In Vitro Modeling of Fatty Acid Synthesis under Conditions Simulating the Zonation of Lipogenic [13C]Acetyl-CoA Enrichment in the Liver*

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In the companion report (Bederman, I. R., Reszko, A. E., Kasumov, T., David, F., Wasserman, D. H., Kelleher, J. K., and Brunengraber, H. (2004) J. Biol. Chem. 279, 43207-43216), we demonstrated that, when the hepatic pool of lipogenic acetyl-CoA is labeled from [13C]acetate, the enrichment of this pool decreases across the liver lobule. In addition, estimates of fractional synthesis calculated by isotopomer spectral analysis (ISA), a nonlinear regression method, did not agree with a simpler algebraic two-isotopomer method. To evaluate differences between these methods, we simulated in vitro the synthesis of fatty acids under known gradients of precursor enrichment, and known values of fractional synthesis. First, we synthesized pentadecanoate from [U-13C3]propionyl-CoA and four gradients of [U-13C6]malonyl-CoA enrichment. Second, we pooled the fractions of each gradient. Third, we diluted each pool with pentadecanoate prepared from unlabeled malonyl-CoA to simulate the dilution of the newly synthesized compound by pre-existing fatty acids. This yielded a series of samples of pentadecanoate with known values of (i) lower and upper limits for the precursor enrichment, (ii) the shape of the gradient, and (iii) the fractional synthesis. At each step, the mass isotopomer distributions of the samples were analyzed by ISA and the two-isotopomer method to determine whether each method could correctly (i) detect gradients of precursor enrichment, (ii) estimate the gradient limits, and (iii) estimate the fractional synthesis. The two-isotopomer method did not identify gradients of precursor enrichment and underestimated fractional synthesis by up to 2-fold in the presence of gradients. ISA uses all mass isotopomers, correctly identified imposed gradients of precursor enrichment, and estimated the expected values of fractional synthesis within the constraints of the data.

In the companion report (1), we demonstrated that the mass isotopomer distributions (MID),1 of fatty acids and sterols isolated from livers of conscious dogs infused with tracer [1,2-13C2]acetate in the portal vein, and (ii) rat livers perfused with 10 mM [1,2-13C2]acetate, are not compatible with a constant enrichment of lipogenic acetyl-CoA across the liver lobule. We concluded that gradients of precursor enrichment occur even in the presence of flooding [1, 2-13C2]acetate concentrations. This probably results from the inverse zonations (2) of the activities of glycolytic (2–5) and lipogenic enzymes (7–9) (perivenous > periportal) versus the activity of cytosolic acetyl-CoA synthase (periportal > perivenous) (10). Gradients of precursor enrichment were detected using isotopomer spectral analysis (ISA) (11). In addition, we found that fractional lipogenesis calculated by the two-isotopomer method (an algebraic method similar to that described by Chinkes et al. (12)) produces lower estimates of fractional synthesis than those produced by the best fit estimates of ISA. Although the “linear gradient” ISA model (see companion report (1) for model definitions) yielded a better fit than the “Single pool” ISA model, it was not possible to evaluate the effect of gradients on estimates of fractional synthesis. Thus, we could not quantitatively evaluate the performance of the ISA in comparison to the two-isotopomer method, because the true rates of fractional synthesis in the liver dog and rat liver perfusion study were unknown.

The goal of the present study was to evaluate the differences in estimates of precursor enrichment and fractional synthesis calculated by the two-isotopomer method and ISA. We used an experimental model where both the gradient in precursor enrichment and the fractional synthesis are known. This was accomplished by in vitro preparations that simulated the zonation of acetyl-CoA enrichment. Lipogenesis from sub-populations of hepatocytes across the liver lobule was simulated, in parallel incubations, by synthesizing a fatty acid using purified fatty acid synthase (13, 14) and [U-13C6]malonyl-CoA of varying enrichment. We used gradients of malonyl-CoA enrichment, because fatty acid synthesis involves the conversion to malonyl-CoA of all acetyl units added to the primer. We used [U-13C6]propionyl-CoA as a primer to avoid the possibility of contamination of our newly synthesized pentadecanoate with unlabeled pentadecanoate. In the presence of unlabeled malonyl-CoA, the process yields M32 [13,14,15-13C3]pentadecanoate. By monitoring the distribution of M3 to M15 isotomers of pentadecanoate, we simulated in vitro the polymerization of six [13C]acetyl units using GC-MS, gas chromatography-mass spectrometry; ISA, isotopomer spectral analysis.

1 The abbreviations used are: MID, mass isotopomer distribution; M, M1, M2 . . . Mi are the intensities of the mass spectrometric signals of the corresponding isotopomers.

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into a C-12 fatty acid, for multiple values of acetyl enrichment. Our goal was to simulate lipogenesis as it occurs in a real liver (i) under gradients of acetyl-CoA $^{13}$C enrichment and (ii) in the presence of unlabeled lipids. To achieve this goal, we monitored the MID of pentadecanoate from (i) sets of incubations with progressively decreasing malonyl-CoA enrichments, (ii) pools of incubations from each set, and (iii) pools of incubations spiked with increasing amounts of "unlabeled" $^{[13,14,15-13]$C$_3$]pentadecanoate. The data were analyzed by the two-isotopomer method and by ISA (11, 15).

**EXPERIMENTAL PROCEDURES**

**Materials**

Chemicals, biochemicals, and enzymes were purchased from Sigma-Aldrich. Pentafuorobenzyl bromide was from Pierce. Bovine serum albumin (fraction V, fatty acid poor) was purchased from Miles Biochemicals, and dialyzed as a 15% solution against Krebs-Ringer bicarbonate buffer for 48 h. [U-$^{13}$C$_3$]Propionate and [U-$^{13}$C$_3$]malonic acid (99% enriched) were from Isotec. [U-$^{13}$C$_3$]Mysiric acid and [U-$^{13}$C$_3$]propionyl-CoA were prepared from the corresponding acids and purified as reported previously (16, 17). Fatty acid synthase was isolated from livers from rats that were first starved for 2 days then re-fed with a high glucose diet for 2 days (13). The enzyme was precipitated with ammonium sulfate from the effluent of an Ultragel AcA-34 column, and the suspension was kept frozen in small aliquots at -80 °C. The enzyme was used as an ammonium sulfate suspension (1 unit/ml).

**In Vitro Synthesis of Pentadecanoate**

Theory—The protocol was conceived to simulate decreasing gradients of $^{13}$C enrichment of lipogenic acetyl-CoA across the liver lobule. Fatty acid synthesis involves the addition to a primer molecule (usually acetyl-CoA) of malonyl-CoA molecules formed by carboxylation of acetyl-CoA. Thus, gradients of acetyl-CoA enrichment can be reflected by gradients of malonyl-CoA enrichment. Because we wanted the acetyl units added to the primer to be labeled on both carbons, we created gradients of [U-$^{13}$C$_3$]malonyl-CoA enrichment. In the process of fatty acid synthesis, carbon 3 of [U-$^{13}$C$_3$]malonyl-CoA is lost as $^{13}$CO$_2$. Four protocols were followed to generate gradients of malonyl-CoA enrichment within four series of incubations. For three series of 15 incubations each, the gradients of M3 enrichment of malonyl-CoA were generated from 65% to 10% with the three profiles shown below in Fig. 1 (continuous lines). Note that the range of values for in vitro gradients from 65% to 10% was not chosen randomly. We observed a similar range of precursor enrichments in our *in vivo* models (see companion report (1)). In a fourth series of seven incubations, the M3 enrichment of malonyl-CoA decreased linearly from 10% to 0%. Control incubations were conducted with unlabeled malonyl-CoA and resulted in the formation of $^{[13,14,15-13]$C$_3$]pentadecanoate. The latter represents an unlabeled species, because it was prepared from unlabeled malonyl-CoA. In our simulation of liver lipogenesis, [U-$^{13}$C$_3$]malonyl-CoA was incorporated into the newly synthesized labeled fatty acid. When pentadecanoate synthesis is conducted with 97% enriched [U-$^{13}$C$_3$]propionyl-CoA and [U-$^{13}$C$_3$]malonyl-CoA of various enrichments, the MID of pentadecanoate ranged from M3 up to M15 (Fig. 2).

Incubations—For each set of incubations, we prepared 15 or 7 solutions of malonyl-CoA of decreasing enrichment by mixing high-performance liquid chromatography-standardized stock solutions of unlabeled and M3 malonyl-CoA. To verify the malonyl-CoA enrichments and precursor enrichments in our experiments, we conducted with unlabeled malonyl-CoA and resulted in the formation of $^{[13,14,15-13]$C$_3$]pentadecanoate.

The equation is constructed so that as $c$ decreases, $D_{\text{max}}$ and $D_{\text{min}}$ are the upper and lower limits for the variable $D(c)$ given by the following relationship,

$$D(c) = D_{\text{min}} - D_{\text{max}}(c/14) + D_{\text{max}}$$ (Eq. 1)

where $c$ is an integer ranging from 0 to 14, and $D_{\text{max}}$ and $D_{\text{min}}$ are the upper and lower limits for the variable $D(c)$. By solving for linear $D(c)$ at each value of $c$, the mixture of labeled $D(c)$ and natural malonyl-CoA (1 - $D(c)$) is specified for each of the 15 samples comprising the gradient. The equation is constructed so that the $c$ increases from 0 to 14, the value of $D(c)$ decreases. $D_{\text{max}}$ and $D_{\text{min}}$ were set at 0.85 and 0.1, respectively. The low limit was set at 0.01, respectively. The low linear gradient was constructed similarly with $c$ ranging from 0 to 5, and $D_{\text{max}}$ and $D_{\text{min}}$ were set at 0.1 and 0.01, respectively.

To compare the effect of gradient shape on the fit of model to data, two additional equations were used to generate concave and convex gradients with 15 distinct values of $D(c)$.
Concave

\[ D_{\text{c}} = (D_{\text{min}} - D_{\text{max}})[1 - e^{\left(\frac{D_{\text{max}}}{D_{\text{max}} - D_{\text{max}}}\right)}] + D_{\text{max}} \quad (\text{Eq. 2}) \]

Convex

\[ D_{\text{c}} = (D_{\text{min}} - D_{\text{max}})[e^{\left(\frac{D_{\text{max}}}{D_{\text{max}} - D_{\text{max}}}\right)}] + D_{\text{max}} \quad (\text{Eq. 3}) \]

where \( k \) specifies the degree of nonlinearity of the concave and convex gradients. \( k \) was set to 5.

ISA Models for Gradients—A key feature of ISA is that it uses all measurable isotopomer data to find the best fit of model to data. As originally designed (11), ISA solves for two unknown parameters, the precursor enrichment, \( D \), and the fraction of new synthesis at the time of sampling \( g(t) \). However, the nonlinear regression feature of ISA allows for models with additional parameters. First, gradients in precursor enrichment are modeled via ISA in discrete steps. We use 15 steps to model the gradients for ISA computed exactly as for the \( \text{in vitro} \) synthesis procedure described above. For each step of the gradient a different value is used for the precursor enrichment, \( D_{\text{c}} \), as indicated by the equations above. The gradient is created by combining the values for all isotopomers for the 15 steps of the gradient and computing the fractional abundances for the combined gradient. Second, as with the conventional form of ISA, the program compares the fractional abundance values for isotopomers between data and model by calculating the weighted sum of square errors. The program searches for the best fit values of the three parameters, \( D_{\text{min}}, D_{\text{max}}, \) and \( g(t) \) yielding the smallest error using the Levenberg-Marquardt algorithm (11). ISA requires no correction for natural \(^{13}\)C abundance, which is included in the model. A spreadsheet is included in the Supplementary Materials to demonstrate how the gradient ISA fractional abundances are created. The algebraic equations describing the steps of the gradient were developed with the assistance of the symbolic algebra facility of Mathcad (Maple) (Mathsoft, Cambridge, MA). Although the spreadsheet provides sample calculations, it does not have the capacity to perform the complete ISA calculations. The ISA program requires additional modeling that is not available in Excel; this allows finding the best-fit solution for all isotopomer equations simultaneously. For additional details about the ISA program, contact one of us (J. K. K.).

The Two-isotopomer Method—We used the following two-isotopomer equations to compute precursor enrichment, \( p \), and fractional synthesis, \( f \), using the notation of Hellerstein (19),

\[ p = \frac{(2M_i)}{(5M_i) + (2M_i)} \quad (\text{Eq. 4}) \]

\[ f = \frac{M_i / \gamma M}{6p(1 - p)} \quad (\text{Eq. 5}) \]

where \( M_i \) is the intensity of the signal for various isotopomers corrected for natural abundance. These equations are identical to those de-
enrichment is identical in all cells and does not change with time. The metabolic zonation of the liver (1), resulting from the organ’s lobular architecture that functions as a plug-flow reactor (29), poses particular challenges to the measurement of fractional synthesis of biopolymers by MID analysis using this assumption. This is because each cell along the liver lobule is in contact with blood of continuously changing composition in terms of substrate concentrations and isotopic enrichment of tracers. Several studies reported that the concentration and enrichment of glyceraldehyde 3-phosphate (30, 31), acetate (32), and acetate (20) markedly decreases across the liver. In addition, the activities of enzymes involved in the synthesis of biopolymers also vary across the lobule. For example, there is an inverse zonation of the enzymes, which fuels lipogenesis (glycolysis (3) and ATP-citrate lyase and fatty acid synthase (7–9)) and cytosolic acetyl-CoA synthetase (which introduces label from $[^{13}\text{C}]$acetyl-CoA in the lipogenic pathway (10)).

In the companion report (1), we demonstrated the existence of translobular gradients of enrichment of lipogenic acetyl-CoA (labeled from $[1,2^{13}\text{C}]$acetate) in the livers of live dogs and in perfused rat livers. In the latter animal preparations, ISA indicated the presence of a gradient of acetyl-CoA enrichment even in the absence of gradients of acetate concentration and enrichment across the liver. The MIDs of fatty acids isolated from the various livers were analyzed by the two-state-of-the-art computation techniques, i.e. the two-isotopomer method (modified from Chinkes (12)) and by ISA (11). The two-isotopomer method, like the more widely applied MID analysis method (19, 33, 34), assumes that the precursor enrichment is constant in all cells and does not change with time. The metabolic zonation of the liver (1), resulting from the organ’s lobular architecture that functions as a plug-flow reactor (29), poses particular challenges to the measurement of fractional synthesis of biopolymers by MID analysis using this assumption. This is because each cell along the liver lobule is in contact with blood of continuously changing composition in terms of substrate concentrations and isotopic enrichment of tracers. Several studies reported that the concentration and enrichment of glyceraldehyde 3-phosphate (30, 31), acetate (32), and acetate (20) markedly decreases across the liver. In addition, the activities of enzymes involved in the synthesis of biopolymers also vary across the lobule. For example, there is an inverse zonation of the enzymes, which fuels lipogenesis (glycolysis (3) and ATP-citrate lyase and fatty acid synthase (7–9)) and cytosolic acetyl-CoA synthetase (which introduces label from $[^{13}\text{C}]$acetyl-CoA in the lipogenic pathway (10)).

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that a sufficient number of isotopomers is detected to test for the occurrence of gradients.

In this study, we modeled the zonation of enrichment of lipogenic acetyl-CoA in liver by setting up four sets of incubations in which pentadecanoate was synthesized from [U-13C3]propionyl-CoA and lots of [U-13C3]malonyl-CoA of decreasing enrichment. Each incubation simulates a population of hepatocytes, which synthesizes pentadecanoate from a pool of malonyl-CoA of defined enrichment. Each set of incubations simulates one gradient of precursor enrichment of a given shape across the liver lobule. We isolated and measured each of the four gradients of [U-13C]malonyl-CoA enrichment used in the study. Fig. 1 (symbols) shows measured gradients of precursor enrichment. Fig. 1 (continuous lines) shows the expected shape of the corresponding gradients. It is evident that data points that fit well with the predicted curves (regression of all data has \( r^2 = 0.99 \)).

Fig. 2 illustrates the influence of varying the enrichment of M3 malonyl-CoA on the MID of pentadecanoate synthesized from M3 propionyl-CoA. As expected, the profile of mass isotopomers of pentadecanoate shifts to the right as the enrichment of malonyl-CoA increases. The small amounts of M to M2 isotopomers correspond to traces of natural pentadecanoate present in the fatty acid synthase preparation. The priming of pentadecanoate synthesis with M3 propionyl-CoA avoids the interference of the M to M2 isotopomers of pentadecanoate with the computations. This is why, in the context of the present study, we consider M3 pentadecanoate prepared from naturally labeled malonyl-CoA as an unlabeled species.

To test whether the MIDs of newly synthesized pentadecanoate match the theoretical distributions of mass isotopomers, we plotted these theoretical distributions in Fig. 3 (continuous lines) and superimposed the measured MIDs of six samples of pentadecanoate synthesized from M3 propionyl-CoA and lots of M3 malonyl-CoA of increasing enrichments. Each symbol corresponds to a lot of pentadecanoate made from a given malonyl-CoA enrichment. The MIDs used for Fig. 3 were taken from the linear gradient experiment (from 65% to 10%). Note that the symbols of each set of data points fall on the theoretical curves. This was expected, because in each incubation, fractional pentadecanoate synthesis is 100%. Indeed, when the data of all individual incubations from the four gradients are computed using the two-isotopomer method, the average fractional synthesis is 0.96 with a coefficient of variation of 3.7%. \((n = 51)\). This reflects the precision of our measurements of isotopomer distributions.

In each of the 51 incubations, we calculated the enrichment of the malonyl-CoA precursor from the measured MIDs of pentadecanoate, using both ISA and the two-isotopomer method. In the case of ISA, we used the “Single pool” model that assumes constant precursor enrichment. Fig. 4 shows the excellent agreement between (i) the enrichments of malonyl-CoA calculated using either the two-isotopomer method (Fig. 4A) or ISA (Fig. 4B) and (ii) the actual (measured) enrichments of malonyl-CoA \((96\% , CV = 3.7\% , \text{for both models})\). This confirms that the MIDs of pentadecanoate were measured under optimal conditions.

In the second phase of the experiment, we simulated the extraction of a real liver by pooling equal aliquots of all incubations from each gradient. The MIDs of the four “pools” were analyzed by the two-isotopomer method and by ISA. In the latter, we used the “Gradient” model, which assumes a range of precursor enrichments. From the conditions imposed in the protocol, we knew the exact limits of the gradients of precursor enrichment \((D_{\text{max}} \text{ and } D_{\text{min}})\), as well as the average values of precursor enrichments \((D_{\text{average}})\). We also knew that fractional synthesis was 100% in each pool.

We had therefore valid reference values for the parameters to be calculated from the two-isotopomer method and the “Gradient” ISA model. The latter model yielded correct limits for all imposed gradients (Table I, compare columns 2 and 4 to columns 3 and 5). The values of precursor enrichment calculated from the gradient ISA model were then used to determine the MIDs of pentadecanoate in each pooled gradient, assuming precursor enrichments from 65% to 10% or to 1% (Low linear gradient). For each gradient, the ISA program was given the gradient shape and the value of \(k\). The dark bars of Fig. 5 show the measured MIDs of pentadecanoate in each of the four pooled gradients. The white bars of Fig. 5 show the tight fitting of the experimental MIDs to the gradient model of ISA.

For all gradients, the two-isotopomer method computations yielded only a single value of precursor enrichment, \(p\). In the case of the three large gradients (Concave, Convex, and Linear), \(p\) was substantially below the average value \((D_{\text{average}})\). For the Low linear gradient, \(p\) was higher than \(D_{\text{average}}\) (Table I, last column). Table II shows the values of fractional synthesis computed by ISA (single-pool model) and the two-isotopomer method from the MIDs of each mixed pool. The expected 100% value of fractional synthesis was slightly underestimated \((<3.5\%)\) by ISA and substantially underestimated \((25\% \text{ to } 54\%)\) by the two-isotopomer method.
In the third phase of the experiment, the four pools of pentadecanoate were increasingly diluted with known amounts of unlabeled M3 pentadecanoate to simulate the dilution of newly synthesized fatty acids by the pre-existing hepatic fatty acids. Therefore, the limits of the gradient, \( D_{\text{max}} \) and \( D_{\text{min}} \), were known as was the expected fractional synthesis, which equaled 1 minus the fraction of the unlabeled pentadecanoate added to the sample. To put the range of fractional synthesis into perspective, note that values of fractional synthesis reported in in vivo experiments are below 10% under normal dietary conditions (see Refs. 12 and 33 and Table I of the companion report (1)) but can exceed 40% under hypercaloric diets (35, 36). Each diluted sample was analyzed by GC-MS, and the MID was analyzed by ISA and by the two-isotopomer method. Each

| Shape of gradient | \( D_{\text{max}} \) | \( D_{\text{min}} \) | \( D_{\text{average}} \) | Apparent PE computed by 2 \(-\text{M}_i\) | Ratio PE\(_{\text{apparent}}\)/\( D_{\text{average}} \) |
|------------------|----------------|----------------|----------------|----------------|----------------|
| Linear           | 64.3           | 65.2           | 9.7            | 8.9            | 36.5           | 29.3           | 0.80          |
| Concave          | 66.8           | 68.4           | 10.7           | 10.4           | 22.9           | 18.7           | 0.82          |
| Convex           | 65.2           | 63.6           | 10.3           | 7.6            | 53.2           | 38.6           | 0.73          |
| Low linear       | 10.4           | 12.1           | 0.9            | 2.6            | 4.7            | 7.9            | 1.68          |

**Fig. 5. Comparisons between the measured MID (solid bars) of pentadecanoate from each gradient pool with the MID of each pool calculated from the gradient model of ISA (light bars).**
The measured MIDs of the pooled fractions of each gradient were introduced into ISA and the two-isotopomer method to calculate values of fractional synthesis. Note that, in all cases, fractional synthesis was 100% as imposed by the experimental protocols.

Next, consider mathematically generated data of fractional abundances assuming a linear gradient of precursor enrichments ranging from $D_{\text{max}} = 0.65$ to $D_{\text{min}} = 0.1$ with $g(t) = 0.7$. The range of possible solutions for each isotopomer is plotted for a model assuming a single value for $D$ (Fig. 8B). Note that the continuous isotopomer lines no longer intersect at a single point. This is because the data are not consistent with a model that assumes a single value of $D$. The two-isotopomer method can find a solution by choosing any of the intersection of pairs of isotopomer lines. However, the “solution” for each pair of adjoining isotopomer lines is different and none are the statistical “best fit.” For example, the M5/M7 pair of lines cross at $p = 0.297$ and $f = 0.495$, which is identical to the solution found using the two-isotopomer equations for pentadecanoate presented above. However, these values for $p$ and $f$ do not produce a good fit to the entire isotopomer spectrum as shown in Fig. 8C. Although they fit M5 and M7 perfectly, this fit overestimates M0 and underestimates masses greater than M7. The effect of this forced fit to M5 and M7 is an underestimation of fractional synthesis computed by the two-isotopomer method. The ISA approach to data is different, because it uses all of the isotopomer data and a regression approach that provides an estimate of how well the model fits the data. In the example of a gradient of precursor enrichment illustrated in Fig. 8B, the ISA model uses a constant value for $D$ to estimate for $D = 0.376$ and $g(t) = 0.628$. Also, ISA produces a statistical evaluation of the computation, indicating that this solution is a poor fit of model to data characterized by a large sum of squares error, 0.005. If a linear gradient ISA model is used instead, this model finds a solution ($D_{\text{max}} = 0.65$, $D_{\text{min}} = 0.1$, and $g(t) = 0.7$) with a very small error, less than $10^{-14}$. Thus, the linear gradient model is a better choice for these data. Fig. 8B also demonstrates that all two-isotopomer equations using adjoining pairs of isotopomers, such as M7/M9 and other pairs, underestimate fractional synthesis when a gradient in precursor enrichment is present. Thus, the poor outcome of the two-isotopomer
The method is independent of the choice of the pair of adjoining isotopomers used for the computation of the above parameters. (See also Supplementary Material for the two-isotopomer approach.)

Other researchers have previously discussed the effects of gradients in precursor enrichment. Hellerstein and Neese (34) acknowledged that gradients in precursor enrichment can be detected by variations in the estimated precursor enrichment as shown in Fig. 8B. Lee et al. (37) also noted that the gradients of precursor enrichment lead to incorrect estimates of fractional synthesis. Here, we show that ISA can both detect gradients and correctly estimate precursor enrichment and frac-
tional synthesis when the data are of high quality, i.e., being close to the theoretical values and providing sufficient number of detectable mass isotopomers. However, note that all reported in vivo measurements of fatty acid and sterol synthesis labeled from \([13\text{C}]\)acetate (12, 19, 33, 36, 38–41) were conducted under conditions that yield low precursor enrichment, and thus low abundances of heavy mass isotopomers of the biopolymers. These conditions are similar to the in vitro synthesis conditions simulated by our Low linear gradient \((D_{\text{max}} = 0.1)\) (Fig. 1).

Indeed, the MID of pentadecanoate synthesized in this gradient includes only low abundances of light mass isotopomers with two or four \(^{13}\text{C}\) atoms derived from \([U-^{13}\text{C}]\)malonyl-CoA (bottom panel of Fig. 5). In most in vivo studies of fatty acid and sterol synthesis, polymers are labeled from plasma \([^{13}\text{C}]\)acetate of low enrichment and are diluted by the abundant endogenous species. Under these conditions, the sampled polymers, i.e. fatty acids and sterols, do not include sufficient amounts of labeled mass isotopomers. Thus, one is unable to evaluate the possibility of gradients (33, 36, 39). However, if gradients are present, our analysis indicates that applying methods that assume constant precursor enrichment results in underestimated fractional lipogenesis.

An unresolved question is the magnitude of the gradient in vivo when the enrichment is low. Assuming that supplying the tracer at high enrichment and concentration yields a linear gradient and 5-fold change in enrichment across the liver, \(D_{\text{max}} = 0.5\) and \(D_{\text{min}} = 0.1\). This does not imply that tracer supplied at low enrichment to yield a \(D_{\text{max}}\) of 0.05 would also produce a linear gradient with \(D_{\text{min}} = 0.01\). If the liver extracts acetate vigorously, at low enrichment, the precursor labeling may decrease to undetectable level at some point across the lobule (20). Then, the newly synthesized polymer in the downstream area of the lobule would be unlabeled and would not be distinguishable from the pre-existing polymer. This scenario would result in large underestimates of fractional synthesis. Additional studies are required to determine whether gradients at low enrichment are simply scaled versions of high enrichment gradients and whether their shape and endpoints are affected by the concentration of acetate.

What are the implications of the above findings on estimates of hepatic fractional lipogenesis by the two-isotopomer and the ISA models in the companion report (1)? In the companion study we imposed high levels of precursor enrichment in dog livers and in perfused rat livers, to ensure the presence of multiple mass isotopomers. Thus, the conditions were similar to those of the in vitro constructed gradients using \(D_{\text{max}} = 0.65\). Also, in the companion report we provide convincing evidence that, in the presence of \([^{13}\text{C}]\)acetate, the enrichment of lipogenic acetyl-CoA follows sharp descending gradients across the liver. The present report validates the ability of ISA to detect gradients of precursor enrichment, to estimate the limits of these gradients (Fig. 6), and to generate reliable values of fractional synthesis (Fig. 7) under gradient conditions. These validations support the conclusions of the companion report.

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