ABSTRACT

Mammary gland tissue slices from three lactating Holstein cows were incubated in Krebs-Ringer bicarbonate-based media with varying concentrations of lactate and other substrates. Conversions of 1- and 2-carbon-14 of lactate to carbon dioxide, fatty acids, citrate, glyceride glycerol, and lactose were determined.

Effects of acetate, glucose, beta-hydroxybutyrate, stearate, and pyruvate on lactate metabolism were evaluated. Oxidation of lactate increased asymptotically with lactate concentration. Low acetate concentration stimulated oxidation of carbon 2 of lactate slightly while higher acetate availability inhibited lactate oxidation. Conversion of lactate to fatty acids increased linearly with lactate concentration. This conversion of lactate was inhibited strongly by acetate. Significant conversion of carbon 2 of lactate to glyceride-glycerol but not lactose was detected.

Bovine mammary glands have the capacity of utilize sizeable quantities of lactate for oxidation and lipogenesis. Both phenomena are highly dependent on availability of acetate. Pyruvate dehydrogenase and citrate lyase could represent important regulatory sites in vivo for determination of tissue preference for acetate over lactate. Results indicate factors other than an inactive malate transhydrogenation cycle likely limit glucose conversion to fatty acids.

INTRODUCTION

Whitehurst et al. (17) and Prior (13) reported significant incorporation of lactate carbon into adipose tissue fatty acids of ruminants in vitro and in vivo. Linzell (12) estimated that 10% of blood lactate is removed by bovine mammary glands. Significant utilization of lactate for fatty acid synthesis contradicts the theory that malate transhydrogenation cycle enzymes are relatively inactive in ruminant tissue and, as such, limit glucose conversion to fatty acids. Lactate conversion to this product is presumably dependent upon adequate activities of these enzymes and adenosine triphosphate (ATP) citrate lyase, in particular. Smith and Prior (15) presented evidence for an active malate transhydrogenation pathway in bovine adipose tissue. In view of these observations on adipose tissue and significant uptake of lactate by mammary glands of ruminants, the role of lactate as a substrate for maintenance and biosynthesis in bovine mammary tissue was undertaken. Specific emphasis was placed on examining possible regulatory phenomena that may establish priorities for utilization of alternate substrates for specific functions in mammary tissue and factors, that may contribute to limited conversion of glucose to fatty acids.

MATERIALS AND METHODS

Methods of tissue collection, preparation, incubation, quantitation of 14C in products and analyses were presented (8). The [2-14C]-lactate (Lac-2-14C) and [1-14C]-lactate (Lac-1-14C) were prepared from [2-14C]pyruvate (Pyr-2-14C) or [1-14C]pyruvate (Pyr-1-14C; Amersham) with lactate dehydrogenase in the presence of excess reduced nicotinamide adenine dinucleotide (NADH2) (Sigma Chemical Co.). L-Lactate thus formed was purified on a Bio-Rad AG-1-X8 column (formate form, 200 to 400 mesh) and stored at −20°C.
Conversion of $^{[14]C}$ lactate to various products was quantified at 1.0, 2.5, 5.0, 10.0, 20.0, and 30.0 mM lactate in the presence of 2.0 mM glucose and 2.0 or 8.0 mM acetate (sodium salt, Mallinckrodt). For quantitation of lactate conversion to fatty acids, a double hexane extraction (6) was required to remove residual $^{[14]C}$ lactate. Conversions of 2.0 mM Pyr-1-$^{14}$C and Pyr-2-$^{14}$C in the presence of 2.0 mM glucose and 0, 2.0, or 16.0 mM acetate also were investigated. Conversion of 30 mM Lac-2-$^{14}$C to products at 2.0 mM glucose and 2.0 or 16.0 mM acetate with a lactate to pyruvate ratio of 40.6 was investigated. Effects of removal of 1.16 mM DL-betahydroxybutyric acid (BHBA; present in all other incubation media to simulate in vivo conditions), addition of 100 molar N,N,N',N'-tetramethyl-p-phenylenediamine dihydrochloride (TMPD) or .6 mM stearate (Sigma Chemical Co.) complexed with defatted bovine serum albumin (Sigma Chemical Co.) also were studied.

RESULTS AND DISCUSSION

Oxidation of $^{[1-14]C}$ lactate and $^{[2-14]C}$ lactate increased in an asymptotic fashion with increased lactate availability (Figure 1). Data presented by Forsberg et al. (8) indicated that lactate did not displace acetate as a substrate for oxidation. Mammary tissue had a higher apparent capacity ($V_{max}$) for lactate C-1 oxidation as compared to lactate C-2 (Table 1, Figure 1). The ratio of Lac-1-$^{14}$C/Lac-2-$^{14}$C oxidation (2.40 ± .32) did not vary systematically with lactate availability. At physiological concentrations, lactate C-2 oxidation would contribute approximately 10 to 20% of total carbon dioxide (CO$_2$) production by mammary tissue when expressed as a percent of total oxidation at near-physiological concentrations of acetate, glucose, and lactate (7, 8, 9).

Acetate concentrations greater than 1.0 mM significantly lowered rates of lactate oxidation (Figures 2 and 3). Low concentrations of acetate (.5 and 1.0 mM) stimulated oxidation but not lipogenesis from Lac-2-$^{14}$C in two of three animals while higher concentrations depressed both oxidation and lipogenesis in all animals (Figure 2). The inhibitory effect of acetate on lactate oxidation was more marked with 5 than 30 mM lactate (7). Glucose (2.0 mM) did not alter Lac-2-$^{14}$C metabolism (Table 2). At lower concentrations, it appears that acetate may be used primarily for fatty acid synthesis with available lactate supporting this function through oxidation and generation of reduced nicotinamide adenine dinucleotide phosphate (NADPH$_2$) via NADP-linked isocitrate dehydrogenase (NADP-IDCH). This is consistent with observations that lactate stimulated lipogenesis from acetate in bovine adipose tissue (13, 15, 16). At higher acetate concentrations (> 1 mM), acetate may provide NADPH$_2$ via the isocitrate cycle as suggested by Bauman et al. (5) and concomitantly lower lactate oxidation through allosteric inhibition of pyruvate dehydrogenase (PDH). Alternatively, the apparent depression in lactate oxidation could be attributed to a decrease in the specific activity of mitochondrial acetyl-Coenzyme A (CoA) by formation of acetyl-CoA from acetate. Lactate does not stimulate acetate conversion to fatty acids as noted above for adipose tissue. This may not reflect a tissue difference, however, because glucose was provided in the mammary tissue study but not in comparable adipose tissue incubations (17).

Robertson et al. (14) speculated that PDH may limit pyruvate flow into the tricarboxylic cycle.
TABLE 1. Estimates of kinetic parameters relating to lactate metabolism.  

<table>
<thead>
<tr>
<th>Product</th>
<th>Label</th>
<th>Acetate</th>
<th>n</th>
<th>$K_{l\text{ac}}$</th>
<th>SE</th>
<th>$V_{\text{max}}$</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbon dioxide</td>
<td>Lac-1$^4$</td>
<td>2</td>
<td>2</td>
<td>2.1</td>
<td>.24</td>
<td>6.6</td>
<td>.16</td>
</tr>
<tr>
<td></td>
<td>Lac-2$^5$</td>
<td>2</td>
<td>2</td>
<td>2.3</td>
<td>.10</td>
<td>2.1</td>
<td>.35</td>
</tr>
<tr>
<td></td>
<td>Lac-2$^7$</td>
<td>8</td>
<td>1</td>
<td>5.2</td>
<td></td>
<td>3.3</td>
<td></td>
</tr>
<tr>
<td>Fatty acids</td>
<td>Lac-1$^8$</td>
<td>2</td>
<td>1</td>
<td>7.2</td>
<td></td>
<td>.024</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lac-2</td>
<td>2</td>
<td>2</td>
<td>16.6</td>
<td>4.8</td>
<td>2.1</td>
<td>.10</td>
</tr>
<tr>
<td></td>
<td>Lac-2$^9$</td>
<td>8</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glyceride</td>
<td>Lac-1$^{10}$</td>
<td>2</td>
<td>1</td>
<td>N/D</td>
<td></td>
<td>N/D</td>
<td></td>
</tr>
<tr>
<td>glycerol</td>
<td>Lac-2$^{10}$</td>
<td>2</td>
<td>1</td>
<td>2.7</td>
<td></td>
<td>.98</td>
<td></td>
</tr>
<tr>
<td>Citrate</td>
<td>Lac-1$^{11}$</td>
<td>8</td>
<td>1</td>
<td>95.0</td>
<td></td>
<td>11.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lac-2</td>
<td>2</td>
<td>1</td>
<td>7.6</td>
<td></td>
<td>90.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lac-2</td>
<td>8</td>
<td>1</td>
<td>33.6</td>
<td></td>
<td>88.0</td>
<td></td>
</tr>
</tbody>
</table>

1 Media contained 2 mM glucose.
2 Millimoles.
3 Apparent capacity ($V_{\text{max}}$) expressed as microgram atoms of lactate traced converted to product per gram tissue wet weight per hour.
4 1-[$^{14}$C] lactate.
5 2-[$^{14}$C] lactate.
6 Not detectable.
7 In two additional studies with 8 mM acetate, the response in lactate oxidation was linear.
8 In an additional study, the conversion of lactate carbon 1 to fatty acids was linear (Figure 4).
9 This response was essentially linear in both studies (Figure 4).
10 In one study data relating to lactate conversion to glyceride-glycerol were discarded because of excessive variability.
11 Citrate formation from lactate carbon 1 was linearly related to lactate concentration.

acid cycle in bovine adipose tissue. The cow mammary data (Figures 2 and 3) suggest that regulation of PDH may be the site at which mammary tissue establishes a priority between lactate and acetate as energy sources for tissue maintenance. Lactate appears to fulfill a secondary role, relative to acetate, in support of mammary tissue maintenance. Data in (8) indicated that lactate does not displace acetate as a substrate for fatty acid synthesis. Conversion of Lac-1-$^{14}$C to fatty acids was low compared with Lac-2-$^{14}$C conversion. This indicates that PDH is the primary route of lactate entry into TCA intermediates with incorporation via pyruvate carboxylase fulfilling a lesser role. This observation agrees with studies that indicated pyruvate carboxylase may be the most limiting enzyme of the malate transhydrogenation cycle in bovine mammary and adipose tissues (5, 10). The relationship between lactate concentration and Lac-2-$^{14}$C conversion to fatty acids was linear within physiological concentrations of lactate (Figure 4). The ratio of lactate tracer conversion to CO$_2$ versus fatty acids increased from 1.53 to 5.78 for Lac-2-$^{14}$C and from 79 to 681 for Lac-1-$^{14}$C as acetate concentration increased from 0 to 8 mM (calculated from data in Figures 2 and 3). Although net metabolism of lactate was depressed, elevation of the ratio of lactate oxidation as compared to conversion to fatty acids indicates that acetate affects lipogenesis from lactate much more strongly than lactate oxidation. Conversion of 30 mM lactate to fatty acids in the absence of acetate exceeded rates of fatty acid synthesis from 16 mM acetate (8). This indicates that the activity of ATP-citrate lyase is sufficient to support high rates of lipogenesis from glucose and lactate. White-
LACTATE METABOLISM IN THE MAMMARY GLAND IN VITRO

Figure 2. Effect of acetate on conversion of lactate carbon 2 (Lac-2-14C) to carbon dioxide (CO₂) and fatty acids. Respective glucose and lactate concentrations were 2 and 30 mM. Means are of three observations ± SE. An equation (fatty acids = intercept - X/(1 + KLaC/Ac)) that describes Lac-2-14C conversion to fatty acids (grams tissue wet weight) -1 h -1 is fatty acids = 1.51 - (1.49/(1 + 11.4/Ac)), P<.005, where intercept and X have units of gram atoms Lac-2-14C converted (grams tissue) -1 h -1 and KLaC (Michaelis-Menten constant) and Ac (acetate concentration) are millimoles.

Whitehurst et al. (17) proposed a mechanism by which lactate metabolism could yield one cytosolic acetyl-CoA and two NADPH₂ required for incorporation of one acetyl-CoA into fatty acid in bovine adipose tissue. They proposed that NADH₂ produced by PDH and NAD-malate dehydrogenase after malate translocation into and oxidation in the mitochondrion could be used to generate NADPH₂ via NADP-ICDH. Cytosolic acetyl-CoA and oxaloacetate for formation of malate using NADPH₂ from lactate would be provided by ATP-citrate lyase. Smith and Prior (16) demonstrated that lactate conversion to fatty acids in bovine adipose tissue was dependent upon malate transport across the mitochondrial membrane and citrate efflux. Published activities of ATP-citrate lyase in bovine mammary tissue (2) indicate sufficient capacity to account for lactate conversion to fatty acids.

Oxidation of lactate was dependent upon availability of acetate (Figures 2 and 3). Increasing acetate concentration from 0 to 2 mM typically halved conversion of lactate to fatty acids. Results were similar in bovine adipose tissue for Whitehurst et al. (17) and Prior (13). Acetate did not alter pyruvate oxidation (Table 2) but lowered significantly pyruvate conversion to fatty acids. Addition of pyruvate to achieve a concentration ratio of lactate/pyruvate of 40.6 with a final concentration of 29.3 mM lactate did not alter lactate conversion to fatty acids or other products [P<.05; (7)].

As noted, the depressing effect of acetate upon oxidation of lactate was much smaller than upon lipogenesis from lactate (Figure 2). Because lactate and acetate share a common pool in cytosolic acetyl-CoA for conversion to fatty acids, dilution of labelled acetyl-CoA from lactate by acetate would affect conversion of lactate tracer to fatty acids more noticeably than oxidation. Alternatively, decreased coenzyme A concentrations due to high acetate concentrations and increased acetyl-CoA could limit the rate of the ATP-citrate lyase reaction. The latter hypothesis is supported by the observation that label from Lac-2-14C accumulated in citrate as acetate concentration increased (Figure 5). Regulation of ATP-citrate lyase may represent a site at which the priority for acetate as a primary lipogenic substrate is established.

Figure 3. Effect of acetate on lactate carbon 1 (Lac-1-14C) conversion to carbon dioxide (CO₂) and fatty acids (FA) in the presence of 2 mM glucose. Lactate concentration was 30 mM. Means are of three observations ± SE. Equations (product synthesized = intercept - [X/(1 + KLaC/Ac)]) that describe these effects are CO₂ = 8.5 -[3.49/(1 + .9/Ac)], P<.1; FA = .12 -[.12 (1 + 2.6/Ac)], P<.001, where intercept and X are expressed as gram atoms Lac-1-14C converted (grams tissue) -1 h -1 and KLaC (Michaelis-Menten constant) and Ac (acetate concentration) are millimoles.
Mammary tissue prefers acetate as a substrate for fatty acid synthesis (8). With physiological concentrations of glucose, lactate, and acetate, lactate provides carbon to account for 5 to 10% of total fatty acid synthesis. If acetate supply was limited, the significance of lactate as a lipogenic substrate would increase.

Conversion of Lac-2-14C but not Lac-1-14C to glyceride glycerol (GG) was observed (Table 1). This indicates incorporation into TCA cycle intermediates via pyruvate dehydrogenase and acetyl-CoA rather than via pyruvate carboxylase.

At Vmax (high lactate concentration) with acetate and glucose present, 15 to 20% of GG was derived from lactate carbon. Other investigators have obtained similar results. Whitehurst et al. (16) observed that lactate conversion to GG in bovine adipose tissue could nearly satisfy the total requirement for this metabolite. Such conversion is presumably dependent on phosphoenolpyruvate carboxykinase (PEPCK) and reverse glycolysis to triose phosphates (TP). Measurements of PEPCK activity in bovine adipose tissue (3) indicate capacity appropriate...
Table 3. Effects on lactate metabolism of removal of glucose or BHBA from or addition of TMPD or stearate to incubation media.1

<table>
<thead>
<tr>
<th>Product</th>
<th>Metabolic effector</th>
<th>Minus glucose2</th>
<th>Minus BHBA3</th>
<th>Plus TMPD4</th>
<th>Plus stearate5</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Carbon dioxide</td>
<td>1.06 .08</td>
<td>1.08 .15</td>
<td>1.08 .15</td>
<td>.98 .24</td>
<td></td>
</tr>
<tr>
<td>Fatty acids</td>
<td>1.13 .13</td>
<td>.81 .11</td>
<td>1.03 .01</td>
<td>.96 .04</td>
<td></td>
</tr>
<tr>
<td>Glycerol</td>
<td>.98 .09</td>
<td>1.11 .10</td>
<td>.52 .28</td>
<td>.78 .23</td>
<td></td>
</tr>
</tbody>
</table>

1 Means for three animals relative to their controls ± SE.
2 Glucose was in all remaining media at 2 mM.
3 β-Hydroxybutyrate (BHBA) was present in all remaining media at 1.16 mM (DL-form).
4 N,N,N',N'-Tetramethyl-p-phenylenediamine dihydrochloride (TMPD) at 100 μM.
5 Stearate (.6 mM) complexed with defatted bovine serum albumin.

Lactate metabolism in the mammary gland in vitro

To this route. Measurements of PEPCK activity in bovine mammary tissue have not been reported.

In our standard incubation media, 1.16 mM BHBA was provided; BHBA is considered a preferred priming molecule for fatty acid synthetase in bovine mammary tissues (5). Removal of this substrate from incubation media did not affect lactate conversion to fatty acids or CO2 (Table 3).

Addition of TMPD to incubation media consistently lowered 30 mM lactate transfer to GG by 50%; however, this effect was not significant (P<.05; Table 3). Oxidation of cytosolic NADH2 by TMPD would be expected to reduce reverse glycolysis and conversion of TP to GG. The TMPD did not alter lactate conversion to other products (P<.05). Addition of stearate complexed with bovine serum albumin did not alter metabolism of lactate (Table 3).

Only trace quantities of lactate or pyruvate tracers were recovered in lactose indicating low amounts of fructose-1,6-diphosphate phosphatase in this tissue.

Mammary tissue utilizes acetate as a primary substrate for lipogenesis and oxidation. Lactate can be utilized at similar rates for both processes in the absence of acetate. Rates of glucose oxidation via the TCA cycle and conversion to fatty acids are much lower (9). Only limited quantities of glucose carbons one and two were recovered in citrate (9). The conversion of both lactate and acetate to GG indicate substantial flux of TCA carbon to TP. This suggests that flux from TP to PEP may limit glucose conversion to fatty acids. Indeed, the reverse flux (PEP to TP) seems more prominent. Effects of high acetate concentrations, mediated through effects of acetyl-CoA, on rates of the PDH and ATP-citrate lyase reactions affect lactate metabolism. However, these are not sufficient to explain the low rates of fatty acid synthesis from glucose (9). This limitation may be due to equilibria of glycolytic reactions beyond TP. Robertson et al. (14) suggested that pyruvate kinase could limit glucose conversion to pyruvate in bovine adipose tissue. Baldwin and Yang (4) reported that this enzyme was not in equilibrium in bovine mammary tissue. This supports the view that carbon flow from PEP to TP is favored to provide TP for GG synthesis, spare glucose for lactose and GG synthesis, and to provide NADPH2 for fatty acid synthesis. This suggestion certainly explains high rates of acetate and lactate conversion to GG and low rates of glucose conversion to TCA cycle intermediates and fatty acids.

Huntington et al. (2, 11) reported that the lactate turnover rate in concentrate fed steers was 339 mmol/h. Only 10.9% of this was accounted for as gluconeogenesis. Arterial concentrations of lactate range from .75 to 1.5 mM (1, 11). This lactate entry rate is close to estimates of acetate entry. Linzell (12) observed that lactate extraction by bovine mammary tissue was 10% of blood lactate. If one-half (remainder oxidized; Table 1) of this lactate
were to be used for lipogenesis, 5% of milk fatty acids would be formed from lactate. This is only an approximation as fluctuations of lactate concentration over the course of lactation in the dairy cow have not been documented. This estimate includes fatty acids derived from the blood. These observations agree with estimates of the relative contribution of lactate to lipogenesis discussed above. The complexity of interactions among substrates as concentrations vary over the physiological range indicates that patterns of substrate utilization by mammary tissue, adipose tissue, and likely hepatic tissue can vary greatly and affect lactational performance and efficiency.

REFERENCES