) from m/z 356–363.

Calculations—Mass isotopomer distribution was calculated from the ion distribution in the mass spectrum by the method of Rosenblatt et al. (13).

The percent of glycogen derived from the flux of \([U-{^{13}\text{C}}]\)glucose via the direct pathway was calculated by a modification of an equation derived for use of \([U-{^{13}\text{C}}]\)Glc as a tracer (14, 15),

\[
\%\text{direct} = 100 \times \left( \frac{P_n}{F} \times \left[ \frac{1}{2} \times \sum_{n=1}^{3} m_n \right] + P_4 \right)
\]

where \(P_n\) is proportional to the number of glucose molecules with \(n\) ( \(n = 1–6\) \(^{13}\)C atoms, and \(F\) is the dilution factor of enrichment in C3 intermediates due to entry of unlabeled metabolites into the indirect pathway.

Alternatively, the percent direct pathway was calculated by a modification of equations previously derived for use of \([U-{^{13}\text{C}}]\)Glc as tracer (11),

\[
\%\text{direct} = 100 \times \left( \frac{\text{Gly}_{m=6}}{\text{C. F.} \times \text{Gly}_{m=6}} \right) (\text{Eq. 2})
\]

where \(\text{Gly}_{m=6}\) represents the corrected enrichment of glycogen with \([U-{^{13}\text{C}}]\)glycogenyl units, and \(\text{Gly}_{m=6}\) is the enrichment of portal vein glycogen with \([U-{^{13}\text{C}}]\)Glc. The correction factor, C, F, adjusts for the time for the infusion to reach isotopic steady state in plasma \([U-{^{13}\text{C}}]\)Glc. The MPE of \([U-{^{13}\text{C}}]\)glycogen is corrected for basal glycogen stores with no \([U-{^{13}\text{C}}]\)glycogenyl units, and

\[
\text{Gly}_{m=6} = \frac{\text{Gly}_{m=6}^{\text{basal}} \times (\text{Gly}_{m} \times (\text{Gly}_{m} - \text{Gly}_{m=6})))}{\text{Gly}_{m=6}^{\text{final}}} (\text{Eq. 3})
\]

where \(\text{Gly}_{m=6}^{\text{basal}}\) equals the m + MPE of glycogen determined by GC-MS, \(\text{Gly}_{m=6}^{\text{final}}\) is the final glycogen concentration, and \(\text{Gly}_{m=6}\) is the basal glycogen concentration.

The probability that two \(^{13}\)C-labeled 3-C precursors combine to form \(^{13}\)C glycol by the indirect pathway, \(P_n\) was calculated as follows:

\[
P_n = \left[ \frac{2}{3} \right] \times \left[ \frac{1}{(1 - \text{MPE}^{\text{ind}}/100)} - P_4 \right] (\text{Eq. 4})
\]

where \(\text{MPE}^{\text{ind}}\) is the m + 3 MPE liver alanine, and \(P_4\) is the m + 3 MPE of glycogen (15).

Calculation of the relative \(^{13}\)C isotopic distribution from the NMR signal intensity of overlapping resonances was calculated as previously derived (16).

The results are expressed as means ± S.E. Comparisons between groups were made by the paired student's t test.

**RESULTS AND DISCUSSION**

From the isotopomer frequency in the mass spectrum of periporal and pericentral liver glycogen, plasma glucose, and infusedate (Table I), the MPE of each mass isotopomer was calculated (Table II). During the basal period, plasma glucose concentration was 6.5 ± 0.1 mEq and rose to a plateau of 9.9 ± 0.1 mEq by 30 min of infusion. Plasma glucose \(m_1+6\) MPE (\([U-{^{13}\text{C}}]\)Glc) plateaued at 28.2 ± 2.0 by ~60 min. The MPE of plasma glucose with one to three \(^{13}\)C atoms continued to rise throughout the study, indicative of some hepatic glucose output from gluconeogenesis despite hyperglycemia. To ensure selec-
molecules with a total of 28,064 metabolism is spatially heterogeneous. We found no significant glycogen and to test the hypothesis that liver carbohydrate glycogen recovered from the different fractions and Equation 1, synthesized during the 2-h infusion protocol (4). From each zone was a few percent of the total hepatic glycogen the pericentral and periportal zones (4). Recovery of glycogen similar to that found previously, reflecting selective sampling of the enzyme activity of glutamine synthetase, which occurs only in the pericentral zone (9). Selective zonal cell permeabilization was further verified by light microscopy from liver following a 20-s pulse of digitonin (5 mg/ml) at 20 ml/min (4). Enzyme activities of glutamine synthetase and lactate dehydrogenase recovered in the eluant following the digitonin pulse were similar to that found previously, reflecting selective sampling of the pericentral and periporal zones (4). Recovery of glycogen from each zone was a few percent of the total hepatic glycogen content. We estimate that ~70% of the total liver glycogen was synthesized during the 2-h infusion protocol (4).

Using the mass isotopomer distribution (Table II) in the glycogen recovered from the different fractions and Equation 1, it is possible to calculate the percent direct flux of glucose into glycogen and to test the hypothesis that liver carbohydrate metabolism is spatially heterogeneous. We found no significant difference in the percent direct flux of glucose into glycogen within the either the periporal (34 ± 4) or pericentral (38 ± 4) zones or the residual glycogen (41 ± 1, p = ns). The probability that two 13C-labeled 3-C precursors combine to form [U-13C] glycogen by the indirect pathway can be determined from the MPE of triple-labeled glycogen and alanine (Table II, Equation 4) and was calculated to reduce the estimate of the percent direct pathway by only ~1%. From the enzyme profiles, we estimate ~10% cross-contamination of cytosol from the two zones. Allowing for 20% cross-contamination (i.e. that the calculated percent direct flux represents a mix of 80% glycogen from the upstream zone and 20% from the downstream zone), we calculate that this will decrease our estimate of the direct flux in the periporal zone to 32% and increase the direct flux in the pericentral zone to 41%. Because of hepatic glucose production during the study, the plasma glucose has mass isotopomers in addition to the m + 6 of the infusate. By definition, the plasma glucose m + 1 → m + 3 isotopomers are derived via flux of glucose through the indirect pathway. We, therefore, have not made any corrections in the observed mass isotopomer distribution to account for the increase in the enrichment of the m + 1 → m + 3 isotopomers in the glycogen from direct flux of plasma glucose enriched in isotopomers other than m + 6. However, if we take the extreme case in which all glycogen in the pericentral zone is synthesized via the direct pathway and...
account for the observed isotope pattern in plasma glucose, the direct flux of plasma glucose into pericentral glycogen will decrease the m+1 → m+3 enrichment from 15.5 to 13.5 (15.5 – (6.9/30.2)*(2.6 + 3.6 + 2.5)) and increase the calculated percent direct by only 3%, from 38 to 41%. The combined effect of 20% contamination and 100% direct flux of labeled glucose increases the estimate of the direct pathway in the pericentral zone to 45%. Thus, over half of the glycogen in both zones is synthesized by way of the indirect pathway, and the differential between the two zones is at most only –13%.

These results are in qualitative and quantitative agreement with those of our earlier study (i.e. direct flux was approximately the same in the two zones; periportal, 29%; pericentral, 35%) as determined from a comparison of [1-13C]glycogen enrichment with plasma [1-13C]glucose enrichment (4). However, there is a subtle distinction in the interpretation of the percent direct flux calculated by these two methods. Using [1-13C]glucose as an infusion, we calculate the fraction of glycogen synthesized directly from plasma glucose, the remainder of the glycogen synthesized by flux through the indirect pathway is inclusive of all carbon sources. In contrast, only glycogen that originated from plasma glucose is considered when a dilution factor of 1 is used in Equation 1 to calculate the percent direct flux from the m+1 → m+6 mass isotope distribution of glycogen synthesized from [U-13C]glucose. Therefore, these values represent the maximum contribution of the direct pathway.

Inclusion of the dilution factor, F, in Equation 1 allows for entry of unlabeled 3-carbon precursors (primarily, flux of unlabeled glycerol into dihydroxyaceton-P (DHAP) at the level of triose isomerase), and refines the estimate of the direct pathway by this method (17, 18). The magnitude of the factor, F, in Equation 1 can be estimated by comparing the calculation of direct flux from the mass isotope distribution within glycogen (Equation 1) with a calculation based on the ratio of m+6 enrichment in glycogen with that of plasma glucose (Equation 2). Using Equation 2, and parameters determined previously (4) under similar experimental conditions (basal glycogen concentration, 0.57 g/100 g of wet liver; glycogen synthesis rate, 0.61 µmol/g of liver/min; steady state correction factor, 0.79), we estimate the direct flux to be ~36% in the perportal zone and ~41% in the pericentral zone. The agreement between the two methods of calculating percent direct flux indicates that the dilution of C-3 labeled isotopes is minor and similar in both zones (periportal, 1.3; pericentral, 1.3; p = ns). The dilution by unlabeled carbon we have determined is approximately half that calculated by Katz and Lee (17) using a similar experimental design. However, they make no allowance for correction of basal glycogen stores (which they determined were negligible) or for the time necessary to reach isotopic steady state (which from our experience is ~0.8–0.9). Using a steady-state correction factor of 0.8 and the data presented by Katz and Lee (17), we calculate a value of 62 percent direct pathway and a value of 1.4 for the dilution of the 3-carbon pool by non-glucose carbon in their experiment. Additional dilution of label can occur as the result of flux of labeled precursors through the tricarboxylic acid cycle, E, and can be calculated from the isotope distribution in the liver glycogen using an

![Image](http://www.jbc.org/)

**FIG. 1. Pathways of glycogen synthesis and tricarboxylic acid cycle parameters in perportal and pericentral hepatic zones.** Fraction of glycogen synthesized by the indirect pathway (ind), degree to which equilibration of oxaloacetate to fumarate is complete (O/F), and pyruvate carboxylase to tricarboxylic acid cycle flux (P/T) were calculated from the mass isotope distribution of perportal and pericentral glycogen (Table II) and a model of the tricarboxylic acid cycle developed to account for mass-isotope distribution in glycogen derived from [U-13C]glucose via the indirect pathway (14). Glycogen was selectively isolated by the dual-digitoxin-pulse technique (4, 5) from livers of rats following a 2-h intraduodenal infusion of [U-13C]glucose.

| C | Isotopomer | M* | Residual | Pericentral | Perportal |
|---|---|---|---|---|---|
| 1 | 1-13C | s | 2.1 ± 0.2 | 1.9 ± 0.4 | 1.9 ± 0.1 |
| 1, 2-13C2 and 1, 2, 3-13C3 | d | 15.2 ± 0.6 | 14.3 ± 2.8 | 13.1 ± 2.8 |
| 2-13C | s | 0.5 ± 0.1 | 1.5 ± 0.4 | 0.9 ± 0.2 |
| 1, 2-13C2 and 2, 3-13C3 | d | 4.4 ± 0.2 | 4.5 ± 0.2 | 4.2 ± 0.5 |
| 1, 2, 3-13C3 | t | 12.7 ± 0.5 | 10.7 ± 1.1 | 12.6 ± 0.5 |
| 3-13C | s | 1.3 ± 0.2 | 0.0 ± 0.6 | 0.2 ± 0.3 |
| 2, 3-13C2 and 3, 4-13C3 | d | 5.1 ± 0.4 | 8.4 ± 1.4 | 5.3 ± 0.4 |
| 2, 3, 4-13C3 | t | 11.8 ± 1.8 | 15.8 ± 1.3 | 13.0 ± 0.8 |
| 4-13C | s | 0.9 ± 0.2 | 0.1 ± 0.6 | 0.0 ± 0.3 |
| 3, 4-13C2 and 4, 5-13C3 | d | 3.7 ± 0.1 | 3.1 ± 0.2 | 3.2 ± 0.3 |
| 3, 4, 5-13C3 | t | 10.2 ± 0.6 | 9.8 ± 1.5 | 11.6 ± 0.5 |
| 5 | Not resolved | 17.4 ± 0.2 | 20.9 ± 1.0 | 20.4 ± 1.3 |
| 6 | Not resolved | 14.8 ± 0.7 | 10.7 ± 0.2 | 11.1 ± 0.6 |

*a Multiplicity: s = singlet, d = doublet, t = triplet.

*b Percent of total integrated area.
isomerase equilibration rather than by flux of unlabeled glyceraldehyde-3-P under these experimental conditions is dominated by triose phosphate fructose 1,6-diphosphate from DHAP and glyceraldehyde-3-P.

where \( P_n \) is proportional to the number of glucose molecules that have \( n \) (n = 1-3) \(^{13}\)C atoms. Comparable to the value of 1.4 determined by Katz and Lee (17), we calculate \( E \) to be 1.6 in both the periporal and pericentral zones. The overall dilution, \( T \) (the product of \( E \) and \( F \)), is then \( ~1.8 \) in both zones. This is similar to a value of 1.9 for the overall total dilution of labeled pyruvate into glycogen using the data of Katz and Lee (17), if we incorporate a steady-state correction factor of 0.8 as above. Thus, in either the periporal or pericentral zones of the glycogen derived via the indirect pathway, approximately 44% of the carbon was from sources other than the infused glucose.

Flux through the tricarboxylic acid cycle results in randomization of label and dilution from equilibration of tricarboxylic acid intermediates with other unlabeled metabolic pools. Using a model of the tricarboxylic acid cycle derived to follow the distribution of mass isotopomers in glycogen originating from [U-\(^{13}\)C]glucose, we can calculate the relative rates of pyruvate carboxylase versus citrate synthase (y) and hence determine the extent of dilution of tracer in OAA (R) (14). Under the conditions of glycogen repletion following a fast, there was no significant periporal/pericentral heterogeneity of glycogen metabolism with respect to any of these tricarboxylic acid parameters: pyruvate carboxylase versus tricarboxylic acid cycle flux (y; periporal, 0.70 ± 0.20; pericentral, 0.68 ± 0.16; p = ns), dilution of label in the conversion of pyruvate to OAA (R; periporal, 0.64 ± 0.07; pericentral, 0.64 ± 0.07; p = ns), and extent of OAA:fumarate equilibration (periporal, 0.54 ± 0.01; pericentral, 0.53 ± 0.01; p = ns) (Fig. 1). We calculate similar values for these parameters for the residual liver glycogen following digitonin treatment (y = 0.58 ± 0.10, R = 0.65 ± 0.05, and OAA:fumarate equilibration = 0.58 ± 0.01; p = ns compared to periporal or pericentral glycogen). Equilibration of tricarboxylic acid intermediates (OAA with aspartate and α-KG with glutamine) with other unlabeled pools as a source of isotopic dilution can be determined from analysis of the isotopic enrichment in alanine, aspartate, and glutamate. We found no significant differences between the two zones in the enrichment of intermediates in equilibration with the tricarboxylic acid intermediates (Table II). Therefore, there was no appreciable heterogeneity in the flux of substrates into the tricarboxylic acid cycle between the periporal and pericentral zones.

An index of the relative flux of unlabeled glycerol into DHAP relative to triose isomerization can be determined from the enrichment of glycogen C1-C3 relative to enrichment of C4-C6. Early studies of gluconeogenesis using either \(^{13}\)C-labeled CO\(_2\) or glyceraldehyde demonstrated asymmetric distribution of label in glycogen, suggesting incomplete equilibration of label at the triose-isomerase step (19, 20). From the NMR spectra, relative enrichment of \(^{13}\)C in C1→C3 and in C4[arrow]C6 was assessed (16). No significant differences were seen in the \(^{13}\)C enrichment of glycogen C1[arrow]C3 (derived from DHAP) compared to C4[arrow]C6 (derived from glyceraldehyde-3-P, GAP) in the glycogen isolated from the periporal or pericentral zones (C1→C3[C4]C6: 1.2 ± 0.04, 1.1 ± 0.01, respectively, p = ns). Therefore, distribution of label into fructose 1,6-diphosphate from DHAP and glyceraldehyde-3-P under these experimental conditions is dominated by triose isomerase equilibration rather than by flux of unlabeled glyceraldehyde in both the periporal and pericentral zones.

Further evidence of the similarity of the carbon source for the indirect pathway can be seen in the \(^{13}\)C-NMR spectrum of glycogen. In the \(^{13}\)C-NMR spectra of hepatic glycogen following an infusion of [U-\(^{13}\)C]glucose, multiple isotopomers are observed due to coupling of magnetic spin between adjacent \(^{13}\)C, making it possible to distinguish labeled atoms that have 0, 1, or 2 \(^{13}\)C as nearest neighbors. Positional isotopomers of \(^{13}\)C-labeled glycogen can thus be characterized by analysis of the splitting patterns in the \(^{13}\)C-NMR spectra (Table III). The splitting pattern in C3-C4 of periporal and pericentral glycogen will differ if there are relative differences in the number of labeled 3-carbon precursors combining to form glycogen. Fig. 2 is typical of the splitting pattern seen in the \(^{13}\)C-NMR spectra of \(\alpha\)-C3 and \(\alpha\), \(\beta\)-C4 of periporal and pericentral glycogen. The triplet at C3 is the superposition of a triplet due to glycogen labeled at C2-C3 and C4, with a singlet from molecules labeled at C3 with no label at either C2 or C3. The doublet at C3 is the resonance due to glycogen molecules with label at C2 and C3 but not C4, and with label at C3 and C4 but not C2. The doublet is due to isotopomers derived solely via the indirect pathway, whereas the triplet resonance (minus the singlet) is due to glycogen derived mainly from the direct pathway. Interpretation of the multiplet structure of C4 is analogous to that of C3. The similarity of the splitting pattern (doublet to triplet) at C3 (periporal, 0.53 ± 0.9; pericentral, 0.41 ± 0.6; p = ns) and C4 (periporal, 0.33 ± 0.4; pericentral, 0.29 ± 0.3, p = ns) indicates that the glycogen derived from the indirect pathway has an equivalent distribution of the C3 and C4 positional isotopomers in the two zones.

In conclusion, we found no isotopic evidence in support of hepatic heterogeneity with respect to either the amount or the
source of the 3-carbon precursors used to synthesize glycogen via the indirect pathway. Furthermore, there was no difference between the periportal and pericentral zones with regard to the flux of the 3-carbon precursors through the tricarboxylic acid cycle.

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