Zonation of Labeling of Lipogenic Acetyl-CoA across the Liver

IMPLICATIONS FOR STUDIES OF LIPOGENESIS BY MASS ISOTOPOMER ANALYSIS*

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Measurement of fractional lipogenesis by condensation polymerization methods assumes constant enrichment of lipogenic acetyl-CoA in all hepatocytes. Mass isotopomer distribution analysis (MIDA) and isotopomer spectral analysis (ISA) represent such methods and are based on the combinatorial analyses of mass isotopomer distributions (MIDs) of fatty acids and sterols. We previously showed that the concentration and enrichment of \(^{13}\text{C}_2\)acetate decrease markedly across the dog liver because of the simultaneous uptake and production of acetate. To test for zonation of the enrichment of lipogenic acetyl-CoA, conscious dogs, perfused with transhepatic catheters, were infused with glucose and \([1,2-^{13}\text{C}_2]\)acetate in a branch of the portal vein. Analyses of MIDs of fatty acids and sterols isolated from liver, bile, and plasma very low density lipoprotein by a variant of ISA designed to detect gradients in precursor enrichment revealed marked zonation of enrichment of lipogenic acetyl-CoA. As control experiments where no zonation of acetyl-CoA enrichment would be expected, isolated rat livers were perfused with 10 mM \([1,2-^{13}\text{C}_2]\)acetate. The ISA analyses of MIDs of fatty acids and sterols from liver and bile still revealed a zonation of acetyl-CoA enrichment. We conclude that zonation of hepatic acetyl-CoA enrichment occurs under a variety of animal models and physiological conditions. Failure to consider gradients of precursor enrichment can lead to underestimations of fractional lipogenesis calculated from the mass isotopomer distributions. The degree of such underestimation was modeled in vitro, and the data are reported in the companion paper (Bederman, I. R., Kasumov, T., Reszko, A. E., David, F., Brunengraber, H., and Kelleher, J. K. (2004) J. Biol. Chem. 279, 43217-43226).

The fractional synthesis rate of polymers can be determined by the mass isotopomer distribution (MID)\(^1\) of the polymer following synthesis in the presence of a \(^{13}\text{C}\)-labeled precursor.

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\(^1\) The abbreviations used are: MID, mass isotopomer distribution; GC-MS, gas chromatography-mass spectrometry; ISA, isotopomer spectral analysis; MIDA, mass isotopomer distribution analysis; MPE, molar percent enrichment; VLDL, very low density lipoprotein.

Mass isotopomer\(^2\) distribution analysis (MIDA) was proposed as a method for estimating the fractional synthetic rate of fatty acids and cholesterol synthesized \textit{in vivo} and \textit{in vitro} in the presence of \([^{13}\text{C}]\)acetate (1-3). MIDA uses the enrichment of two mass isotopomers to calculate precursor enrichment and fractional synthesis. Another approach, isotopomer spectral analysis (ISA) was presented by Kelleher and Masterson (4); for reviews, see Refs. 5 and 6). These and other approaches (3, 7) provide estimates of the precursor enrichment and the fractional synthesis rate.

MIDA (2), the other two-isotopomer method presented by Chinkes et al. (7), and the basic form of ISA (4) yield a single value for the enrichment of the acetyl-CoA monomer precursor. It is therefore assumed that the precursor enrichment is constant in all cells that synthesize the polymer. When the enrichment of the precursor is not constant, models using single values for precursor enrichment cannot provide a measure of this variation in precursor enrichment and may incorrectly estimate fractional synthesis. The reason for this is that the equations of the models are not linear in terms of precursor enrichment. Thus, models based on average values for acetate enrichment cannot be guaranteed to provide the best estimates of the parameters. (For a detailed discussion, see Ref. 5.) In contrast, the equations of the models are linear in terms of fractional synthesis and thus variations in fractional synthesis are correctly estimated as the average value. In this work, we modified the equations of ISA to allow for variations in precursor enrichment, and tested the hypothesis that gradients of lipogenic \([^{13}\text{C}]\)acetyl-CoA occur \textit{in vivo}.

In a previous study, we found that, in dogs infused with \([1,2-{^{13}\text{C}_2}]\)acetate in a peripheral vein, the concentration and enrichment of acetate decrease 3- and 7-fold, respectively, as blood passes through the liver (8). This suggested that the enrichment of lipogenic acetyl-CoA also decreases across the liver lobule. The goal of the project reported here was to probe the homogeneity of the acetyl-CoA labeling by the ISA analyses of polymeric compounds derived from acetyl-CoA, i.e. fatty acids (myristate and palmitate) and sterols (lathosterol and cholesterol). Because ISA requires computations of the abundances of multiple mass isotopomers, conditions were chosen to ensure fairly high labeling of lipogenic acetyl-CoA. Two animal models were used: (i) conscious dogs infused with \([1,2-{^{13}\text{C}_2}]\)acetate in a branch of the portal vein and (ii) livers, isolated from rats fed either a control diet or a triacetin-enriched diet, perfused with 10 mM \([1,2-{^{13}\text{C}_2}]\)acetate. The second

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\(^2\) Mass isotopomers are designated as M, M\(_1\), M\(_2\) . . . M\(_i\), where \(i\) is the number of mass units above that of the unlabeled isotopomers M. The subscripted notations M\(_i\), M\(_{i-1}\), . . . M\(_1\) are the intensities of the mass spectrometric signals of the corresponding isotopomers.
model was used as a control where the enrichment of lipogenic acetyl-CoA was expected to be nearly constant across the liver because of the high concentration of [1,2-\(^{13}\)C]\(^2\)acetate in the portal vein. When we unexpectedly found evidence of zonation of labeling of acetyl-CoA despite the 10 mM [1,2-\(^{13}\)C]\(^2\)acetate concentration, we attempted to abolish this zonation by using livers isolated from rats fed a diet containing triacetin, the triglyceride of acetate. ISA was used to evaluate the hypothesis that gradients of lipogenic acetyl-CoA enrichment better characterize the observed isotopomer data for these models. Our data indicate that a decrease in the enrichment of lipogenic acetyl-CoA across the liver of conscious dogs. Unexpectedly, similar zonation of enrichment of acetyl-CoA occurs in livers perfused with a high concentration of [1,2-\(^{13}\)C]\(^2\)acetate. The data of all experiments indicated that the fractional synthetic rates calculated from the MIDs of lipids were underestimated to an unknown degree. This is why this study was complemented by an in vitro modeling of fatty acid synthesis under conditions simulating the zonation of the enrichment of lipogenic acetyl-CoA in liver (see companion report (9)).

EXPERIMENTAL PROCEDURES

Materials—Chemicals were purchased from Sigma-Aldrich. Pentfluorobenzyl bromide was from Pierce. Sodium [1,2-\(^{13}\)C]\(^2\)acetate, [U-\(^{13}\)C]malonic acid, and sodium [1-\(^{13}\)C,2\(^{2}\)H\(_3\)]acetate (99%) were from Isotec.

Live Dog Experiments—Mongrel dogs (18–26 kg) of either gender, that had been fed a standard diet (Kal Kan beef dinner, Vernon, CA and Wayne Lab Blox: 51% carbohydrate, 31% protein, 11% fat, and 7% fiber based on dry weight, Allied Mills, Inc., Chicago, IL), were studied. The dogs were housed in a facility that met Association for Assessment of Laboratory Animal Care guidelines, and the protocols were approved by Vanderbilt University Institutional Animal Care and Use Subcommittee. The Silastic catheters were installed for infusion of labeled acetate and for blood sampling across the liver as described previously (8).

The Silastic catheters were inserted in the vena cava for infusions. Silastic catheters (0.04-inch inner diameter) were inserted into the portal vein and left common hepatic vein for blood sampling (10). The gallbladder was removed, and a catheter placed in the common bile duct to allow sampling of primitive bile. Incisions were also made in the neck region for the placement of a sampling catheter in the carotid artery. The carotid artery was isolated, and a Silastic catheter (0.04-inch inner diameter) was inserted so that its tip rested in the aortic arch. After insertion, the catheters were filled with saline containing heparin (200 U/ml\(^{-1}\), Abbott Laboratories, North Chicago, IL), and their free ends were knotted.

Ultrasonic flow probes (Transonic Systems Inc., Ithaca, NY) were used to measure portal vein and hepatic artery blood flows. Briefly, a small section of the portal vein, upstream from its junction with the gastroduodenal vein, was cleared of tissue, and a 7.0-mm inner diameter flow cuff was placed around the vessel and secured. The gastroduodenal vein was isolated and then ligated proximal to its confluence with the portal vein. A section of the main hepatic artery lying proximal to the portal vein was isolated, and a 3.0-mm inner diameter flow cuff was placed around the vessel and secured. The Doppler probe leads and the knotted free catheter ends, with the exception of the carotid artery, were stored in a subcutaneous pocket in the abdominal region so that complete closure of the skin incision was possible. The free end of the carotid artery catheter was stored under the skin of the neck.

On the day of the experiment, the subcutaneous ends of the catheters were fed through small skin incisions made. For local anesthesia (2% lidocaine, Astra Pharmaceutical Products, Worcester, MA) over the subcutaneous pockets in which catheters were stored. The contents of each catheter were aspirated, and catheters were flushed with saline. Silastic tubes were connected to the exposed catheters and brought through the skin incision and connected to the external collection system. The dogs were housed in a facility that met Association for Assessment of Laboratory Animal Care guidelines, and the protocols were approved by Vanderbilt University Institutional Animal Care and Use Subcommittee. The Silastic catheters were installed for infusion of labeled acetate and for blood sampling across the liver as described previously (8).

At least 16 days before each experiment, a laparotomy was performed under general anesthesia (0.04 mg/kg pentothal sodium presurgery, and 1.0% isofluorane inhalation anesthesia during surgery). Silastic catheters (0.03-inch inner diameter) was inserted so that its tip rested in the aortic arch. After insertion, the catheters were filled with saline containing heparin (200 U/ml\(^{-1}\), Abbott Laboratories, North Chicago, IL), and their free ends were knotted.

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min to 320 °C, then hold 9 min. The transfer line was held at 320 °C, and source was held at 200 °C (ammonia-negative chemical ionization). Cholesterol elutes at 13.5 min and lathosterol at 14.6 min. For both compounds, ions were monitored from m/z 580 to 607, and the dwell time was 10 ms. Because the abundance of lathosterol is much lower than that of cholesterol in the biological samples, the MIDs of the two analytes were determined separately by injecting the sample in splitless versus 20:1 split mode for lathosterol and cholesterol, respectively.

The following procedures were carried out for fatty acid analysis: an HP-5 50-m capillary column was used, injector at 250 °C, start at 100 °C, hold for 1 min at 100 °C, ramp 20 °C/min to 280 °C, and hold for 3 min at 280 °C, ramp 10 °C/min to 300 °C, then hold for 6 min. The transfer line was held at 300 °C, and the source was at 200 °C (ammonia-negative chemical ionization). Myristate elutes at 16 min (ions at m/z 227–241, and the dwell time is 25 ms) and palmitate at 18 min (ions at m/z 255–271, with dwell time of 25 ms). Note that the pentafluorobenzyl group splits off in the ion source, so the ions monitored are the undervatized acyl groups. In all cases, GC-MS analyses were conducted with double or triple injections, and the data are averaged for each sample. In selected perfusate and tissue samples from perfused rat livers, we assayed the MIDs of lactate.

Calculations—In live dog experiments, the concentration and enrichment of acetate in the mixed blood entering the liver were calculated (8) using Equations 1 and 2, in which P and H represent the portal vein and the hepatic artery, respectively. Blood flows are expressed in m/min kg⁻¹.

\[
[A_{acetate, mixed}] = \frac{(HA flow)\cdot [acetate]_H + (PV flow)\cdot [acetate]_PV}{HA flow + PV flow} \quad (Eq. 1)
\]

\[
MPE_{mixed} = \frac{(HA flow)\cdot [acetate]_H\cdot MPE_{acetate} + (PV flow)\cdot [acetate]_PV\cdot MPE_{acetate}}{HA flow + PV flow} \quad (Eq. 2)
\]

Note that the numerator of Equation 1 represents the nanomoles of total acetate (unlabeled plus labeled) that enter the liver per minute. The numerator of Equation 2 represents the nanomoles of total acetate (unlabeled plus labeled) that exit the liver per minute.

ISA Model and Two Isotopomer Calculations—Calculations of the parameters of fatty acid and sterol biosynthesis (precursor enrichment and fractional synthesis) were conducted using two models: (i) isotopomer spectral analysis (ISA) (4, 17) and (ii) the two-isotopomer method, an algebraic method similar to that described by Chinkes (7). ISA uses fractional abundance of observed isotopomers as the input and requires estimates of all isotopomers of a product. Like all forms of regression, ISA finds the best fit solution when there are more equations than unknowns. ISA uses weighted non-linear least squares regression to find the best fit of the model to the entire experimental mass isotopomer spectrum. Usually ISA models seek the best fit solution using a set of equations with two unknowns, the precursor enrichment, and the fractional synthesis g(time). However, a more complicated model, with additional parameters, may be used when a larger number of mass isotopomers of the product lipid are generated. Additional mass isotopomers allow for a statistical test of whether the more complicated model is justified. The studies reported here were designed to produce a highly 13C-enriched lipogenic acetyl-CoA pool. This generated a large number of labeled mass isotopomers of each lipid. Each mass isotopomer detected is associated with a unique equation in the ISA model. Thus this experimental approach, producing a large number of isotopomers of each compound, allows for testing two specific ISA models.

The traditional ISA model ("single D model") with a constant value for precursor enrichment was compared with a "gradient D model" where the precursor enrichment varied linearly between two values Dmax and Dmin (Fig. 1). Although the single D model contained two unknowns, D and g(t), the more complicated gradient D model contained two additional parameters, Dmax and Dmin (Fig. 2). A more detailed description of the gradient ISA model is presented in the accompanying report (9). To decide whether the gradient D model provided a better description of the data, we used an F test to compare the two models following a stepwise regression procedure. The more complicated model is justified as a better description if it reduces the residual sum of squares sufficiently (18). Adding a parameter to any model with a residual error will likely reduce the error slightly. The F test requires a larger reduction in the sum of squares to indicate that the model containing the additional parameter truly provides a better description of the data. The sum of squares was used to support the hypothesis that the biosynthesis occurred under conditions where there was a gradient in precursor enrichment (see accompanying report (9)).

ISA models were compared to a "two-isotopomer" method for estimating the two parameters, precursor enrichment (p) and fractional synthesis (f). The two-isotopomer method uses fractional abundances and probability-based equations for only two isotopomers to solve for the two parameters. The two-isotopomer method used here requires the availability of a program to fit the data, and the program monitored is described in more detail in the accompanying report.

Equations 3 and 4 yield the enrichment of acetyl-CoA (p) and the fractional synthesis of the fatty acid analyzed (f). These equations were used to compute parameters in Table I for palmitate, columns 8 and 9.

\[
p = \frac{(2M_p) - (n-1)M_p + (2M_H)}{n p (1 - p)^{n-1}} \quad (Eq. 3)
\]

\[
f = \frac{M_p/2M}{np(1 - p)^{n-1}} \quad (Eq. 4)
\]

For these equations, n represents the number of subunits of a polymer; e.g. n equals 8 for palmitate, and 7 for myristate. The terms M_p and M_H refer to intensities of the mass isotopomer signal corrected for natural abundance. 2M refers to the sum of all corrected intensities. These equations were derived from probability-based equations with the assistance of the symbolic algebra program, MAPLE, as implemented in Mathcad (MathSoft, Cambridge, MA). Comparable equations for sterol synthesis are more complicated and were not derived for this study.

Both ISA and the two isotopomer method used here require multiple isotopomer measurements (up to 24 masses for sterols, and up to 16 for palmitate). This requirement increases the number of measurement cycles that can be performed across the GC peak. The impact of the number of measurement cycles on the magnitude of the mass cycling error has been extensively studied by Matthews and Hayes (19). They calculated the number of measurement cycles required for specific levels of accuracy. Using Table I of their report and the number of measurement cycles, we assessed the following levels of accuracy of our measurements for each mass abundance: <0.1% for lathosterol and cholesterol, <2% for myristate and palmitate, and <1% for pentadecanoate (assayed in the companion report (9)). Despite the different levels of precision of the measured MIDs of fatty acids versus sterols, the ISA gradient modeling yielded similar low values for the sum of squares error, which represents the best-fit of the MIDs (see below).

RESULTS

Dog Experiments—The concentrations and enrichments of acetate in the artery, portal vein, hepatic vein, as well as in the mixed (arterial plus portal) blood entering the liver (see "Experimental Procedures") are shown in Fig. 2 (A and B). The concentration of acetate (Fig. 2A) increases markedly in the portal vein when the infusion of [1,2-13C]acetate into a small branch of this vein is started. This increase in concentration (about 200 μM) matches well the ratio (150 μmol of [1,2-13C]acetate infused per minute)/0.8 liter of portal blood flow per minute) = 188 μM. Note that the concentration of acetate in the hepatic vein did not increase when [1,2-13C]acetate was infused into the portal vein. This reflects the immediate adjustment of the rate of acetate uptake upon increased portal supply. There was a 6-fold decrease in the concentration of acetate across the liver (compare concentrations in mixed inflowing and hepatic vein bloods).

The enrichment of acetate in the portal vein was high (55–60%) as expected, given the site of infusion. There was a 6-fold decrease in the enrichment of acetate across the liver (compare enrichments in mixed inflowing and hepatic vein bloods). This reflects the production of unlabeled acetate in the liver, presumably from acetyl-CoA hydrolysis (20, 21). The higher enrichment of acetate in the hepatic vein compared with the artery results from the uptake of labeled acetate and the release of unlabeled acetate by peripheral tissues (22). The 6-fold decrease in both acetate concentration and enrichment across the liver strongly suggests that the enrichment of lipogenic acetyl-CoA also decreases across the liver lobule. This was
investigated through ISA of fatty acids and sterols isolated from liver, bile, and VLDL.

The measured MID of lathosterol isolated from the liver of a dog infused with [1,2-13C2]acetate in a small branch of the portal vein is shown as dark bars in Fig. 3A. Note that the spectrum of unlabeled lathosterol (not shown) contains only M to M4 mass isotopomers. The MID of lathosterol isolated from dog liver includes mass isotopomers from M to M25. These data were analyzed using ISA to fit the data to a single value of acetyl-CoA enrichment. The best fit of acetyl-CoA enrichment was 47%, which would correspond to a MID of newly synthesized lathosterol represented by the white bars of Fig. 3A.

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A linear gradient of acetyl-CoA enrichment Fig. 3B shows the best fit of the measured MID (dark bars, same as in Fig. 3A) to a linear gradient of acetyl-CoA enrichment varying from 70 to 14%. As evident, the standard error is smaller when the measured data are fitted to a gradient of acetyl-CoA enrichment (Fig. 3B) rather than to a single acetyl-CoA enrichment (Fig. 3A). The fractional synthesis of lathosterol calculated by ISA (g(t)) was very similar when the fitting assumed a constant versus a varying enrichment of acetyl-CoA (73% versus 77%, respectively).

Fig. 3A and B reports the measured and computed data on lathosterol labeling in one dog liver. Table I (line 1) presents the statistics of the computation of lathosterol labeling in five dog livers. Three types of calculations are presented: (i) the best fitting by ISA of the data to a single pool of acetyl-CoA enrichment (showing the precursor enrichment D and the fractional synthesis g(6 h)), (ii) the best fitting by ISA of the data to a linear gradient of acetyl-CoA enrichment (showing the maxi-
mal and minimal values of the enrichment, $D_{\text{max}}$ and $D_{\text{min}}$, as well as the fractional synthesis $g(t)$, and (iii) the computation by the two-isotopomer method of the acetyl-CoA enrichment ($p$) and fractional synthesis ($f$). Similar computations were conducted for cholesterol isolated from dog liver (Table I, line 2). Note that $D_{\text{max}}$ and $D_{\text{min}}$ for cholesterol and lathosterol are significantly different, suggesting that they were formed from different precursor pools.

The same type of data treatment was conducted for myristate extracted from the same dog liver as in Fig. 3 (Fig. 4, A and B). The measured MID of myristate (Fig. 4, dark bars) could not be well fitted to a single enrichment of acetyl-CoA (42%, Fig. 4A, white bars), but was quite well fitted to a linear gradient of acetyl-CoA from 73% to 3%. Table I, lines 3 and 4 present the statistics of these computations for myristate and palmitate isolated from dog livers. Note that $D_{\text{max}}$ and $D_{\text{min}}$ for both myristate and palmitate are almost identical, suggesting that the two compounds are formed from the same precursor pools.

Similar computations were conducted for total lathosterol, cholesterol, myristate, and palmitate isolated from dog bile (Table I, section 2), as well as for myristate and palmitate isolated from dog plasma VLDL at the end of the experiments (Table I, section 3). All computations of MID of sterols and fatty acids isolated from dog liver, bile, and plasma VLDL revealed marked gradients of acetyl-CoA enrichment across the liver (compare $D_{\text{max}}$ and $D_{\text{min}}$ in sections 1–3 of Table I). Similar to what was found in dog livers, sterols and fatty acids isolated from dog bile and VLDL have $D_{\text{max}}$ and $D_{\text{min}}$ values that are not statistically different between members of lipid classes (cholesterol versus lathosterol and palmitate versus myristate) supporting the hypothesis that biliary pools of lipids are formed from similar precursor pools. The $F$ test indicated that the linear gradient (three-parameter model) reduced the sum of squares error sufficiently to warrant using the more complicated model. Note that the fractional synthesis values $g(t)$ are very similar for both versions of ISA. However, fractional syntheses estimated by either ISA model, $g(t)$, are larger than those estimated by the two-isotopomer method, $f$. Also, all ISA estimates by single or variable $D$ are significantly higher than the estimates of $p$ computed by the two-isotopomer method ($p < 0.05$).

**Perfused Rat Liver Experiments**—In an orientation experiment, a liver from a chow-fed rat was perfused with recirculating buffer containing initially $10 \text{ mM} [1,2-\text{\textsuperscript{13}C}_2]\text{acetate}$ (98% M2). During the 2-h experiment, the concentration and MPE of acetate decreased from 10 to 8 mM and from 96% to 83%, respectively. In all subsequent perfusion experiments, the uptake of acetate was compensated by a constant infusion of 7.5 $\mu\text{mol}$ of [1,2-\text{\textsuperscript{13}C}_2]\text{acetate} per minute into the perfusate reservoir. Then, the concentration and MPE of acetate plateaued at $10 \pm 0.3 \text{ mM}$ and $91 \pm 0.3\%$, respectively (not shown).

Fig. 5 (A and B) reports the measured and computed data on lathosterol labeling in one perfused rat liver. Table I (section 4, line 1) presents the statistics of the computation of lathosterol labeling in six perfused rat livers. Comparison of Figs. 5A and 3A shows that the fitting of the MID of lathosterol to a single acetyl-CoA enrichment (59%) is better in the case of a rat liver.

![Figure 2](image-url)
perfused with a high concentration of \([1,2-^{13}C_2]\)acetate (Fig. 5A) than in the case of a live dog liver infused with \([1,2-^{13}C_2]\)acetate via the portal vein (Fig. 3A). However, the MID of lathosterol isolated from a perfused rat liver still fits much better to a gradient of acetyl-CoA enrichments ranging from 72 to 46%. Note that this gradient is shallower than that of Fig. 3B (from 70% to 14%). The more shallow character of the acetyl-CoA enrichment gradient in perfused rat livers is also found when computing the MIDs of cholesterol, myristate, and palmitate (compare \(D_{max}\) and \(D_{min}\) in sections 1 and 4 of Table I). As was the case for the dog data, the \(D_{max}\) values of sterols and fatty acids are not significantly different among the classes of lipids, whereas the \(D_{min}\) values differ in the case of rat liver fatty acids and rat bile sterols.

The MID of lipids in bile secreted by perfused rat livers also showed evidence for gradients of acetyl-CoA enrichment in the liver (Table I, section 5). In addition, the ISA analysis of the MIDs of bile sterols and fatty acids revealed the existence of a small time-gradient of enrichment of lipogenic acetyl-CoA (Fig. 6, solid symbols). The precursor enrichments at 120 min are greater than those at 30 min (\(p < 0.05\) for cholesterol and lathosterol). The precursor enrichments for cholesterol and lathosterol are not different. The time gradient in precursor enrichment is also visible from the time profile of the mass isotopomer pattern of cholesterol excreted in bile by perfused rat livers (Fig. 7). The vertical arrows point to the most abundant mass isotopomer during each time interval. Over the 2-h bile collection, the most abundant mass isotopomer shifts from M14 to M15, M16, and M17. This reflects the progressive increase in acetyl-CoA enrichment during the experiment. Similar time increases in acetyl-CoA enrichment were found for total bile cholesterol, myristate, and palmitate (not shown). Evidence for a progressive increase in acetyl-CoA enrichment was not observed in the MID of lipids isolated from the bile of dogs infused with \([1,2-^{13}C_2]\)acetate (not shown).

We tested whether the progressive increase in acetyl-CoA enrichment detected in perfused rat livers resulted from the formation of secondary tracers derived from the primary labeled substrate \([1,2-^{13}C_2]\)acetate. We did not detect the accumulation of M1 acetate in the perfusate. In a previous study in monkeys infused with \([1,2-^{13}C_2]\)acetate for 5 h, we observed the accumulation of M1 and M2 lactate in plasma (Fig. 1B of Ref. 23). Because labeled lactate can generate labeled acetyl-CoA, we assayed the MID of lactate in the perfusate of the livers, the data of which are presented in Fig. 6. The mass spectrum of lactate di-t-butyl olomethylislyl includes a fragment (m/z 159) corresponding to C2 plus 3 of lactate. The M1 and M2 enrichments of this fragment increased progressively reaching 9.2 ± 1.3% and 3.8 ± 0.3% (\(n = 5\)) after 2 h (Fig. 8). Thus, if some perfusate lactate were converted to liver lipogenic acetyl-CoA, it could enrich the latter with M1 and M2 species. The extent of this enrichment is not known.

In an attempt to abolish transhepatic gradients of acetyl-CoA enrichment, we fed rats for 10 days with a synthetic food containing either (i) 30% of the calories as triacetin and 50% as carbohydrates or (ii) no triacetin and 80% of the calories as carbohydrates (see “Experimental Procedures”). We perfused livers from these two series of rats under the same conditions as livers from chow-fed rats. ISA analysis of the MIDs of the lipids from the two groups of livers still revealed the presence of the gradients of acetyl-CoA enrichment across the liver (Table I, sections 6 and 7). Note that, in all cases, \(D_{max}\) and \(D_{min}\) values for fatty acids and sterols isolated from rat liver and bile samples were not significantly different among the lipid classes.

**DISCUSSION**

The zonated architecture of the liver lobule, recognized since the pioneering work of Jungermann (24, 25), has had a major impact on the regulation of metabolic processes occurring in the liver (ureagenesis, gluconeogenesis, glycolysis, fatty acid synthesis, and others). Two major factors contribute to the zonation process. First, the activities of a number of key enzymes follow descending or ascending gradients across the lobule. Second, the concentrations of a number of substrates also follow descending or ascending gradients across the lobule. The zonation of substrate concentrations is particularly marked for compounds that reach the liver at low concentrations and that are both taken up and generated in the liver, for example glycerol (26, 27), \(NH_3\) (28), and acetate (8). Previous work from this and other laboratories has demonstrated major decreases in the concentration and isotopic labeling of these three metabolites across the liver. When \([1,2-^{13}C_2]\)acetate was infused in the peripheral vein of dogs fitted with transhepatic catheters, the concentration and molar percent enrichment of acetate decreased 3- and 7-fold, respectively, across the liver (8).

Transhepatic gradients of concentration and enrichment of labeled substrates are likely to result in gradients of labeling of the intracellular products of metabolism of the labeled substrates. This was demonstrated in the case of \([2-^{13}C]\)glycerol and \([U-^{13}C_3]\)glycerol by the analysis of the mass isotopomer distribution of glucose released by the liver (29–31). This isotopomer distribution was incompatible with a uniform enrichment of the triose phosphates across the liver. The heterogeneity of triose phosphate labeling can be overcome by flooding the liver with a high concentration of labeled glycerol (31).
Table I
Computations of acetyl-CoA enrichment and fractional synthesis using ISA and the two-isotopomer method

Data are presented as ± S.E. (N). Data were compared using paired t-test (*p versus D₁, **f versus gᵢ, ***f versus gⱼ, p < 0.05 (paired t-test).

| Compound | Single pool model (ISA) | Gradient model (ISA) | Two-mass isotopomer model |
|----------|-------------------------|----------------------|--------------------------|
|          | D₁ | gᵢ(5) | Dᵢᵡ | gᵫ(5) | p | Δf |
| 1) Dog liver | Lathosterol | 48.9 ± 6.5 (5) | 65.0 ± 2.8 (5) | 65.3 ± 1.9 (4) | 9.9 ± 2.1 (4) | 50.8 ± 7.8 (4) | NC | NC |
|            | Cholesterol | 31.6 ± 1.5 (5) | 27.0 ± 0.1 (5) | 50.9 ± 0.5 (5) | 26.6 ± 3.5 (5) | 2.8 ± 0.1 (5) | NC | NC |
|            | Myristate | 42.1 ± 3.2 (5) | 28.2 ± 3.5 (5) | 51.1 ± 1.6 (5) | 8.7 ± 0.7 (5) | 29.7 ± 2.2 (5) | 23.3 ± 1.4 (5) | 16.0 ± 3.0 (5) |
|            | Palmitate | 36.0 ± 4.6 (5) | 12.4 ± 2.3 (5) | 57.2 ± 3.2 (5) | 8.5 ± 0.6 (5) | 12.7 ± 1.5 (5) | 17.2 ± 0.7 (5) | 5.4 ± 1.7 (5) |
| 2) Dog bile (last time point) | Lathosterol | 64.2 ± 2.4 (3) | 63.3 ± 1.9 (3) | 68.5 ± 3.4 (3) | 18.1 ± 5.7 (3) | 52.0 ± 6.5 (3) | NC | NC |
|            | Cholesterol | 53.1 ± 2.9 (3) | 2.9 ± 0.1 (3) | 57.0 ± 3.3 (3) | 16.6 ± 4.1 (3) | 2.4 ± 0.5 (3) | NC | NC |
|            | Myristate | 55.3 ± 2.4 (5) | 33.5 ± 2.8 (5) | 61.8 ± 2.1 (5) | 9.9 ± 0.6 (5) | 35.6 ± 3.0 (5) | 22.9 ± 1.7 (5) | 9.7 ± 1.1 (5) |
|            | Palmitate | 51.7 ± 4.4 (5) | 14.3 ± 2.7 (5) | 52.8 ± 5.8 (5) | 8.0 ± 0.2 (5) | 14.3 ± 3.8 (5) | 13.7 ± 2.9 (5) | 4.3 ± 0.2 (5) |
| 3) Dog VLDL | Cholesterol | 46.5 ± 5.6 (5) | 1.2 ± 0.2 (5) | 49.1 ± 6.6 (5) | 30.4 ± 1.9 (5) | 1.4 ± 0.2 (5) | NC | NC |
|            | Myristate | 51.6 ± 2.0 (3) | 8.0 ± 0.8 (3) | 56.4 ± 5.1 (4) | 9.2 ± 0.2 (4) | 9.0 ± 0.8 (4) | 17.5 ± 2.1 (4) | 3.8 ± 0.5 (4) |
|            | Palmitate | 36.8 ± 5.7 (3) | 3.7 ± 1.1 (3) | 56.3 ± 2.1 (4) | 7.6 ± 0.3 (4) | 4.3 ± 0.2 (4) | 20.2 ± 2.1 (4) | 1.9 ± 0.3 (4) |
| 4) Perfused livers from chow-fed rats | Lathosterol | 61.5 ± 1.2 (6) | 39.8 ± 1.8 (6) | 75.4 ± 0.9 (6) | 47.4 ± 1.8 (6) | 40.2 ± 1.8 (6) | NC | NC |
|            | Cholesterol | 19.8 ± 1.7 (6) | 6.9 ± 1.2 (6) | 77.8 ± 1.1 (5) | 44.6 ± 2.3 (5) | 6.2 ± 0.7 (5) | NC | NC |
|            | Myristate | 54.7 ± 2.8 (9) | 24.1 ± 1.7 (9) | 84.0 ± 1.6 (7) | 25.6 ± 2.7 (7) | 27.1 ± 1.6 (7) | 33.3 ± 1.0 (9) | 10.7 ± 1.4 (9) |
|            | Palmitate | 62.4 ± 1.9 (9) | 7.9 ± 0.7 (9) | 79.6 ± 0.9 (7) | 45.9 ± 1.6 (7) | 8.8 ± 0.3 (7) | 36.6 ± 4.1 (9) | 3.0 ± 0.5 (9) |
| 5) Bile from perfused livers (section 4) (final time point) | Lathosterol | 66.4 ± 1.1 (6) | 93.1 ± 0.8 (6) | 80.5 ± 1.8 (3) | 45.9 ± 2.9 (3) | 64.9 ± 2.0 (3) | NC | NC |
|            | Cholesterol | 62.5 ± 2.0 (6) | 27.3 ± 5.2 (6) | 79.8 ± 2.0 (3) | 26.3 ± 1.3 (3) | 35.3 ± 4.6 (3) | NC | NC |
|            | Myristate | 59.6 ± 1.1 (5) | 28.9 ± 0.7 (5) | 86.8 ± 0.7 (5) | 24.3 ± 4.1 (5) | 30.6 ± 0.8 (5) | 32.8 ± 2.8 (5) | 8.5 ± 0.3 (5) |
|            | Palmitate | 61.8 ± 1.2 (7) | 13.6 ± 0.7 (7) | 82.3 ± 1.2 (7) | 38.1 ± 1.3 (7) | 13.7 ± 0.7 (7) | 29.4 ± 2.2 (7) | 1.9 ± 0.3 (7) |
| 6) Perfused livers from rats on control synthetic diet | Lathosterol | 40.8 ± 2.0 (4) | 35.4 ± 3.6 (4) | 54.8 ± 2.5 (6) | 27.1 ± 1.7 (6) | 35.7 ± 3.7 (6) | NC | NC |
|            | Myristate | 39.1 ± 1.5 (4) | 27.4 ± 1.6 (4) | 65.6 ± 3.9 (7) | 14.4 ± 1.0 (7) | 29.3 ± 1.8 (7) | 31.7 ± 0.7 (7) | 17.4 ± 1.4 (7) |
|            | Palmitate | 37.6 ± 1.2 (4) | 13.7 ± 1.2 (4) | 59.5 ± 1.9 (4) | 16.9 ± 0.8 (4) | 14.0 ± 1.2 (4) | 36.5 ± 1.8 (4) | 10.9 ± 0.8 (4) |
| 7) Perfused livers from rats on triacetin diet | Lathosterol | 42.3 ± 2.1 (7) | 37.4 ± 4.5 (7) | 55.9 ± 2.5 (6) | 26.5 ± 2.1 (6) | 34.4 ± 3.5 (6) | NC | NC |
|            | Myristate | 40.7 ± 1.8 (7) | 32.1 ± 2.1 (7) | 63.7 ± 2.1 (7) | 18.5 ± 1.5 (7) | 33.8 ± 2.3 (7) | 34.2 ± 1.3 (7) | 21.4 ± 1.4 (7) |
|            | Palmitate | 39.9 ± 1.8 (7) | 15.2 ± 0.9 (7) | 62.5 ± 2.0 (3) | 12.5 ± 1.7 (3) | 14.7 ± 1.1 (3) | 39.7 ± 1.6 (3) | 12.8 ± 0.8 (3) |

*a t is 6 h for dog data and 2 h for rat data.
*b NC, not computed.
However, this strategy affects the metabolic status of the liver and the rate of gluconeogenesis. The large decreases in the concentration and $^{13}$C enrichment of acetate across the liver (Fig. 2) strongly suggest that the labeling of the cytosolic and mitochondrial pools of acetyl-CoA decreases from the periportal to the perivenous area of the lobule. This suggestion is reinforced by the inverse zonations of glycolytic (32–35) and lipogenic (36–38) enzymes (mostly perivenous), versus cytosolic acetyl-CoA synthetase (mostly periportal) (39). The combination of (i) a periportal to perivenous decrease in the formation of labeled acetyl-CoA from $[1,2-^{13}C_2]$acetate with (ii) a periportal to perivenous increase in the formation of unlabeled acetyl-CoA from glucose and glycogen might result in a gradient of enrichment of lipogenic acetyl-CoA that is steeper than the gradient in acetate enrichment.

The literature provides some information on the absolute periportal versus perivenous activities of cytosolic acetyl-CoA synthetase (1.0 versus 0.5 unit/g in hepatocytes fractionated on Percoll gradients (39)) and of glucokinase (1.5 versus 2.0 units/g in hepatocytes isolated after selective destruction of one zone with digitonin (40); 1.0 versus 1.5 units/g based on quantitative histochemistry (35)). These data suggest that, in the periportal zone, the absolute activity of glucokinase is greater than that of acetyl-CoA synthetase. Also, in livers from fed rats, fatty acid synthesis is fueled mostly by glycogenolysis (13). So one would expect, in first analysis, that even in the periportal zone, the labeling of lipogenic acetyl-CoA from $[1,2-^{13}C_2]$acetate would be greatly diluted by unlabeled acetyl-CoA derived from glucose and glycogen.

In fact, the MIDs of fatty acids and sterols isolated from livers and bile reveal that the enrichment of cytosolic acetyl-CoA is very high in a sub-population of hepatocytes located presumably in the periportal zone. In the presence of $[1,2-^{13}C_2]$acetate, the enrichment of cytosolic acetyl-CoA results from the combination of two processes. First, $[1,2-^{13}C_2]$acetate...
entering the hepatocyte is activated by a cytosolic acetyl-CoA synthetase forming acetyl-CoA molecules, which, initially, should be almost as enriched as the incoming labeled substrate. Second, $[1,2$-$^{13}$C$_2$]acetate is activated in mitochondria to acetyl-CoA derived from carbohydrate (glycogen) and fatty acid catabolism. When mitochondrial acetyl-CoA is transferred to the cytosol via citrate and ATP-citrate lyase, it dilutes the enrichment of acetyl-CoA molecules formed by cytosolic acetyl-CoA synthetase. Thus, depending on the relative contributions of the sources of cytosolic acetyl-CoA, the enrichment of the pool of lipogenic acetyl-CoA should be intermediate between the enrichment of the incoming $[1,2$-$^{13}$C$_2$]acetate and that of mitochondria acetyl-CoA.

Table I compares parameters of lipogenesis (precursor enrichment and fractional synthesis) calculated from MIDs of fatty acids by the two-isotopomer method versus the “Single pool” and “Gradient” ISA models. For the latter model, best-fit computations were run assuming a linear gradient of enrichment of lipogenic acetyl-CoA across the liver. In all cases, the fitting of the experimental data to the model was better with a gradient of acetyl-CoA enrichment than with a constant acetyl-CoA enrichment. Because many different shapes of gradients are possible in a biological environment, we chose the linear gradient because it is the simplest mathematical gradient, adding only one additional parameter to the model. The companion paper (9) illustrates the ISA computations based on non-linear gradients.

The two-isotopomer computations yield a single value for the precursor enrichment and fractional synthesis. This is because this model uses two measurements of isotopomer fractional abundances (here, M2 and M4) to compute two parameters and does not allow variations in either parameter. In contrast, ISA is an over-determined model, because the two parameters are calculated from a large number of mass isotopomer abundances (as many as 15 for myristate, and 26 for lathosterol). A consequence of the over-determined model is that ISA not only estimates parameters but provides an estimate of how well these parameters fit the entire MID (Figs. 3–5). Because the equations for the probability of each isotopomer contain both terms, i.e. the precursor enrichment and fractional synthesis, the parameters are not independent (17). As illustrated by the two-isotopomer equations above, the value for $f$, fractional synthesis, is very sensitive to small changes in abundance of M2 or M4. In contrast, ISA calculates parameters $(D$ and $g(t))$ by non-linear regression using all isotopomer data and may be more robust to small changes in single isotopomers abundance. For example, the two ISA models compared in Table I and Figs. 3–5, yielded very similar values for fractional synthesis despite different approaches to measuring precursor enrichment.

In most cases, the values of precursor enrichments and fractional syntheses computed from the two-isotopomer method were lower than those calculated with ISA, the latter assuming a single value for precursor enrichment. However, because we do not know the real values of these parameters, a more detailed analysis of the two methods is not feasible. This is addressed in the companion paper.

However, the ISA computations reported here clearly demonstrate the occurrence of major gradients of enrichment of lipogenic acetyl-CoA in the livers of dogs infused with $[1,2$-$^{13}$C$_2$]acetate. This was indeed predicted from the zonation of the concentration and enrichment of acetate across the liver (Fig. 2 and Ref. 8). However, in rat livers perfused with a high concentration of $[1,2$-$^{13}$C$_2$]acetate, where there is no gradient of concentration or enrichment of acetate across the organ, we still show evidence of a gradient of acetyl-CoA enrichment, albeit more shallow than in the in vivo experiments. This gradient cannot be prevented by feeding the rats with a diet enriched with triacetin in the hope of evening out the activity of cytosolic acetyl-CoA synthetase across the liver.

The computation of the MIDs of bile fatty acids and sterols reveal the existence of an increasing time gradient of acetyl-CoA enrichment in rat livers perfused with a high concentration of $[1,2$-$^{13}$C$_2$]acetate (Figs. 6 and 7). It appears that, as the duration of the experiment increases, there is a slight decrease in the dilution of the enrichment of acetate by unlabeled carbon derived presumably from glucose and glycogen. One possible explanation of the time gradient is the progressive activation of acetyl-CoA synthetase by a high concentration of acetate. A second possible explanation is the formation of secondary tracers, such as lactate, from the primary $[1,2$-$^{13}$C$_2$]acetate substrate. We observed substantial M1 and M2 labeling of C2 plus 3 of lactate (Fig. 8) in the perfusate of the livers described in Fig. 6. The small magnitude of the time gradients observed in

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**Table I**

Comparing parameters of lipogenesis (precursor enrichment and fractional synthesis) calculated from MIDs of fatty acids by the two-isotopomer method versus the “Single pool” and “Gradient” ISA models. For the latter model, best-fit computations were run assuming a linear gradient of enrichment of lipogenic acetyl-CoA across the liver. In all cases, the fitting of the experimental data to the model was better with a gradient of acetyl-CoA enrichment than with a constant acetyl-CoA enrichment. Because many different shapes of gradients are possible in a biological environment, we chose the linear gradient because it is the simplest mathematical gradient, adding only one additional parameter to the model. The companion paper (9) illustrates the ISA computations based on non-linear gradients.

The two-isotopomer computations yield a single value for the precursor enrichment and fractional synthesis. This is because this model uses two measurements of isotopomer fractional abundances (here, M2 and M4) to compute two parameters and does not allow variations in either parameter. In contrast, ISA is an over-determined model, because the two parameters are calculated from a large number of mass isotopomer abundances (as many as 15 for myristate, and 26 for lathosterol). A consequence of the over-determined model is that ISA not only estimates parameters but provides an estimate of how well these parameters fit the entire MID (Figs. 3–5). Because the equations for the probability of each isotopomer contain both terms, i.e. the precursor enrichment and fractional synthesis, the parameters are not independent (17). As illustrated by the two-isotopomer equations above, the value for $f$, fractional synthesis, is very sensitive to small changes in abundance of M2 or M4. In contrast, ISA calculates parameters $(D$ and $g(t))$ by non-linear regression using all isotopomer data and may be more robust to small changes in single isotopomers abundance. For example, the two ISA models compared in Table I and Figs. 3–5, yielded very similar values for fractional synthesis despite different approaches to measuring precursor enrichment.

In most cases, the values of precursor enrichments and fractional syntheses computed from the two-isotopomer method were lower than those calculated with ISA, the latter assuming a single value for precursor enrichment. However, because we do not know the real values of these parameters, a more detailed analysis of the two methods is not feasible. This is addressed in the companion paper.

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**FIG. 7.** Time course of the MID of cholesterol in the bile excreted by a perfused rat liver. There are four abundances for each mass isotopomer corresponding to the four periods of bile collection. Note the time shift of the most abundant cholesterol mass isotopomer in each bile collection (arrows).

**FIG. 8.** Labeling of the C2 plus 3 fragment of lactate in rat livers perfused with 10 mM $[1,2$-$^{13}$C$_2$]acetate. Solid symbols represent M1 MPE, open symbols represent M2 MPE of lactate. Data are presented as mean ± S.E. (n = 5).
rat liver perfusions and the lack of evidence for time gradients in dog livers is most consistent with the concept that the large gradients in precursor enrichment observed in most plasma lipids are not time gradients and reflect gradients produced by the spatial architecture and metabolic zonation of the liver.

In conclusion, we demonstrated that gradients of enrichment of lipogenic acetyl-CoA exist in various in vivo animal models. Such gradients do not appear to be caused by substrate exhaustion or induction of enzyme activities. Most likely the gradients are formed because of highly zonated architecture of the liver. During the extractions of the liver samples, all the product pools were mixed together, making it impossible to infer the possible gradient shape or size. The ISA calculations estimated the fractional synthesis and the range of the precursor enrichments that formed the product during synthesis. Due to its limited nature, the two-isotopomer method estimates one value for each precursor enrichment and fractional synthesis and cannot determine how well these parameters fit the entire set of isotopomers.

In the companion report (9), we address issues described above. Namely, we set up perfect conditions of polymerization of isotopomers. We cannot determine how well these parameters fit the entire set of isotopomers. In the companion report (9), we address issues described above. Namely, we set up perfect conditions of polymerization of isotopomers. We cannot determine how well these parameters fit the entire set of isotopomers. In the companion report (9), we address issues described above. Namely, we set up perfect conditions of polymerization of isotopomers. We cannot determine how well these parameters fit the entire set of isotopomers. In the companion report (9), we address issues described above. Namely, we set up perfect conditions of polymerization of isotopomers. We cannot determine how well these parameters fit the entire set of isotopomers. In the companion report (9), we address issues described above. Namely, we set up perfect conditions of polymerization of isotopomers. We cannot determine how well these parameters fit the entire set of isotopomers.

REFERENCES

1. Hellerstein, M. K., Kletec, C., Kaempfer, S., Wu, K., and Shackleton, C. H. (1999) Am. J. Physiol. 59, 417–427.
2. Hellerstein, M. K., and Neese, R. A. (1992) Am. J. Physiol. 59, 5986–5991.
3. Lee, W. N., Byerley, L. O., Bergner, E. A., and Edmond, J. (1999) Am. J. Physiol. 59, 417–427.
4. Kelleher, J. K., and Masterson, T. M. (1992) Eur. J. Biochem. 596, 458–468.
5. Brunnengraher, H., Kelleher, J. K., and Des Rosiers, C. (1992) Am. J. Physiol. 59, 417–427.
6. Hellerstein, M. K., and Neese, R. A. (1992) Am. J. Physiol. 59, 417–427.
7. Hellerstein, M. K., and Neese, R. A. (1992) Am. J. Physiol. 59, 417–427.
8. Puchowicz, M. A., Bederman, I. R., Comte, B., Yang, D., David, F., Stone, R., Jabbeur, K., Wasserman, D. H., and Brunengraher, H. (1999) Am. J. Physiol. 59, 417–427.
9. Bederman, I. R., Kasumov, T., Reszko, A. R., David, F., Brunengraher, H., and Kelleher, J. K. (2004) J. Biol. Chem. 59, 417–427.
10. Keller, U., Cherrington, A. D., and Liljenquist, J. (1978) Am. J. Physiol. 263, 417–427.
11. Irutin, O., Martini, W. Z., Orkan, O., and Wolfe, R. R. (2001) Metabolism 50, 417–427.
12. Brunengraher, H., Weinstock, S. B., Story, D. L., and Kopito, R. R. (1981) J. Lipid Res. 22, 916–920.
13. Brunengraher, H., Kelleher, J. K., and Des Rosiers, C. (1997) J. Biol. Chem. 248, 4586–4590.
14. Robins, S. J., and Brunengraher, H. (1982) J. Lipid Res. 23, 464–468.
15. Powers, L., Osborne, M. K., Kien, C. L., Murray, R. D., and Brunengraher, H. (1983) J. Biol. Chem. 290, 747–750.
16. Otslund, E. R., Jr., Hsu, F. F., Bosmer, M. S., Stenson, W. F., and Hachey, D. L. (1996) J. Mass Spectrom. 31, 1291–1296.
17. Kelleher, J. K., Kharrubbi, A. T., Abdaliahs, T. A., Shambat, I. B., Kennedy, K. A., Halle, A. L., and Masters, T. M. (1984) Am. J. Physiol. 266, E384–E395.
18. Glantz, S. A., and Siekert, B. D. (1984) Primer of Applied Regression and Analysis of Variance. McGraw-Hill, New York.
19. Matthews, D. E., and Hayes, J. M. (1976) Anal. Chem. 58, 375–382.
20. Crabtree, B., Gordon, M. J., and Christie, S. L. (1990) Biochem. J. 270, 219–225.
21. Prass, R. L., Ishiashi, F., and Utter, M. F. (1980) J. Biol. Chem. 265, 5215–5223.
22. Mittendorfer, B., Sidossis, L. S., Walser, E., Chinkes, D. L., and Wolfe, R. R. (1998) Am. J. Physiol. 274, E978–E983.
23. Yang, D., Previs, S. F., Fernandez, C. A., Dagelay, S., Soloviev, M. V., Haze, J. W., Agarwal, K., Levine, W. C., David, F., Rinaldo, P., Beylot, M., and Brunengraher, H. (1996) Am. J. Physiol. 264, E569–E573.
24. Jungermann, K. (1986) Enzyme 57, 161–165.
25. Jungermann, K., and Kretzmann, T. (1986) Annu. Rev. Nutr. 59, 179–203.
26. Landau, B. R., Wahren, J., Previs, S. F., Eikberg, K., Chandramouli, V., and Brunengraher, H. (1999) Am. J. Physiol. 271, E1110–E1117.
27. Previs, S. F., Martin, S. K., Haze, J. W., Soloviev, M., Keating, A. P., Lucas, D., David, F., Kasby, J., Kirschbaum, D., Tseng, K. Y., and Brunengraher, H. (1999) Am. J. Physiol. 271, E1118–E1124.
28. Yang, D., Haze, J. W., David, F., Singh, J., Rice, R., Stremm, J., Halperin, M. L., and Brunengraher, H. (2000) Am. J. Physiol. 278, E469–E476.
29. Landau, B. R., Fernandez, C. A., Previs, S. F., Eikberg, K., Chandramouli, V., Wahren, J., Halperin, M. L., and Brunengraher, H. (1995) Am. J. Physiol. 269, E18–E20.
30. Previs, S. F., Fernandez, C. A., Yang, D., Soloviev, M. V., David, F., and Brunengraher, H. (1999) J. Biol. Chem. 270, 1806–1809.
31. Previs, S. F., Cline, G. W., and Shulman, G. I. (1999) Am. J. Physiol. 277, E154–E160.
32. Fischer, W., Eikberg, K., and Katz, N. R. (1982) Hoppe Seylers. Z. Physiol. Chem. 580, 453–455.
33. Katz, N., Teutsch, H. F., Jungermann, K., and Sasse, D. (1977) FEBS Lett. 83, 272–276.
34. Mietheke, H., Wittig, B., Nauth, A., Zierz, S., and Jungermann, K. (1985) Biol. Chem. Hoppe-Seylers 580, 453–455.
35. Trus, M., Zawalich, K., Gaynor, D., and Matschinsky, F. (1980) J. Histochem. Cytochem. 59, 579–581.
36. Katz, N., Thiele, J., and Gifhorn-Katz, S. (1989) Eur. J. Biochem. 180, 185–189.
37. Katz, N. R., Fischer, W., and Gifhorn-Katz, S. (1989) Eur. J. Biochem. 135, 103–107.
38. Katz, N. R., Fischer, W., and Eikberg, K. (1989) J. Biol. Chem. 130, 297–301.
39. Knudsen, C. T., Immerdal, L., and Quistorff, B. (1998) Eur. J. Biochem. 265, 359–362.
40. Guzman, M., and Castro, J. (1989) Biochem. J. 264, 107–113.