Formate metabolism in fetal and neonatal sheep

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Submitted 29 January 2015; accepted in final form 17 March 2015

Formate is a critical component of folate-mediated, one-carbon metabolism; it plays a key role in the production of one-carbon groups for the synthesis of purines and of thymidine and for the provision of methyl groups for transmethylation reactions (11). These are all processes that are likely to occur at elevated rates in growing tissues, particularly in fetal and neonatal tissues. Purine synthesis is required for the synthesis of nucleotides and nucleic acids. Thymidine synthesis is required for the synthesis of DNA. Methylenetetrahydrofolate (THF) is the scaffold on which most one-carbon intermediates are built. The provision of methyl groups occurs via the reduction of 5,10-methylene-THF to 5-methyl-THF by NADPH catalyzed by methylenetetrahydrofolate reductase (MTHFR), followed by the transfer of the methyl group from 5-methyl-THF to homocysteine to form methionine; this is catalyzed by methionine synthase, one of only two mammalian enzymes known to require vitamin B12 for the synthesis of their prosthetic groups (21). Methionine is used for the synthesis of S-adenosylmethionine (SAM), the principal biological methylation agent. It has been computed that the human genome contains more than 200 methyltransferases that use SAM to methylate a variety of acceptors, including DNA, RNA, and proteins, regulating gene expression, mRNA processing, and protein function (28). In addition, methyltransferases are responsible for the synthesis of such key molecules as creatine, phosphatidylcholine, and epinephrine (25) and for the detoxification of inorganic arsenic ions (12). Recently, it has been shown that folate metabolism plays a key role in the reduction of NADP+ to NADPH (10).

Folate is critically important in development, particularly in pregnancy. Low folate status, particularly in the context of the TT genotype of MTHFR, is a risk factor for the development of neural tube defects such as spina bifida and anencephaly (32). Mandatory folic acid fortification of the food supply in the US, Canada, and some other countries has greatly reduced the incidence of neural tube defects (8). The mechanism by which folate supplementation acts is not fully understood.

Formate is produced primarily in mitochondria from serine, glycine, and two intermediates of choline catabolism, sarcosine and dimethylglycine. Mitochondrial 10-formyl-THF can give rise to formate, which leaves the mitochondrion and is incorporated into the cytosolic pool of 10-formyl-THF, where it may be used for purine synthesis (29). Cytosolic 10-formyl-THF may also be reduced to 5,10-methylene-THF and then to 5-methyl-THF. Its formyl group may also be oxidized to CO2, providing an exit for excess one-carbon groups from folate metabolism (16). Formate may also arise from a number of other metabolic reactions, such as methanol metabolism, phtyanic acid α-oxidation, tryptophan catabolism, and cholesterol synthesis (19). Figure 1 shows an outline of one-carbon and formate metabolism. Although intermediates in one-carbon metabolism occur primarily within cells, formate can be found in appreciable quantities in plasma; this may provide insights into cellular one-carbon metabolism.

MATERIALS AND METHODS

Animals. All aspects of the surgical and experimental protocols were approved by the Texas A & M University Institutional Animal Care and Use Committee. Suffolk ewes aged 2–5 yr were obtained...
from a commercial supplier. Upon arrival at the animal facility, each ewe received an intramuscular injection of Covexin 8 (Merck Animal Health, Summit, NJ) and an oral bolus of Valbazen (Zoetis, Kalamazoo, MI). Ewes received progesterone-impregnated vaginal implants (EAZI-BREED, CIDR; Zoetis). Implants were removed 11 days after placement, at which time prostaglandin F₂α (20 mg, LUTALYSE; Zoetis) was administered intramuscularly. The following day, ewes were placed with a ram fitted with a marking harness for a period of 4 h. Marked ewes were presumed pregnant until they were confirmed pregnant ultrasonographically on gestation days (GD) 25 and 92.

Ewes were fed TAMU Ewe Ration, a custom ration (Nutrena; Zoetis) was administered intramuscularly. The following day, ewes were placed with a ram fitted with a marking harness for a period of 4 h. Marked ewes were presumed pregnant until they were confirmed pregnant ultrasonographically on gestation days (GD) 25 and 92.

Six pregnant ewes were surgically implanted with indwelling cannulas so as to permit simultaneous sampling from the fetal and maternal circulations; five pregnant ewes were allowed to lamb naturally (no implanted cannulas) to measure ewe and lamb plasma and milk formate levels. In addition, seven nonpregnant ewes were sampled for comparison.

Ewes were allowed to lamb naturally, and shortly after birth the lambs were weighed and ear-tagged, and their navels were dipped in 7% iodine. They were weighed weekly thereafter and remained with their mothers until weaning at 8 wk age. All lambs were allowed ad libitum access to water. Daily feed consumption was monitored; all subjects consumed all of the food offered.

Six pregnant ewes were surgically implanted with indwelling cannulas so as to permit simultaneous sampling from the fetal and maternal circulations; five pregnant ewes were allowed to lamb naturally (no implanted cannulas) to measure ewe and lamb plasma and milk formate levels. In addition, seven nonpregnant ewes were also sampled for comparison.

Ewes were allowed to lamb naturally, and shortly after birth the lambs were weighed and ear-tagged, and their navels were dipped in 7% iodine. They were weighed weekly thereafter and remained with their mothers until weaning at 8 wk age. All lambs were allowed ad libitum access to water. Daily feed consumption was monitored; all subjects consumed all of the food offered.

Sample collection. From the cannulated ewes, maternal and fetal samples were collected into lithium heparin tubes via the surgically implanted catheters at GD 117 ± 1 days; amniotic fluid was collected into polystyrene tubes with no additives. From the noncannulated group, plasma samples were collected by jugular venipuncture into lithium heparin tubes. Samples were collected from the ewes and lambs shortly after lambing occurred, and at 1 wk, 2 wk, 1 mo, and 2 mo after lambing. Milk samples were collected from the ewes by hand-milking into polystyrene tubes with no additives. Milk samples were obtained shortly after lambing occurred and at 1 wk, 2 wk, 1 mo, and 2 mo later (time of weaning). Plasma samples were immediately separated by centrifugation, and all samples were stored at −80°C until analysis.

Cannulation. On GD 117 ± 1 the pregnant sheep were anesthetized, and aseptic surgery was performed. Methods have been described previously (31, 37). In brief, anesthesia was induced using intravenous administration of diazepam (0.2 mg/kg body wt; Abbott Laboratories, North Chicago, IL) and ketamine (4 mg/kg body wt; Ketaset, Fort Dodge, IA). The ewes were intubated and ventilated, and anesthesia was maintained with isoflurane (0.5–2.5%, WaseFlo; Abbott Laboratories) and oxygen. The abdomen was opened at the midline, the uterus was externalized and incised, and a fetal hindlimb was exteriorized. An incision was made over the cranialateral aspect of the fetal hindlimb midway between the hock and stifle. A catheter (0.030 in. inner diameter, 0.050 in. outer diameter polyvinyl chloride) was advanced from the fetal cranial tibial artery into the abdominal aorta to the level of the diaphragm; the procedure was then repeated on the other hindlimb. The fetus was returned to the uterus, and the uterus and maternal midline were closed. The maternal femoral vessels were isolated and cannulated, and catheters (0.050 in. inner diameter, 0.090 in. outer diameter polyvinyl chloride) were advanced from the maternal femoral artery and vein to the level of the diaphragm of the abdominal aorta and vena cava, respectively. Fetal and maternal catheters were passed through the abdominal wall, where they were stored in a cloth pouch.

Analyses. Formate was analyzed by the gas chromatography-mass spectrometry method of Lamarre et al. (17). Total plasma homocysteine (Hcy) was measured as described by Vester and Rasmussen (35). Total plasma and amniotic folate were measured using the Lactobacillus casei microbiological assay (14). Total plasma and amniotic vitamin B₁₂ were analyzed using the Advia Centaur XP Immunoassay (Siemens Canada) as described by the manufacturer. Amino acids were derivatized with o-phthaldehyde and assayed by HPLC as described (38). Choline and its derivatives dimethylglycine and betaine were quantified by liquid chromatography-mass spectrometry/mass spectrometry (13, 36), with modifications based on available instrumentation (41, 42).

Expression and analysis of data. Data are expressed as means ± SD, with the number of observations in parentheses. Statistical anal-
FORMATE METABOLISM IN THE FETAL LAMB

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Table 1. Plasma levels of folate, vitamin B12, and homocysteine in pregnant and fetal sheep and their controls

<table>
<thead>
<tr>
<th>Precursor</th>
<th>Pregnant Ewes</th>
<th>Amniotic Fluid</th>
<th>Nonpregnant Ewes</th>
<th>2- to 3-Mo-Old Lambs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Folate, nmol/l</td>
<td>0.757 ± 0.411 (n = 6)</td>
<td>1.20 ± 0.56 (n = 4)</td>
<td>0.988 ± 0.199 (n = 4)</td>
<td>0.705 ± 0.262 (n = 7)</td>
</tr>
<tr>
<td>Vitamin B12, pmol/l</td>
<td>870 ± 416 (n = 5)</td>
<td>1,720 ± 776 (n = 6)</td>
<td>2,250 ± 828 (n = 5)*</td>
<td>2,600 ± 704 (n = 7)*</td>
</tr>
<tr>
<td>Homocysteine, μmol/l</td>
<td>2.47 ± 0.91 (n = 6)</td>
<td>4.25 ± 1.57 (n = 6)</td>
<td>7.13 ± 3.39 (n = 6)*</td>
<td>18.4 ± 4.5 (n = 7)*</td>
</tr>
</tbody>
</table>

Results are given as means ± SD. Analysis by 1-way ANOVA with Dunnett’s multiple-comparison posttest. *Results that are significantly different from the fetus.

Table 2. Plasma levels of principal formate precursors in pregnant and fetal sheep and their controls

<table>
<thead>
<tr>
<th>Precursor</th>
<th>Pregnant Ewes</th>
<th>Amniotic Fluid</th>
<th>Nonpregnant Ewes</th>
<th>2- to 3-Mo-Old Lambs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serine</td>
<td>1,660 ± 384 (n = 3)</td>
<td>188 ± 39 (n = 3)*</td>
<td>3,490 ± 1,236 (n = 3)*</td>
<td>157 ± 28 (n = 3)</td>
</tr>
<tr>
<td>Histidine</td>
<td>67.9 ± 36.5 (n = 3)</td>
<td>64.6 ± 18.0 (n = 3)</td>
<td>81.3 ± 24.6 (n = 3)</td>
<td>86.2 ± 10.0 (n = 3)</td>
</tr>
<tr>
<td>Glycine</td>
<td>948 ± 276 (n = 3)</td>
<td>582 ± 42 (n = 3)</td>
<td>612 ± 314 (n = 3)</td>
<td>1,710 ± 154 (n = 3)*</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>129 ± 50 (n = 3)</td>
<td>58.1 ± 17.7 (n = 3)</td>
<td>41.7 ± 6.0 (n = 3)</td>
<td>38.8 ± 5.4 (n = 3)*</td>
</tr>
<tr>
<td>Methionine</td>
<td>36.1 ± 8.6 (n = 3)</td>
<td>30.2 ± 17.9 (n = 3)</td>
<td>17.4 ± 3.5 (n = 3)</td>
<td>20.8 ± 3.9 (n = 3)</td>
</tr>
<tr>
<td>Choline</td>
<td>19.5 ± 5.5 (n = 6)</td>
<td>5.47 ± 1.11 (n = 6)</td>
<td>25.2 ± 18.8 (n = 6)</td>
<td>7.41 ± 1.18 (n = 7)</td>
</tr>
<tr>
<td>Betaine</td>
<td>166 ± 66 (n = 6)</td>
<td>95.9 ± 42.6 (n = 6)</td>
<td>190 ± 110 (n = 6)</td>
<td>138 ± 42 (n = 7)</td>
</tr>
<tr>
<td>Dimethylglycine</td>
<td>48.9 ± 25.5 (n = 6)</td>
<td>1.55 ± 0.90 (n = 6)*</td>
<td>124.0 ± 54.0 (n = 6)*</td>
<td>16.0 ± 0.45 (n = 7)*</td>
</tr>
</tbody>
</table>

Results are given as means ± SD. Analysis by 1-way ANOVA with Dunnett’s multiple-comparison posttest. *Results that are significantly different from the fetus.

The analysis was carried out with the GraphPad 3 statistical package. Data were analyzed by means of a t-test, a one-way ANOVA, or a repeated-measures ANOVA, followed by Dunnett’s multiple-comparison post hoc test, as indicated in the figures and tables.

RESULTS

Figure 2 shows plasma formate levels in maternal and fetal plasma and in amniotic fluid taken from six pregnant ewes between GD 119 and 121 (full term is 147 ± 2 days), corresponding to mid-third trimester in humans. Very high levels of formate were evident in fetal plasma (191 ± 62 μM) and in amniotic fluid (296 ± 154 μM). Both of these were significantly higher than the formate concentration in maternal plasma (33 ± 13 μM). We then compared the maternal formate concentrations with those of a separate group of nonpregnant ewes; the formate concentration in the pregnant ewes was significantly higher (P < 0.01) than in nonpregnant ewes (6 ± 7 μM). We also compared the formate concentrations in the fetal lambs with those in a separate group of 2- to 3-mo-old lambs; the formate concentration in the fetal lambs was significantly higher (P < 0.01) than in plasma obtained from 2- to 3-mo-old lambs (62 ± 11 μM).

Previously, we have reported elevated formate levels in rats that were deficient in either vitamin B12 or folate (18). Although it is highly unlikely that these pregnant mothers or developing fetuses are deficient in either of these vitamins, we felt it was important to obtain a measurement of their folate and vitamin B12 status. A deficiency in either vitamin B12 or folate leads to hyperhomocysteinemia due to impaired function of methionine synthase. This enzyme is one of only two known vitamin B12-requiring enzymes in mammals; in addition, it employs 5-methyl-THF as its source of methyl groups. Therefore, we assayed for these two vitamins as well as tHcy. Table 1 shows tHcy levels in fetal plasma to be significantly lower (P < 0.01) than in plasma from 2- to 3-mo-old lambs. tHcy in plasma from the pregnant ewes was significantly (P < 0.001) lower than that in nonpregnant ewes. Table 1 also provides data on folate and vitamin B12 levels. There were no significant differences in folate concentrations among the groups, including between fetal and maternal plasma. Fetal plasma vitamin B12 was significantly lower than amniotic fluid vitamin B12. The 2- to 3-mo-old lambs also had significantly lower (P = 0.004) plasma vitamin B12 than the nonpregnant ewes.

Homocysteine, which leads to hyperhomocysteinemia due to impaired function of methionine synthase, was very low (1–2 μM) in both the pregnant and the nonpregnant plasma. Fetal plasma vitamin B12 was significantly lower than amniotic fluid vitamin B12. The 2- to 3-mo-old lambs also had significantly lower (P = 0.004) plasma vitamin B12 than the nonpregnant ewes.

Formate can arise in a number of ways. Within mitochondria it may be produced from one-carbon groups arising from serine or glycine metabolism or from the catabolism of the choline metabolites sarcosine and dimethylglycine. Betaine, which can either be provided in the diet or arise from choline catabolism, is used by betaine-homocysteine methyltransferase (BHMT) to remethylate homocysteine to methionine; the other product of BHMT is dimethylglycine. Conversion of methionine to SAM follows by methylation of glycine by SAM can also provide sarcosine. In addition, formate may be produced in the cytoplasm via the catabolism of tryptophan and histidine; it also arises during cholesterol synthesis in the demethylation of lanosterol (19).

Table 2 provides information on the concentrations of the major precursors of formate in amniotic fluid and in fetal and maternal plasma as well as in plasma from nonpregnant ewes and 2- to 3-mo-old lambs. The most remarkable finding in Table 2 is the very high serine concentration in both amniotic fluid and fetal plasma. There was a ninefold concentration gradient for serine across the placenta, with the fetal circulation having the highest concentration. High glycine concentrations in all of the plasmas and in amniotic fluid were observed. Betaine levels were also appreciable. Dimethylglycine levels were very low (1–2 μM) in both the pregnant and the nonpregnant ewes; however, there were appreciable levels of dimethylglycine (~50 μM) in the fetal plasma and amniotic fluid (~120 μM) such that there was an ~30-fold concentration gradient between the fetal and maternal plasmas.

We carried out a separate set of experiments to determine formate levels in postpartum ewes and in lambs. These sheep had not been cannulated for sampling fetal plasma or amniotic fluid. Initially, we studied five such sheep, but one delivered a full-term, dead lamb. We removed this animal from our study.
stable level of ewes’ milk from lambing to 8 wk of age. Milk formate was postpartum sheep. Glycine increased to quite high concentrations in 8-wk-old lambs (42.3 ± 3.2 µM), Table 4 provides data on the plasma concentrations of the principal formate precursors in lambs from the day of lambing to 8 wk of age. Serine 649, Glycine 1,140, Tryptophan 219, Methionine 177, Choline 20.0 ± 6.9 (n = 4), Betaine 281 ± 48 (n = 4), Dimethylglycine 13.9 ± 3.3 (n = 4) were appreciable precursors in the day of lambing. Table 3 provides data on the plasma concentrations of the principal formate precursors in the sheep. The pattern of brain growth in the sheep effectively models that in humans, and the body mass of the sheep and fetal lamb is similar to the body mass of the pregnant woman and human fetus (1, 9). Thus the sheep, which is often used as a model for human pregnancy, is an ideal model for answering the questions posed in this study. Although not a great deal is known about one-carbon metabolism in sheep, its broad features appear to be similar to that in other animals. A few studies have addressed specific aspects of one-carbon metabolism in lambs. In particular, Xue and Snoswell (39) have reported that hepatic BHMT activity rapidly increases after birth but then drops to low activity when the lambs reach the ruminant stage; they maintain this low activity into adulthood. This may reflect the availability of betaine in sheep milk; we found that milk betaine averaged 196 ± 70 µM for the first 4 wk and dropped to 104 ± 25 µM in the 8th wk.

We investigated formate metabolism, as well as other aspects of one-carbon metabolism, in pregnant and nonpregnant ewes as well as in fetal sheep and in neonatal lambs. There is strong evidence that formate plays an important role in mammalian development. Pike et al. (29) have shown robust expression in mouse embryos of mRNA for MTHFD1L (methylene-THF dehydrogenase 1-like), the enzyme responsible for mitochondrial formate production, from at least embryological day 4.5 to parturition. They have further shown in an embryonic cell line that mitochondrially derived one-carbon units are the single largest source of methyl groups for cytoplasmic methylation reactions. Formate also appears to be

**Table 3. Plasma levels of the principal formate precursors in lambs from the day of lambing to 8 wk of age**

<table>
<thead>
<tr>
<th>Precursors</th>
<th>Lambing</th>
<th>1</th>
<th>2</th>
<th>4</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serine</td>
<td>649 ± 71 (n = 3)</td>
<td>394 ± 69 (n = 3)</td>
<td>590 ± 203 (n = 3)</td>
<td>243 ± 8 (n = 3)</td>
<td>261 ± 34 (n = 3)</td>
</tr>
<tr>
<td>Histidine</td>
<td>512 ± 275 (n = 3)</td>
<td>289 ± 74 (n = 3)</td>
<td>379 ± 49 (n = 3)</td>
<td>279 ± 40 (n = 3)</td>
<td>286 ± 28 (n = 3)</td>
</tr>
<tr>
<td>Glycine</td>
<td>1,140 ± 402 (n = 3)</td>
<td>1,320 ± 271 (n = 3)</td>
<td>1,390 ± 280 (n = 3)</td>
<td>1,120 ± 187 (n = 3)</td>
<td>1,100 ± 130 (n = 3)</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>219 ± 19 (n = 3)</td>
<td>245 ± 8 (n = 3)</td>
<td>239 ± 28 (n = 3)</td>
<td>208 ± 10 (n = 3)</td>
<td>186 ± 5 (n = 3)</td>
</tr>
<tr>
<td>Methionine</td>
<td>177 ± 29 (n = 3)</td>
<td>144 ± 33 (n = 3)</td>
<td>144 ± 16 (n = 3)</td>
<td>135 ± 14 (n = 3)</td>
<td>143 ± 9 (n = 3)</td>
</tr>
<tr>
<td>Choline</td>
<td>20.0 ± 6.9 (n = 4)</td>
<td>18.5 ± 1.4 (n = 4)</td>
<td>19.7 ± 6.9 (n = 4)</td>
<td>13.7 ± 2.6 (n = 4)</td>
<td>11.4 ± 2.1 (n = 4)</td>
</tr>
<tr>
<td>Betaine</td>
<td>281 ± 48 (n = 4)</td>
<td>352 ± 158 (n = 4)</td>
<td>359 ± 114 (n = 4)</td>
<td>259 ± 36 (n = 4)</td>
<td>189 ± 28 (n = 4)</td>
</tr>
<tr>
<td>Dimethylglycine</td>
<td>13.9 ± 3.3 (n = 4)</td>
<td>28.5 ± 13.7 (n = 4)</td>
<td>23.7 ± 6.2 (n = 4)</td>
<td>8.98 ± 3.33 (n = 4)</td>
<td>1.89 ± 0.73 (n = 4)</td>
</tr>
</tbody>
</table>

Results are given as means ± SD. Analysis by repeated-measures ANOVA with Dunnett’s multiple-comparison posttest. *Results that are significantly different from the day of lambing.

**Fig. 3. Plasma formate levels in lambs on the day of lambing until 8 wk of age. Analysis by repeated-measures ANOVA, followed by Dunnett’s multiple-comparison posttest.**

**Fig. 4. Plasma formate levels in ewes on the day of lambing until 8 wk of age. Analysis by repeated-measures ANOVA, followed by Dunnett’s multiple-comparison posttest.** There were no statistical differences between the groups, but there was a strong trend (P = 0.07) for a difference between the formate level at 4 wk and at lambing.

**AJP-Endocrinol Metab • doi:10.1152/ajpendo.00046.2015 • www.ajpendo.org**
critical for neural tube closure. Knockout of MTHFD1L is embryonic-lethal, with embryos exhibiting growth impairment and both neural tube and craniofacial defects. These developmental disorders may be attenuated by providing the pregnant dams with sodium formate in their drinking water (23). Very recently, Pai et al. (27) have found an appreciable incidence of neural tube defects in mice in which the glycine decarboxylase subunit of the glycine cleavage system has been knocked out. Remarkably, these abnormalities were completely prevented when the sodium formate was added to the dams’ drinking water. Serine is generally regarded as the principal source of one-carbon units for folate metabolism. Many cells contain both cytosolic and mitochondrial isoforms of serine hydroxymethyltransferase (SHMT). However, a critical finding is that both cytosolic and mitochondrial isoforms of serine hydroxymethyltransferase (SHMT). However, a critical finding is that knockout of the cytosolic SHMT in mice yields viable offspring with relatively minor abnormalities (20). This focuses attention on the only known vehicle by which mitochondrially derived one-carbon groups. Formate is with relatively minor abnormalities (20). This focuses attention on the mitochondrial production of one-carbon groups. Formate is probably a folate-linked, one-carbon compound, but this was never identified (2). Given recent advances in our knowledge of formate as a critical one-carbon intermediate, as carbon was probably a folate-linked, one-carbon compound, but this was never identified (2). Given recent advances in our understanding of formate and DNA methylation). Dasarathy et al. (7) have reported markedly higher transmethylation rates in pregnant women during the third trimester; they attribute this to higher methylation demands. Formate in fetal plasma may be available to all fetal tissues; we hypothesize that circulating formate may be a means of providing one-carbon groups to many tissues. Nevertheless, we have not experimentally addressed the metabolic fate of formate, and it is possible that the elevation in formate may reflect a decrease in its utilization. We also did not directly address the issue of the metabolic origin of fetal formate. However, we do draw attention to experiments in chronically cannulated pregnant sheep by Cetin and colleagues (3–5) and Chung et al. (6), who reported a unique pattern of interorgan serine metabolism in the sheep fetus. They observed an appreciable placental uptake of maternal serine, but little of this serine is transferred to the fetal circulation. Rather, it is converted to glycine, which is released to the fetal circulation. In addition, the placenta takes up fetal serine and converts it to glycine. Serine contains three carbons, and glycine contains two. Battaglia speculated that the missing carbon was probably a folate-linked, one-carbon compound, but this was never identified (2). Given recent advances in our knowledge of formate as a critical one-carbon intermediate, as well as the very high levels of formate that we find in fetal sheep, we speculate that formate is the much-sought, one-carbon molecule produced by the ovine placenta from serine. Some support for this proposal is provided by recent experiments in our laboratory that found that rat placental mitochondria can produce formate from serine (Quilty R, Brosnan JT, and Brosnan ME, unpublished results). It should also be noted that ovine placenta displays a high activity of the mitochondrial SHMT throughout pregnancy (26). Additional support is provided by Prasannan et al. (30), who reported that of 10 human tissues surveyed, the placenta expressed the highest mRNA abundance of the mitochondrial bifunctional protein (containing 5,10-methylene-THF dehydrogenase and 5,10-methenyl-THF cyclohydrolase) required for formate synthesis.

Although the placenta may be a significant source of formate for the sheep fetus, it cannot provide formate to the postpartum lamb. Yet circulating formate levels remain elevated for some time, decreasing to basal levels after ~8 wk. Therefore, it is possible that formate production from unknown substrates and in unidentified tissues continues at a relatively high rate in the postpartum lamb, although direct isotopic experiments are required to definitely establish whether this is so. Our measurements in milk raise the possibility that this may be an

Table 4. Principal formate precursors in ewe plasma immediately after giving birth (lambing) to 8 wk postlambing

<table>
<thead>
<tr>
<th>Precursors, μM</th>
<th>Lambing</th>
<th>Postlambing, wk</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Serine</td>
<td>51.7 ± 20.1 (n = 3)</td>
<td>83.2 ± 23.0 (n = 3)</td>
</tr>
<tr>
<td>Histidine</td>
<td>82.1 ± 5.5 (n = 3)</td>
<td>70.5 ± 4.0 (n = 3)</td>
</tr>
<tr>
<td>Glycine</td>
<td>1.260 ± 63 (n = 3)</td>
<td>2.790 ± 310 (n = 3)*</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>64.1 ± 11.4 (n = 3)</td>
<td>52.8 ± 5.5 (n = 3)</td>
</tr>
<tr>
<td>Methionine</td>
<td>53.7 ± 7.1 (n = 3)</td>
<td>48.8 ± 8.6 (n = 3)</td>
</tr>
<tr>
<td>Choline</td>
<td>6.94 ± 0.59 (n = 4)</td>
<td>6.79 ± 1.22 (n = 4)</td>
</tr>
<tr>
<td>Betaine</td>
<td>150 ± 22 (n = 4)</td>
<td>144 ± 12 (n = 4)</td>
</tr>
<tr>
<td>Dimethylglycine</td>
<td>2.75 ± 1.57 (n = 4)</td>
<td>1.30 ± 0.31 (n = 4)*</td>
</tr>
</tbody>
</table>

Results are given as means ± SD. Analysis by repeated-measures ANOVA with Dunnett’s multiple-comparison posttest. *Results that are significantly different from the day of lambing.

Fig. 5. Formate levels in ewes’ milk from lambing to 8 wk. Analysis by repeated-measures ANOVA, followed by Dunnett’s multiple-comparison posttest. *Results that are significantly different from the day of lambing.
important source of formate for suckling lambs. Cardellino and Benson (2) reported that ewes rearing singleton lambs produced ~2.6 kg of milk/day for 9 wk. We measured milk formate in the fat-free infranatant. Therefore, we can calculate that ewes’ milk may provide as much as 37 μmol formate/day to the lamb. We require isotopic measurements of new formate appearance in lambs, along the lines of our recent studies in rats (24), to determine what fraction of formate may be provided via the milk.

In addition to serine and glycine, the choline oxidative metabolites may be important sources of formate. Approximately 30 times higher concentrations of dimethylglycine, a choline metabolite produced when betaine is used as methyl donor, were detected in the fetal (vs. maternal) compartment. The use of choline in the generation of formate may also be important in humans. Plasma concentrations of choline-derived, one-carbon donors decrease markedly across pregnancy (40). In general, there was an approximate equivalence between fetal plasma and amniotic fluid concentrations of many metabolites, with the concentrations in amniotic fluid tending to be somewhat higher (41), which is due in part to enhanced use of betaine as a source of one-carbons in this reproductive state (40). In general, there was an approximate equivalence between fetal plasma and amniotic fluid concentrations of many metabolites, with the concentrations in amniotic fluid tending to be somewhat higher (41), which is due in part to enhanced use of betaine as a source of one-carbons in this reproductive state (40).

The principal finding in this work is the very high concentrations of formate that are found in the ovine fetus in both fetal plasma and amniotic fluid. The animals were in good folate and vitamin B12 status and had no evidence of folate deficiency. Indeed, the equivalent of folate and vitamin B12 concentrations in maternal and fetal plasma suggest that formate from this source. There were also more modestly elevated maternal levels of formate in lambs and ewes for some time after lambing. These high levels of plasma formate in fetal and neonatal animals are not a peculiarity of the ovine model, as we have found the same phenomenon in rats (Harnett B, unpublished results).

The second most important finding is the very high concentrations of serine in fetal plasma and amniotic fluid. It is possible that the fetus is a small nutrient sink for serine and that this reflects a role for serine as a precursor of formate, but this remains to be determined. Future studies are required to establish the tissue and substrate sources of fetal formate, the role of formate in fetal one-carbon metabolism, and the sources, including milk, of formate to the nursing lamb.

ACKNOWLEDGMENTS

We give special thanks to Lance Wheeler, Dillon Johnson, and Sarah Baker for their assistance.

GRANTS

This work was supported by grants from the Canadian Institutes for Health Research (CIHR) and the Research Development Corporation of Newfoundland and Labrador (RDC; to J. T. Brosnan and M. E. Brosnan) and the National Institutes of Health (NIH K08AA18166-2; to S. E. Washburn), graduate scholarship support from School of Graduate Studies, the Memorial University of Newfoundland, and CIHR-RDC (to L. MacMillan), and National Science and Engineering Council of Canada Undergraduate Summer Research Awards (to B. Harnett).

DISCLOSURES

None of the authors have any conflicts of interest, financial or otherwise, to declare.

AUTHOR CONTRIBUTIONS


REFERENCES


