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Lactate Production and Absence of Gluconeogenesis from Placentally Transferred Substrates in Fetuses from Fed and 48-H Starved Rats

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ABSTRACT. Fed and 48-h starved rats were infused on day 21.5 of gestation for 20 min through the left uterine artery with [U-14C]-d-glucose, [U-14C]-glycerol, or [U-14C]-l-alanine. The mother and fetuses from both uterine horns were processed separately for radioactivity measurements in plasma and liver. Differences in radioactivity values between fetuses from the left and the right sides are used as indexes of placental transferance of the infused tracers prior to their distribution and transformation in the maternal circulation. After infusion of [U-14C]-d-glucose, [U-14C]-glycerol, or [U-14C]-l-alanine, plasma radioactivity values and specific activities corresponding to the respective infused tracer appeared much higher in fetuses from the left than the right uterine side. Plasma 14C-lactate values also were higher in the left than the right fetuses indicating that fetoplacental structures produced lactate from those placently transferred 14C-metabolites. No difference in plasma 14C-glucose between left and right uterine horn fetuses was observed after maternal infusion with either [U-14C]-glycerol or [U-14C]-l-alanine, either in fed or 48-h starved rats. In the mother both [U-14C]-glycerol and [U-14C]-l-alanine were efficiently converted to 14C-glucose, and this process was significantly enhanced with starvation.

14C-fatty acids present in fetal liver after maternal infusions with either [U-14C]-glycerol or [U-14C]-glycerol were decreased by starvation whereas no fatty acid synthesis from [U-14C]-l-alanine was detected. Much less 14C-glyceride glycerol was found in fetal liver after maternal infusions of [U-14C]-d-glucose than [U-14C]-glycerol, and its incorporation was unaffected by maternal starvation. Results show the significant production by the fetoplacental unit of lactate from transferred maternal substrates and the absence of gluconeogenesis in the rat fetus even after 48 h of maternal food deprivation. Lack of gluconeogenesis occurred even above the triose phosphate step and despite the fact that glycerol phosphorylation is active in fetal liver, indicating that maternal glucose is the only source of this metabolite for the normal rat fetus. (Pediatr Res 22: 6–10, 1987)

In the rat fetus unlike several other species including the guinea pig (1), rabbit (2), sheep (3), and man (4) gluconeogenesis is absent and develops only after birth (5–8) as a consequence of the induction of cytosolic phosphoenolpyruvate carboxykinase (1, 9). Due to the conversion of administered [1-14C]-glycerol to 14C-glucose (10) it has been proposed that the gluconeogenetic pathway is functional in the rat fetus above the triose phosphate step, but in other studies gluconeogenesis from glycerol was not detected in in vitro (11) or in vivo (12) rat fetus preparations. Maternal fasting has been reported to induce gluconeogenesis prematurely in the rat fetus (13–15), but this finding has not been consistent and no change in fetal liver gluconeogenic activities were reported after 48 h of maternal fasting (16). In addition, no changes were detected in fetal liver lipogenic enzymes after maternal fasting (16) whereas fetal lipogenesis from 3H2O was inhibited (17). In order to clarify these controversies, the present study was performed to determine the comparative in vivo metabolic fate of d-glucose, l-alanine, and glycerol in fetuses from fed and 48-h starved late pregnant rats. Labeled substrates were infused through the maternal left uterine artery according to our recently described technique (18) for placental transfer studies which allows determination of the metabolic fate of substrates in the rat fetus independently of interconversions occurring in the mother.

METHODS

Wistar female rats, mated when weighing 170–190 g, were studied at day 21.5 of gestation (estimated by the appearance of spermatozoaids in vaginal smears) comparing fed and 48-h food-deprived animals. Rats were anesthetized with sodium pentobarbital (33 mg/kg body weight, intravenous) and operated according to our previously reported surgical procedure (18). Briefly, a PE-10 cannula (Intramedic) was introduced counter current into the left external iliac artery to the beginning of the superior vesical artery which was clamped distal to the left uterine artery exit level. The infusion medium introduced through the cannula therefore combines with the blood circulating through the left uterine artery. Rats were infused for 20 min at a constant rate of 12.5 µl/min with 250 µl of a 0.9% NaCl solution containing 10 µCi of [U-14C]-d-glucose, [U-14C]-l-alanine, or [U-14C]-glycerol (The Radiochemical Center, Amersham, England) (specific activity 257, 10 and 171 µCi/mmol, respectively).

After collection of blood from the maternal aorta into heparinized syringes, placentas and fetuses from the left and right uterine horns were immediately excised. Fetuses were decapitated and blood collected into heparinized receptacles. All fetal blood from each uterine side was pooled separately. Fetal livers were immediately placed in liquid N2. Plasma aliquots were deproteinized with 10% perchloric acid and neutralized with saturated potassium bicarbonate. Protein-free supernatants were used for esti-
mation of glucose (19), alanine (20), glycerol (21), and lactate (22). In experiments with infusions of [U-14C]-D-glucose or [U-
14C]-L-alanine, labeled plasma glucose, alanine, and lactate were
purified (23) from 100-ml aliquots of deproteinized supernatants,
using microcolumns made with AG 1-X8 200-400 chloride and
AG 50 W-8 200-400 H+ (Bio-Rad Laboratories, Richmond, CA)
rinsed with distilled water, 0.5 M formic acid, and 2 N ammo-
nium hydroxide for 14C-glucose, 14C-lactate and 14C-alanine
eutions, respectively. Eluates were collected in counting vials
and lyophilitized for counting. Recoveries for [U-14C]-D-glucose
added to plasma before precipitation were 97 ± 2% in the first
eulate, 88 ± 3% for [U-14C]-L-alanine in the second eluate,
and 91 ± 2% for [U-14C]-L-alanine in the third eluate. Tracer recov-
eries in the noncorresponding eluates were always below 0.4%.

In experiments with [U-14C]-glycerol infusions, labeled plasma
glycerol, glucose, and lactate were purified by ascending paper
cromatography, the eluent being the upper phase resulting from
the butanol-water-methanol-formic acid mixture (320:320:80:1)
(v/v/v) (24). The spots were identified by running purified stan-
dards in parallel and cutting them into small pieces for introduc-
tion into counting vials. Recoveries of labeled glycerol, glucose,
and lactate were 86 ± 0.5%, 92 ± 1%, and 90 ± 0.5%, respec-
tively. Labeled lipids in fetal liver were extracted (25) and sapon-
fied in ethanolic 1 N potassium hydroxide for fatty acid and
glyceride glycerol fractionation (26). Radioactivity counting was
done by means of a Normascint-22 cocktail (Scharflau, Barcelona,
Spain) in a LS 3800 Beckman counter. Radioactivity values were
corrected by considering 1 × 106 dpm as the total infused
radioactivity per rat. Results were expressed as means ± SD and
statistical comparison between groups was performed by the
Student's t test.

RESULTS

Pregnant rats in the 21.5 day of gestation were infused for 20
min with tracer amounts of three different labeled metabolites
through the left uterine artery to determine their appearance and
potential transformation in the respective fetuses. The steady
state concentration in plasma of these metabolites and lactate
was measured in mothers and their fetuses at the end of the
infusions and values are shown in Table 1. Plasma glucose and
glycerol concentrations were higher and alanine and lactate levels
were lower in fed and 48-h starved mothers than in their respec-
tive fetuses. Maternal starvation caused a decrease in maternal
plasma glucose and alanine and a slight increase in plasma
glycerol concentrations whereas in fetuses the only change de-
tected with starvation was a significant reduction in plasma
alanine (Table 1).

After infusion of labeled metabolites through the left maternal
uterine artery, radioactivity in fetuses from the left uterine side
comprised the amounts transferred from directly infused tracer
and coming from the maternal circulation; whereas in fetuses
from the right uterine side radioactivity corresponded only to
the amount coming from maternal circulation, e.g. the infused
tracer diluted in general maternal circulation and labeled prod-
ucts of metabolism. The difference between radioactivity values
in left and right fetuses therefore provides an index of
direct availability to the fetus of placenta-transferred substrates
from the infused medium.

These values and those of maternal plasma after the infusion
with [U-14C]-D-glucose, [U-14C]-glycerol, or [U-14C]-L-alanine
through the left uterine artery are shown in Tables 2 to 4,
respectively. After maternal infusion with [U-14C]-D-glucose,
plasma 14C-glucose level and its specific activity were much
higher in fetuses from the left uterine side than from the right
side (Table 2). Similarly, the amount of 14C-lactate and its specific
activity in fetal plasma were significantly greater in the left than
in the right fetuses. Interestingly, 14C-lactate plasma values in left
fetuses were much higher than the mother's (Table 2). Maternal
starvation did not modify these relationships although it signifi-
cantly increased the amount of 14C-glucose present in maternal
plasma and its specific activity (Table 2). As shown in Table 3,
when [U-14C]-glycerol was the tracer infused through the mater-
nal left uterine artery both 14C-glycerol and 14C-lactate plasma
values and their specific activities were significantly higher in
the left than in the right fetuses whereas 14C-glucose levels and
14C-glucose specific activity did not differ between fetuses from
both sides (Table 3).

Much more radioactivity appeared as 14C-glucose than as 14C-
glycerol in maternal plasma after maternal infusion with [U-14C]-
glycerol and this difference was even greater in the 48-h starved
rat (Table 3). Plasma 14C-glucose values did not differ between
fetuses from either side whereas both 14C-glycerol and 14C-lactate
levels and their respective specific activities were significantly
higher in fetuses from the left versus the right uterine side in
both the fed and the starved condition after maternal infusion
with [U-14C]-glycerol (Table 3). As shown in Table 4, after mater-
nal infusion of [U-14C]-L-alanine through the left uterine
artery, plasma 14C-alanine levels appeared higher in fetuses from
the left uterine side than in their mothers either fed or starved.
Left fetuses showed higher plasma 14C-alanine and 14C-lactate
values as well as 14C-alanine specific activity than the right ones
after maternal infusion with [U-14C]-L-alanine when either fed
or starved. On the contrary, 14C-glucose levels in fetuses were
very low and no differences were observed between those from
the left and the right uterine sides (Table 4). As it occurred with
[U-14C]-glycerol, after the infusion with [U-14C]-L-alanine, ma-
ternal 14C-glucose values and specific activity were significantly
higher in 48-h starved than in fed rats (Table 4).

Labeled lipid levels in fetal liver after maternal infusion of the
labeled metabolites were measured and values are summarized
in Table 5. [U-14C]-D-glucose and [U-14C]-glycerol produced
higher radioactivity values of both 14C-fatty acids and 14C-glycer-
side glycerol in the liver of fetuses from the left versus the right
uterine horn (Table 5). Maternal starvation diminished 14C-fatty

Table 1. Glucose, glycerol, alanine, and lactate1 concentrations in mother's and fetus' plasma after maternal infusion with D-[U-
14C]-glucose, [U-14C]-glycerol, or L-[U-14C]-alanine, respectively, in 21.5-day pregnant rat

<table>
<thead>
<tr>
<th></th>
<th>Fed rats</th>
<th>48-h starved rats</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mothers</td>
<td>Fetuses</td>
</tr>
<tr>
<td>Glucose (mmol/liter)</td>
<td>4.9 ± 0.7 (6)</td>
<td>3.9 ± 0.4 (6)</td>
</tr>
<tr>
<td>Glycerol (mmol/liter)</td>
<td>155 ± 40 (6)</td>
<td>113 ± 29 (6)</td>
</tr>
<tr>
<td>Alanine (mmol/liter)</td>
<td>479 ± 47 (6)</td>
<td>1667 ± 425 (6)</td>
</tr>
<tr>
<td>Lactate (mmol/liter)</td>
<td>3.2 ± 1.2 (18)</td>
<td>13.2 ± 4.1 (18)</td>
</tr>
</tbody>
</table>

1 Lactate values correspond to all animals studied.
2 Statistical comparisons between mothers and fetuses.
3 Mean ± SD. Numbers in parentheses are numbers of rats/group.
4 Statistical comparisons between fed and 48-h starved rats: p < 0.05; || p < 0.01.
Table 2. Plasma radioactivity values in mothers and fetuses after maternal infusion through left uterine artery with D-[U-14C]-glucose in 21.5-day pregnant rat

<table>
<thead>
<tr>
<th></th>
<th>Fed rats</th>
<th>48-h starved rats</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mothers</td>
<td>Left fetuses</td>
</tr>
<tr>
<td>14C-glucose (dpm/ml)</td>
<td>4479 ± 562†</td>
<td>4495 ± 1238</td>
</tr>
<tr>
<td>14C-glucose specific activity (dpm/μmol)</td>
<td>915 ± 161</td>
<td>1289 ± 562</td>
</tr>
<tr>
<td>14C-lactate (dpm/ml)</td>
<td>818 ± 112</td>
<td>5296 ± 807</td>
</tr>
<tr>
<td>14C-lactate specific activity (dpm/μmol)</td>
<td>305 ± 94</td>
<td>495 ± 156</td>
</tr>
</tbody>
</table>

* Statistical comparisons between left and right fetuses.
† Mean ± SD of six rats/group.
Statistical comparisons between fed and 48-h starved rats: ‡ p < 0.05.

Table 3. Plasma radioactivity values in mothers and fetuses after maternal infusion through left uterine artery with [U-14C]-glycerol in 21.5-day pregnant rat

<table>
<thead>
<tr>
<th></th>
<th>Fed rats</th>
<th>48-h starved rats</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mothers</td>
<td>Left fetuses</td>
</tr>
<tr>
<td>14C-glycerol (dpm/ml)</td>
<td>630 ± 212†</td>
<td>569 ± 366</td>
</tr>
<tr>
<td>14C-glycerol specific activity (dpm/μmol)</td>
<td>4045 ± 2139</td>
<td>4780 ± 2393</td>
</tr>
<tr>
<td>14C-glycerol (dpm/ml)</td>
<td>3387 ± 386</td>
<td>691 ± 120</td>
</tr>
<tr>
<td>14C-glycerol specific activity (dpm/μmol)</td>
<td>685 ± 69</td>
<td>170 ± 45</td>
</tr>
<tr>
<td>14C-lactate (dpm/ml)</td>
<td>878 ± 185</td>
<td>1954 ± 299</td>
</tr>
<tr>
<td>14C-lactate specific activity (dpm/μmol)</td>
<td>373 ± 149</td>
<td>129 ± 18</td>
</tr>
</tbody>
</table>

* Statistical comparisons between left and right fetuses.
† Mean ± SD of six rats/group.
Statistical comparisons between fed and 48-h starved rats: ‡ p < 0.01; § p < 0.001; ‖ p < 0.05.

Table 4. Plasma radioactivity values in mothers and fetuses after maternal infusion through left uterine artery with L-[U-14C]-alanine in 21.5-day pregnant rat

<table>
<thead>
<tr>
<th></th>
<th>Fed rats</th>
<th>48-h starved rats</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mothers</td>
<td>Left fetuses</td>
</tr>
<tr>
<td>14C-alanine (dpm/ml)</td>
<td>1021 ± 321†</td>
<td>6697 ± 2533</td>
</tr>
<tr>
<td>14C-alanine specific activity (dpm/μmol)</td>
<td>2152 ± 664</td>
<td>3647 ± 1628</td>
</tr>
<tr>
<td>14C-alanine (dpm/ml)</td>
<td>627 ± 76</td>
<td>286 ± 89</td>
</tr>
<tr>
<td>14C-alanine specific activity (dpm/μmol)</td>
<td>97 ± 38</td>
<td>51 ± 16</td>
</tr>
<tr>
<td>14C-lactate (dpm/ml)</td>
<td>861 ± 96</td>
<td>6535 ± 2611</td>
</tr>
<tr>
<td>14C-lactate specific activity (dpm/μmol)</td>
<td>212 ± 47</td>
<td>341 ± 167</td>
</tr>
</tbody>
</table>

* Statistical comparisons between left and right fetuses
† Means ± SD of (fed) or five (starved) rats/group.
Statistical comparisons between fed and 48-h starved rats: ‡ p < 0.01; § p < 0.001; ‖ p < 0.05.

acids without affecting 14C-glyceride glycerol (Table 5). Following infusion of [U-14C]-D-glucose in fed pregnant rats, the radioactivity level was higher in liver fatty acids than in glyceride glycerol of left fetuses whereas this relationship was reversed when the mother was starved. Infusion of [U-14C]-glycerol produced a much higher proportion of 14C-glyceride glycerol than 14C-fatty acids in fetal liver; this difference was more pronounced in fetuses from the left than from the right uterine horn (Table 5). Negligible amounts of radioactivity were found in fetal liver when the infused tracer was [U-14C]-L-alanine, and no differences in values were detected in fetuses from the left and right uterine horns.

**DISCUSSION**

Present results in rat fetuses from 21.5-day pregnant fed or 48-h starved mothers support three basic conclusions: 1) significant gluconeogenesis does not occur from either glycerol or L-alanine; 2) a high proportion of these substrates and also D-glucose, when transferred from maternal circulation, reach the fetus in the form of lactate; and 3) the fetus responds to maternal starvation by decreasing liver fatty acid synthesis from transferred D-glucose and glycerol but not from L-alanine which is practically negligible as a lipogenic substrate for the rat fetus. Differences in radioac-
Table 5. Labeled fatty acids and glyceride glycerol in fetal livers after maternal infusion through left uterine artery with D-[U-14C]-glucose, [U-14C]-glycerol, or L-[U-14C]-alanine in 21.5-day pregnant rat

<table>
<thead>
<tr>
<th></th>
<th>Left fetuses</th>
<th>Right fetuses</th>
<th></th>
<th>Left fetuses</th>
<th>Right fetuses</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fed rats</strong></td>
<td></td>
<td></td>
<td>48-h starved rats</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infusion with D-[U-14C]-glucose</td>
<td></td>
<td></td>
<td>48 ± 20</td>
<td></td>
<td>21 ± 11</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>[14C]-fatty acids (dpm/g)</td>
<td>86 ± 49†</td>
<td>24 ± 16</td>
<td></td>
<td>4 ± 4‡</td>
<td>1 ± 2‡</td>
<td>NS</td>
</tr>
<tr>
<td>[14C]-glyceride glycerol (dpm/g)</td>
<td>48 ± 20</td>
<td>21 ± 11</td>
<td></td>
<td>34 ± 7</td>
<td>8 ± 48</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Infusion with [U-14C]-glycerol</td>
<td></td>
<td></td>
<td>91 ± 42</td>
<td></td>
<td>40 ± 16</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>[14C]-fatty acids (dpm/g)</td>
<td>385 ± 149</td>
<td>86 ± 60</td>
<td></td>
<td>19 ± 18‡</td>
<td>5 ± 2‡</td>
<td>NS</td>
</tr>
<tr>
<td>[14C]-glyceride glycerol (dpm/g)</td>
<td>27 ± 29</td>
<td>19 ± 20</td>
<td>NS</td>
<td>471 ± 252</td>
<td>45 ± 13</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Infusion with L-[U-14C]-alanine</td>
<td></td>
<td></td>
<td>23 ± 13</td>
<td></td>
<td>22 ± 13</td>
<td>NS</td>
</tr>
<tr>
<td>[14C]-fatty acids (dpm/g)</td>
<td>50 ± 42</td>
<td>28 ± 26</td>
<td>NS</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Statistical comparisons between left and right fetuses.
† Mean ± SD of five to six rats/group.

Statistical comparisons between fed and 48-h starved rats: † p < 0.01; ‡ p < 0.05.

Lack of [14C]-glycerol synthesis in the rat fetus is clearly in contrast with the efficient incorporation of [14C]-atoms from either labeled glucose, glyceraldehyde, and alanine into fetal lactate which was shown in the present study by the much higher levels of [14C]-lactate in the plasma of fetuses from the left than in those from the right side after maternal infusions through the left uterine artery. Findings indicate that [14C]-lactate is synthesized from maternal transferred substrates by fetoplacental structures. Recently we reported the ability of rat placenta to synthesize lactate from both D-glucose (31) and L-alanine (32). Production of placental lactate from L-alanine seems to be involved in the mechanism of the maternal-fetal transfer of the amino acid (32) and is consistent with the reported presence of alanine aminotransferase in rat placenta (16, 33). The level of labeled lactate in fetal plasma after infusion through the maternal left uterine artery was much lower with [U-14C]-glycerol than with the other substrates studied. This may be due to the limited placental transfer of glyceraldehyde as compared with either D-glucose or L-alanine (34) as a result of the low concentration of glyceraldehyde in maternal circulation. Because of the rapid and efficient conversion of maternal glyceraldehyde to glucose (29), only a limited amount of glyceraldehyde is consequently available for transfer to the fetus, as previously proposed (35). Placental production of lactate from maternal transferred substrates is physiologically important as it contributes to the high lactate levels present in fetal plasma, and also because lactate is used by the rat fetus as an energy fuel (36, 37). As pointed out by Kraan and Dias (38), the excess of lactate produced by fetal structures is efficiently cleared through the placenta to the mother and converted into glucose.

In view of the quality change detected in fetal plasma after maternal infusion of any labeled compound studied, it is not possible to attribute the appearance of labeled lipids in fetal liver to their direct and unique use as lipogenic substrates. It is evident from present results, however, that [U-14C]-L-alanine is not used by the rat fetus as a substrate for labeled fatty acid or glyceraldehyde glycerol synthesis, perhaps due to the low alanine aminotransferase activity found in fetal liver (16). This hypothesis is not in disagreement with the observed appearance of [14C]-lactate in fetus plasma after maternal [14C]-alanine infusion since this conversion occurs in the placenta rather than in fetal tissues (32). Unlike L-alanine, after maternal infusions through the left uterine artery of either [14C]-D-glucose or [14C]-glycerol, labeled lipids appeared in fetal liver. Since this value was higher in livers of fetuses from the left than from the right uterine horn it may be concluded that the lipids were directly synthesized from the placental transferred substrate. Maternal starvation caused a reduction in the synthesis of [14C]-fatty acids from both labeled D-glucose and glycerol, in agreement with the similar effect reported using H2O as tracer (17). This effect seems to occur independently of a
change in fetal liver lipogenic activities which have been shown to be unmodified by maternal starvation (16). It may therefore be caused by the reduced fetal availability of maternal substrates, cosubstrates, and/or effectors for this pathway which occurs in fetuses of starved mothers as a result of diminished placental metabolite transfer, which depends on maternal circulating levels and umbilical cord blood.

In contrast with lipogenesis, 14C-glyceride glycerol synthesis was much greater in liver of fetuses from mothers infused with [U-14C]-glycerol than with [U-14C]-d-glucose and it was unmodified by maternal starvation. This finding indicates that this process occurs through the direct esterification of glycerophosphate formed by the glycerol kinase catalyzed reaction, which is known to be active in fetus liver at late gestation (11), rather than by glycerologenesis. In adults, glycerologenesis from different substrates is directly correlated with gluconeogenesis (23, 39). Therefore the negligible glycerologenesis found in fetal liver further supports the main conclusion of the present study concerning the absence of gluconeogenesis from either l-alanine and glycerol and even from lactate in the fed and 48-h starved rat fetus.

Acknowledgment. The authors thank Angela Murúa for excellent technical assistance.

REFERENCES