Zonation of acetate labeling across the liver: implications for studies of lipogenesis by MIDA

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PUCHOWICZ, Michelle A., Ilya R. Bederman, Blandine Comte, Dawei Yang, France David, Eric Stone, Kareem Jabbour, David H. Wasserman, and Henri Brunengraber. Zonation of acetate labeling across the liver: implications for studies of lipogenesis by MIDA. Am. J. Physiol. 277 (Endocrinol. Metab. 40): E1022–E1027, 1999.—Measurement of fractional lipogenesis by mass isotopomer distribution analysis (MIDA) of fatty acids or cholesterol labeled from [13C]acetate assumes constant enrichment of lipogenic acetyl-CoA in all hepatocytes. This would not be the case if uptake and release of acetate by the liver resulted in transhepatic gradients of acetyl-CoA enrichment. Conscious dogs, prefitted with transhepatic catheters, were infused with glucose and [1,2-13C2]acetate. Stable concentrations and enrichments of acetate were measured in artery (17 µM, 36%), portal vein (61 µM, 5.4%), and hepatic vein (17 µM, 1.0%) and were computed for mixed blood entering the liver (53 µM, 7.4%). We also measured balances of propionate and butyrate across gut and liver. All gut release of propionate and butyrate is taken up by the liver. The threefold decrease in acetate concentration and the sevenfold decrease in acetate enrichment across the liver strongly suggest that the enrichment of lipogenic acetyl-CoA decreases across the liver. Thus fractional hepatic lipogenesis measured in vivo by MIDA may be underestimated.

acetyl-coenzyme A; mass spectrometry; fatty acid synthesis; cholesterol synthesis

MASS ISOTOPOMER DISTRIBUTION ANALYSIS (MIDA) was proposed (11, 17) as a method for estimating the fractional synthetic rate of fatty acids and cholesterol synthesized in vivo and in vitro in the presence of [13C]acetate that labels acetyl-CoA. Computation of the mass isotopomer distribution (MID) of either fatty acids isolated from very low-density cholesterol (VLDL)- triacylglycerols or cholesterol yields the fractional synthetic rates and the enrichment of lipogenic acetyl-CoA. A variant of MIDA, isotopomer spectral analysis (ISA), was presented by Kelleher and Masterson (13).

The validity of MIDA and ISA requires that the enrichment of the acetyl-CoA monomeric precursor be constant in all cells that synthesize the polymer. When the enrichment of the precursor is not constant, computation of the MID of the polymer yields erroneous values for the enrichment of the monomeric precursor and for the fractional synthesis. In fact, the calculated fractional synthesis is not the integrated average enrichment of the precursor. This is because the precursor enrichment appears in the equations for the amount of each isotopomer as a nonlinear parameter. For a detailed discussion, see Ref. 3.

The goal of the project reported here was to test, in dogs infused with [13C]acetate, whether the concentration and 13C enrichment of acetate vary across the liver. The study was prompted by the recognition that acetate is generated in the liver by hydrolysis of cytosolic, peroxisomal, and mitochondrial acetyl-CoA (4–5, 17a, 25, 29). One can therefore wonder whether, during infusion of [13C]acetate, the enrichment of blood acetate decreases across the liver lobule because of the production of unlabeled acetate in the liver. Because acetate is also generated by intestinal fermentation (4, 22), it was necessary to monitor acetate concentrations and enrichments in arterial and portal venous blood. This allowed us to calculate the concentration and enrichment of acetate in the mixed blood that enters the liver via the portal vein and hepatic artery. To simulate conditions where lipogenesis is usually measured, the experiments were conducted under hyperglycemic clamp (8–10 mM). Our data show a marked decrease in the enrichment of acetate across the liver.

METHODS
Materials. Chemicals were purchased from Sigma-Aldrich. Sodium [1,2-13C2]acetate and sodium [1-13C,2H5]acetate (99%) were from Isotec.

Animal 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, Chicago, IL) were studied. The dogs were housed in a facility that met the American Association for Accreditation of Laboratory Animal Care guidelines, and the protocols were approved by Vanderbilt University’s Institutional Animal Care and Use Subcommittee. At least 16 days before each experiment, a laparotomy was performed under general anesthesia (0.04 mg/kg of atropine and 15 mg/kg pentothal sodium presurgery, and 1.0% isoflurane inhalation anesthetic during surgery). Silastic catheters (0.03 in. ID) were inserted into the vena cava for infusions. Silastic catheters (0.04 in. ID) were inserted into the portal vein and left common hepatic vein for blood sampling. 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 in. ID) was inserted so that its tip rested in the aortic arch. After insertion, the catheters were filled with saline containing heparin (200 U/ml, Abbott Labo-
aboral 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 freed through small skin incisions made while the dogs were under 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 tubing was connected to the exposed each catheter were aspirated, and catheters were flushed with saline. Silastic tubing was connected to the exposed end of each catheter, which was secured with quick-drying glue. Saline was infused in the catheters and brought to the back of the dog, where they were stored under the skin of the neck.

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 freed through small skin incisions made while the dogs were under 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 tubing was connected to the exposed end of each catheter, which was secured with quick-drying glue. Saline was infused in the catheters and brought to the back of the dog, where they were stored under the skin of the neck.

Indocyanine green (ICG) was infused at a rate of 0.1 mg·min⁻¹·kg⁻¹. ICG was purchased from Hynson, Westcott, and Dunning (Baltimore, MD). ICG was used as an independent back-up measurement of hepatic blood flow measured using flow probes and as a means of confirming hepatic vein catheter placement. Flow probe measurements of portal vein and hepatic artery blood flows were continuously monitored on-line.

From 0 to 6 h, the dogs were infused intravenously with glucose at variable rates (0.034 to 0.24 mmol·min⁻¹·kg⁻¹) to set up and maintain a stable plasma glucose concentration of 10 mM. Also, from 0.5 to 6 h, 99% enriched [1,2-13C₂]acetate was infused at 1.5 µmol·min⁻¹·kg⁻¹ as a 150 mM solution. Blood was sampled from the carotid artery, portal vein, and hepatic vein at regular intervals, treated with heparin, and centrifuged, and plasma was frozen until analysis. Care was taken to withdraw hepatic vein blood very slowly to prevent contaminating the sample with blood drawn from the inferior vena cava.

Analytical procedures. One set of plasma samples (0.25 ml) was spiked with internal standards of [1-13C₂,2H₃]acetate (50 nmol), [2H₃]propionate (10 nmol), and [3H₂]butyrate (10 nmol). The concentrations of plasma acetate, propionate, and butyrate were determined by gas chromatography-mass spectrometry of the 2,4-difluorobenzaldehyde derivative (24). A second set of plasma samples without internal standards was used to assay the molar percent enrichments (MPE) of acetate (24). To avoid contaminations by ubiquitous acetate, we dedicated an isolated laboratory to the assays. For more details, see Ref. 24. Glucose was assayed enzymatically.

Calculations. Isotopic enrichments are expressed as MPE, that is, the percentage of molecules that are labeled. Some authors (18) express enrichments as tracer-to-tracee ratios (TTR). The two expressions of enrichment are related by Eq. 1.

\[
\text{MPE} = \frac{\text{tracer}}{\text{tracee}} 
\]

Note that the numerator of Eq. 2 represents the nanomoles of total acetate (unlabeled + labeled) that enter the liver per minute. The numerator of Eq. 3 represents the nanomoles of labeled acetate that enter the liver per minute. The denominator of Eq. 3 represents the nanomoles of total acetate (unlabeled + labeled) that enter the liver per minute. The extent of dilution of acetate labeling across the liver and the hepatic acetate uptake were calculated from Eqs. 4 and 5, where HV represents the hepatic vein.

fold dilution of acetate MPE across liver

\[
\text{fold dilution} = \frac{\text{MPE}_{\text{mixed}}}{\text{MPE}_{\text{hv}}} 
\]

fold decrease in acetate concentration across liver

\[
\text{fold decrease} = \frac{\text{[acetate]}_{\text{art}}}{\text{[acetate]}_{\text{hv}}} 
\]

hepatic total acetate uptake

\[
\text{hepatic uptake} = \frac{\text{[acetate]}_{\text{art}}}{\text{[acetate]}_{\text{art}} + \text{[acetate]}_{\text{hv}}}
\]

acetate release from the gut

\[
\text{acetate release} = \frac{\text{[acetate]}_{\text{art}}}{\text{[acetate]}_{\text{art}} + \text{[acetate]}_{\text{hv}}}
\]

Equations 2, 5, 6, and 8 are also used to calculate parameters of propionate and butyrate metabolism.

RESULTS

The variable glucose infusion resulted in a stable arterial glucose concentration of 10–10.4 mM during the last 4.5 h of the experiment (not shown). The mean glucose infusion rate necessary to achieve this glucose concentration was 4.5 ± 0.9 mmol·min⁻¹·kg⁻¹ (SE, n = 6). Total hepatic blood flow averaged 37 ± 2 ml·min⁻¹·kg⁻¹, and the portal vein flow contributed 80–85% of the total hepatic flow.

Figures 1-3 show the measured concentrations of acetate, propionate, and butyrate, respectively, in the artery, portal vein, and hepatic vein, as well as the
calculated concentrations in the mixed blood entering the liver (Eq. 2). The concentrations of the three short-chain fatty acids are markedly higher in the portal vein compared with the artery. For this reason, and because most of the blood supply to the liver is through the portal vein, the concentrations of these compounds in the mixed liver influent are close to those in the portal vein. For each compound, the concentrations in the artery and hepatic vein are very similar. For butyrate, the arterial concentration was slightly, but significantly, higher than in the hepatic vein. Figure 4 shows the fluxes of acetate, propionate, and butyrate in and out of the liver, as well as the percentages of the rates of inflowing substrates that are released in the hepatic vein. Figure 5 compares the releases of acetate, propionate, and butyrate by the gut to the uptake of these substrates by the liver. Within experimental errors, all the gut production of the three compounds is taken up by the liver in a single pass of the blood through the organ.

Figure 6 shows the measured MPE of acetate in the artery, portal vein, and hepatic vein, as well as the calculated MPE of acetate in the mixed blood entering

![Fig. 1. Profile of acetate concentration in artery (ART), portal vein (PV), hepatic vein (HV), and mixed liver influent (MIXED) (Eq. 2). Data are presented as means ± SE (n = 6).](image1)

![Fig. 2. Profile of propionate concentration in artery, portal vein, hepatic vein, and mixed liver influent (Eq. 2). Data are presented as means ± SE (n = 6).](image2)

![Fig. 3. Profile of butyrate concentration in artery, portal vein, hepatic vein, and mixed liver influent (Eq. 2). Data are presented as means ± SE (n = 6).](image3)

![Fig. 4. Fluxes of acetate (unlabeled and labeled), propionate, and butyrate in and out of liver. Fluxes of substrate entering liver (IN) were calculated from numerator of Eq. 2. In the case of acetate, this represents the total flux of unlabeled + labeled substrate. The flux of [1,2-13C2]acetate entering the liver (IN) is calculated from the numerator of Eq. 3. Fluxes of substrate exiting the liver (OUT) are calculated from hepatic vein concentrations and blood flows. Percentages of inflows that are released in the hepatic vein, indicated above OUT bars, were calculated using Eqs. 6–8.](image4)
the liver (Eq. 3). The highest acetate enrichment was in arterial blood (36.1 ± 2.5% from 270 to 360 min, n = 6). The acetate enrichment in the portal vein (5.4 ± 0.8%) was 6.7 times lower than in arterial blood. The enrichment of acetate in the mixed liver influent (7.4 ± 1.0%) was only 1.4 times that in the portal vein. The enrichment of acetate in the hepatic vein (1.0 ± 0.3%) was 7.4 times lower than in the mixed liver influent and 36 times lower than in the artery.

Figure 4 (left) shows another aspect of the dilution of acetate enrichment across the liver. Although 32% of the total acetate entering the liver left the organ through the hepatic vein, the corresponding value for [1,2-13C2]acetate was 4.7%.

**DISCUSSION**

Balances of acetate, propionate, and butyrate across the dog gut and liver (Fig. 5) confirm the notion (4, 22) that the gut is a major site of production of these short-chain fatty acids. The very similar concentrations of these compounds in the arterial and hepatic venous blood (Figs. 1–3), as well as the equivalence between net gut production and net hepatic uptake (Fig. 5) could lead one to believe that these compounds are made only by the gut and taken up only by the liver. However, the sevenfold decrease in acetate enrichment across the liver (Fig. 4) reveals that the liver also generates unlabeled acetate. Furthermore, because the acetate concentration in the hepatic vein is one-third of that of the liver influent, most of the unlabeled acetate generated in the liver is taken up by the same organ.

In mammalian cells, the only known mechanisms for acetate production are the hydrolysis of acetyl-CoA (4, 5) and the metabolism of acetone in the liver by the C2 pathway (8). In our dogs infused with glucose, concentrations of ketone bodies were very low; thus acetyl-CoA hydrolysis was probably the only source of acetate in liver. Our data confirm that the liver is the site of an intense acetate → acetyl-CoA → acetate substrate cycle (5). Indeed, acetyl-CoA synthetases and hydrolyases have been described in liver mitochondria and cytosol (4, 5, 25, 29). In perfused rat liver and hepatocyte experiments conducted in the presence of nonacetate14Co or 13C tracers that label acetyl-CoA in liver mitochondria, cytosol, or peroxisomes, labeled acetate was released (5, 8, 14, 19, 30). Thus acetate → acetyl-CoA → acetate substrate cycles occur in liver cytosol and mitochondria.

Our data do not address the production of acetate by peripheral tissues other than the gut. Bleiberg et al. (1) infused [1-14C]acetate in normal dogs and demonstrated the simultaneous uptake and release of acetate in intestine, liver, kidney, and hindlimb. Mittendorfer et al. (18) infused [1,2-13C2]acetate in humans and found that the acetate enrichment and concentration in the femoral vein were one-half of those in the artery. Thus muscle is also a site of acetate cycling. The physiological role of this substrate cycle is not known.

Based on the enrichment of acetate in arterial blood, the apparent turnover rate of acetate was 2.8 ± 0.3 µmol·min⁻¹·kg⁻¹, which is similar to values obtained by Mittendorfer et al. in humans (3.3 µmol·min⁻¹·kg⁻¹, Ref. 18) and by others using [14C]acetate (1, 28). Pouteau et al. (23) reported much higher rates measured with [13C]acetate. The apparent turnover rate we calculated is most likely underestimated because of the combination of 1) the very short apparent half-life of plasma acetate, which is of the order of the circulatory
time, ~30 s (6), and 2) the intravenous route of tracer infusion with arterial blood sampling (V-A mode, Ref. 12). The contribution of the gut to this apparent acetate turnover (Fig. 5) is ~50%.

Mittendorfer et al. (18) infused [1,2-13C2]acetate in normal humans at the same rate as we did in dogs (1.5 μmol·min⁻¹·kg⁻¹). The enrichment of arterial acetate they observed (0.447 TTR, equivalent to 30.9 MPE) was similar to ours (36.1 MPE, Fig. 6). However, they reported much higher acetate enrichment in the hepatic vein (0.134 TTR, equivalent to 11.2% MPE) compared with our data (1% MPE). Therefore, their artery-to-hepatic vein acetate enrichment ratio was ~3, whereas ours was ~36. The reason for the discrepancy between data by Mittendorfer et al. and ours is not clear. In addition to the species difference of human vs. dog, the Mittendorfer et al. subjects were studied after an overnight fast without glucose infusion. Our dogs were infused with glucose so that the extent of acetate dilution across the liver could be studied under lipogenic conditions. Conceivably, the infusion of glucose increased acetyl-CoA production in the liver and may have resulted in increased acetate → acetyl-CoA → acetate cycling. This would be a possible explanation of the difference in acetate dilution across the liver between the Mittendorfer et al. report and ours. However, against this interpretation are data from one experiment we conducted on an overnight-fasted, anesthetized dog without glucose infusion. There, the artery-to-hepatic vein enrichment ratio was 27, and acetate enrichment across the liver decreased 7.2-fold, as in our experiments on conscious dogs infused with glucose.

The 7.4-fold decrease in acetate enrichment across the liver (Fig. 6) must result in some decrease in the enrichment of lipogenic (cytosolic) acetyl-CoA between periportal and perivenous hepatocytes. The extent of decrease in acetyl-CoA enrichment and the shape of this decrease cannot be deduced from the overall drop in acetate enrichment across the liver. Although there is evidence that the activity of cytosolic acetyl-CoA synthetase is two times greater in periportal than in perivenous hepatocytes (15), we could find no information on the degree of zonation of the activities of the mitochondrial acetyl-CoA synthetase and mitochondrial/cytosolic/peroxisomal acetyl-CoA hydrolases. There is evidence that both cytosolic and mitochondrial acetyl-CoA synthetases participate in the activation of labeled acetate that is incorporated into fatty acids and cholesterol (9). Cytosolic acetyl-CoA synthetase labels directly the lipogenic acetyl-CoA pool. In contrast, mitochondrial acetyl-CoA synthetase forms acetyl-CoA that mixes with unlabeled acetyl-CoA derived from pyruvate and fatty acid oxidations. The resulting mitochondrial acetyl-CoA is transferred to the cytosol via citrate and ATP-citrate lyase (9).

Our calculation of the enrichment of acetate that enters the liver assumes that the portal venous and hepatic arterial influents mix perfectly at the entrance of the liver sinusoid. Mixing of the arterial and portal influents might be incomplete, resulting in streaming of portal and arterial blood through part of the length of the sinusoid. If this were the case, acetyl-CoA would be much more enriched in hepatocytes in contact with arterial blood than in adjacent hepatocytes in contact with portal venous blood.

What are the implications of our data for measurements of fractional lipogenesis (11) by MIDA of fatty acids isolated from VLDL-triglycerides or of plasma cholesterol after infusion of [13C]acetate? An absolute condition of validity of this technique requires that the enrichment of lipogenic acetyl-CoA be constant in all hepatocytes that synthesize lipids (3). Our data suggest that this condition may not be met when acetyl-CoA is labeled from a tracer of acetate. MIDA probably underestimates the degree of fractional hepatic lipogenesis traced with [13C]acetate unless fatty acid synthesis is confined to a narrow band of hepatocytes in the liver lobule. This is unlikely to be the case, because the activity of the lipogenic enzyme acetyl-CoA carboxylase is only about twofold higher in periportal than in pericentral hepatocytes (7). Thus it is likely that lipogenesis extends, to some degree, into the perivenous area of the lobule. Also, there is no variation in VLDL-triacylglycerol secretion between periportal and perivenous hepatocytes (10).

Conceptually, the possible underestimation of fractional lipogenesis traced with [13C]acetate is similar to the underestimation of fractional gluconeogenesis traced with [13C]glycerol. The latter, like [13C]acetate, undergoes considerable dilution across the liver (27). In the case of fractional gluconeogenesis, the degree of underestimation can be estimated in humans and animals whose liver glycogen has been depleted by fasting. Then the apparent contribution of gluconeogenesis to glucose production, calculated from MIDA of glucose labeled from [13C]glycerol, ranges from 27 to 90% (16, 20, 21, 26, 27) instead of 100%. High apparent values for fractional gluconeogenesis result from very high rates of [13C]glycerol infusion that blunt the periporal-to-perivenous gradient of glyceral enrichment (20, 21).

In the case of lipogenesis traced with [13C]acetate, the technique just described is not applicable, because it is impossible to set up conditions where all the fatty acids in VLDL-triglycerides would be derived from de novo synthesis. However, a decrease in enrichment of lipogenic acetyl-CoA across the liver could be detected by ISA (13) of fatty acids isolated from VLDL-triglycerides. This requires that the enrichment of acetate in the portal venous blood be sufficiently high that at least three labeled mass isotopomers are present in the long-chain fatty acids isolated from VLDL-triglycerides. If these conditions are met, ISA could reveal nonhomogeneity in labeling of lipogenic acetyl-CoA. We are presently testing this strategy.
REFERENCES


