Ovine fetal leucine kinetics and protein metabolism during decreased oxygen availability

J. ROSS MILLEY
Division of Neonatology, Department of Pediatrics,
University of Utah School of Medicine, Salt Lake City, Utah 84132

MILLEY, J. Ross. Ovine fetal leucine kinetics and protein metabolism during decreased oxygen availability. Am. J. Physiol. 274 (Endocrinol. Metab. 37): E618–E626, 1998.—The fetus depends on an uninterrupted supply of oxygen to provide energy, not only for basal metabolism but also for the metabolic costs of growth. By curtailing the metabolically expensive processes of protein turnover, the fetus could conserve energy when oxygen availability is limited. Therefore, this investigation was performed to find whether protein synthesis and breakdown are diminished during decreased fetal oxygen availability. Furthermore, if these conditions reduce fetal growth, protein synthesis should be affected more than breakdown so that protein accretion, an important component of fetal growth, also falls. In eight chronically prepared fetal lambs, we compared leucine kinetics (reciprocal pool model) during control conditions with measurements made during maternal hypoxia, a condition that limits fetal oxygen availability. Decreased fetal oxygen availability (−43% P < 0.001) reduced fetal oxygen consumption (−16% P < 0.01), as well as both the uptake of leucine across the placenta (−48% P < 0.001) and its rate of decarboxylation (−30% P < 0.001). Fetal protein synthesis decreased (−32% P < 0.001) to a greater extent than proteolysis (−22% P < 0.001). Consequently, fetal protein accretion, an important component of fetal growth, also decreased (−62% P < 0.001). We calculate that the reduction in fetal protein synthesis and breakdown, both processes that require intracellular expenditure of ATP, decreased fetal energy needs sufficiently to account for most, if not all, of the decrease measured in fetal oxygen consumption.

METHODS

Animals. Time-dated pregnant ewes (6 singleton, 2 twin pregnancies), with gestational ages ranging from 114 to 119 days, were obtained from Torell Ranch (Ukiah, CA). Maternal weight averaged 55.8 ± 2.3 (SE) kg and ranged from 49 to 68 kg. The ewes were sedated with intravenous ketamine (10 mg/kg) and then intubated and anesthetized with inhaled isoflurane (−2%). The uterus was exposed through a midline incision in the maternal abdomen, and a hysterotomy was performed over the area of the fetal neck. An incision in the fetal skin was made over a jugular vein, and two catheters were placed in the fetal superior vena cava. The fetal and uterine incisions were then closed, and the uterus was reopened over the area of the fetal hindlimbs. Catheters were placed by way of a fetal hindlimb artery and two hindlimb veins into the abdominal aorta and inferior vena cava, respectively. Subsequently, all incisions, including the maternal abdominal incision, were closed and the catheters tunneled underneath the maternal skin to the maternal flank, where they were stored in a pouch until needed.

Each animal received 200 mg of trimethoprim and 1,000 mg of sulfadiazine (Di-Trim, 48% injection; Syntex Animal
ments (arrowheads) were made after [1-14C]leucine infusion, 8% of original fetal weight) (31) over the 21/3 days of this study. We did not, however, correct for fetal growth (estimated to be 18% to allow comparison of these data with other published data. The data contained in this report were normalized, where necessary, to allow comparison of these data with other published data.

The animals described in this experiment all fed and drank normally during recovery. After the experimental protocols (to be described in Experimental design) were completed, both the fetus and ewe were killed by barbiturate overdose (Beuthanasia-D Special; Schering, Kenilworth, NJ). All catheter positions were confirmed by direct examination at necropsy. The data contained in this report were normalized, where appropriate, to fetal weight at necropsy [3.23 ± 0.14 (SE) kg] to allow comparison of these data with other published data. We did not, however, correct for fetal growth (estimated to be 8% of original fetal weight) (31) over the 21/3 days of this study because the actual daily rate of growth was unknown. However, our inability to correct for fetal weight could not have influenced the conclusions of this study, because its crossover design allowed separation of those experimental effects due to hypoxia from those due to the duration of the study (such as fetal growth). This study was approved by the University of Utah Institutional Animal Care and Use Committee.

Experimental design. This study protocol includes 2 days of experimentation on each animal (Fig. 1). The first protocol (day 1) included measurements made during control conditions and then during decreased fetal oxygen availability. Following a washout period (≥36 h), a second experiment (day 2) was done with the experimental order reversed (i.e., measurements were made during decreased oxygen availability and then during control conditions). This experimental design was chosen so that the effects of decreased fetal oxygen availability could be separated from those due to the duration or order of the experiment. Day 1 experiments were initiated by starting an infusion of [1-14C]leucine at −5.5 × 10⁶ dpm/min into the fetal inferior vena cava catheter. This infusion was continued for 180 min before blood samples were taken. This duration is sufficient for leucine specific activity to become constant (25). After [1-14C]leucine had been infused for 2 h, an intravenous infusion of antipyrine (24 mg/ml in sterile 0.9% saline) was started at 0.2 ml/min to measure umbilical blood flow. One hour later, blood was drawn simultaneously from the fetal umbilical vein and aorta for analysis of whole blood contents of glucose, antipyrine, oxygen, leucine, and α-ketosocaproate as well as radioactivity as 14CO2 and [1-14C]leucine. In addition, a sample of arterial plasma was obtained after an infusion of α-ketosocaproate specific activity. A second similar set of blood samples was drawn 15 min later. Data obtained from these two samples were averaged before subsequent calculations were made. As the second set of samples was obtained, arterial blood was also taken for analysis of hematocrit, pH, PCO2 and PO2. After each set of blood samples, the fetus was transfused with an amount of maternal blood equal to that removed from the fetus.

After the first set of two blood samples was obtained, the [1-14C]leucine and antipyrine infusions were stopped and fetal oxygen availability was diminished by delivering 7.6–9.8% O2 and 3–4% CO2 to a bag enclosing the ewe’s head. During this and all subsequent periods of hypoxia, maternal fractional inspired O2 (FIO2) was continuously monitored. Blood for measurement of fetal pH and blood gases was drawn every 30 min. One hour after the start of hypoxia, the [1-14C]leucine infusion was restarted, followed 2 h later by a restart of the antipyrine infusion. After an additional hour of both infusions, a second set of two fetal blood samples, identical to the first, was obtained. On the second experimental day (day 2), to reverse the order of the experiment, the ewe was allowed to breathe an inspired oxygen concentration identical to that used to induce hypoxemia on day 1. Fetal [1-14C]leucine and antipyrine infusions were begun at 60 and 180 min, respectively, after the induction of maternal hypoxemia, again at rates identical to those on day 1. After the first set of fetal blood samples had been drawn, the [1-14C]leucine and antipyrine infusions were stopped, and the mother was allowed to breathe room air. Thirty minutes later, we restarted the fetal [1-14C]leucine infusion, followed after an additional 2 h by the antipyrine infusion. After an additional hour of both infusions, blood samples identical to those of the first set were again obtained.

Chemical analyses. The methodology for measurement of fetal whole blood concentrations of antipyrine, leucine, α-ketosocaproate, leucine whole blood radioactivity, fetal plasma specific activity of α-ketosocaproate and blood 14CO2 has previously been described (25). Whole blood glucose concentration was measured by the glucose oxidase method (Sigma Chemical, St. Louis, MO). Methods for measurement of whole blood concentrations of lactate and α-amino nitrogen-containing substances have also been previously described (20). Maternal inspired oxygen concentration was measured by direct analysis (MiniOX O2 monitor; Catalyst Research, Owings Mills, MD). Blood oxygen concentration was calculated from measurements of oxygen saturation and hemoglobin content (Radiometer Copenhagen Hemoximeter, OSM 3). Blood gases and pH were measured at 39°C with standard electrodes (Radiometer Copenhagen ABL 30 Acid Base Analyzer). Hematocrits were measured using the microhematocrit technique.

Calculations. Umbilical blood flow was calculated by the steady-state diffusion method. The fetal availability of oxygen and other substrates was calculated as the umbilical blood
flow times the umbilical venous oxygen or substrate content. Fetal oxygen uptake was calculated by the Fick principle (umbilical blood flow times the umbilical venoarterial oxygen concentration difference). Fetal oxygen extraction was the fetal oxygen uptake divided by fetal oxygen availability.

We have previously described the methods used to calculate fetal protein metabolism (21, 25). Briefly, the total fetal leucine flux (i.e., the sum of all fates for leucine in the fetus) was calculated as the leucine infusion rate corrected for transumbilical loss of tracer divided by the specific activity of plasma α-ketoisocaproate during the protocol. Analysis of variance for crossover experiments was used to test for the effects of decreased fetal oxygen availability as separate from the effects of the duration of the experiment. This analysis allows calculation of a single difference between control and experimental conditions that can be ascribed specifically to decreased oxygen availability rather than the order of the experiment. Comparison with the t distribution was used to find whether such differences were significant (P < 0.05).

RESULTS

Maternal FIO2 and fetal arterial blood gases during hypoxia. Analysis of maternal inspired oxygen concentration during maternal hypoxemia revealed a rapid decrease in maternal inspired FIO2 to ~9% when first measured after 30 min, with no significant variation throughout the remainder of the experimental protocol (Fig. 2). In response to the decrease in maternal inspired oxygen concentration, fetal arterial PO2 fell from initial values of 19.6 ± 0.6 and 20.0 ± 0.4 mmHg on days 1 and 2, respectively, to 11.4 ± 1.1 and 11.1 ± 0.4 mmHg on days 1 and 2, respectively (P < 0.001) by 30 min (Fig. 2). There were no significant changes in fetal PO2 after 30 min of decreased inspired maternal oxygen concentration.

Maternal hypoxia decreased fetal pH within 60–90 min (Fig. 3). After 60 min (day 1) or 90 min (day 2) fetal pH remained constant for the duration of the hypoxia.

![Fig. 2. Maternal inspired oxygen concentration (●) and fetal PO2 (○) during hypoxic conditions on day 1 (A) and day 2 (B) of experiment. Values are means ± SE. If no SE bar is evident, SE is within symbol.](http://ajpendo.physiology.org/)
Because there were no consistent changes in fetal PCO₂ during maternal hypoxia (results not shown), there was no evidence of respiratory acidosis. Measurement of fetal base excess confirmed that the acidosis was metabolic. On day 1, base excess was significantly lower than baseline by 90 min of hypoxia, after which no further significant changes occurred (Fig. 3). On day 2, base excess was significantly negative by 60 min of hypoxia, and no further significant changes occurred after 120 min.

Fetal hematocrit, pH, and blood gas values during sampling periods. Fetal hematocrit rose 4% (average for both study days; P < 0.05; Table 1). As expected, decreased maternal inspired oxygen concentration resulted in a 42% decrease in fetal arterial PO₂ (P < 0.001). In contrast, 4 h of decreased maternal inspired oxygen concentration had no significant effect on fetal PCO₂. Fetal pH, however, decreased (P < 0.001). As would be expected given decreased pH in the presence of normal Pco₂, there was increased metabolic acidosis (base excess decreased on both study days (P < 0.001; Table 1)).

Fetal oxygenation. Hypoxia increased umbilical blood flow by 17% (P < 0.001; Table 2). Fetal arterial oxygen concentration also decreased (65%) in response to decreased maternal inspired oxygen concentration. Indeed, the decrease in fetal blood oxygen concentration was sufficient that fetal oxygen availability (the product of umbilical venous oxygen concentration and umbilical blood flow) fell by 43% (P < 0.001) despite the above noted increase in umbilical blood flow. Hypoxia decreased the venoarterial concentration difference for oxygen across the umbilical circulation by 30% (P < 0.001), a decrease that resulted in a 16% decrease in fetal oxygen uptake (umbilical blood flow times umbilical oxygen venoarterial difference) during hypoxia (P < 0.01). This decrease in fetal oxygen uptake was, however, less severe than the decrease in fetal oxygen availability, so that fetal oxygen extraction increased during hypoxia (P < 0.001).

Fetal oxidative substrate concentrations and availability. Hypoxia had no effect on fetal arterial glucose concentration (Table 3). The availability of glucose to the fetus through the umbilical vein, however, did increase slightly (14%; P < 0.025), primarily because of the above noted increase in umbilical blood flow. In contrast to glucose concentration, fetal lactate concentration markedly increased during reduced fetal oxygen availability (P < 0.001). Fetal lactate concentration increased not only in the umbilical artery but also in the umbilical vein (results not shown). The marked increase in umbilical venous lactate concentration was primarily responsible for a similar increase in fetal lactate availability (P < 0.001; Table 3). In response to decreased maternal inspired oxygen concentration there was a 22% increase in blood arterial amino nitrogen concentration (P < 0.001). Increased fetal amino nitrogen concentration and the increase in umbilical blood flow noted above increased fetal amino nitrogen availability by 35% (P < 0.001; Table 3).

Fetal leucine kinetics. Decreased fetal oxygen availability increased fetal arterial leucine concentration by 37% (Table 4). Fetal umbilical venous leucine concentration also rose, although to a lesser extent (31%). Consequently, there was a significant decrease in the umbilical venoarterial blood concentration difference for leucine, a decrease that was sufficient to account for a decrease (53%) in leucine uptake (Table 4) even though umbilical blood flow increased. Because of the rapid equilibration between leucine and its transamination product, α-ketoisocaproate, transplacental uptake of this substance represents an additional source of leucine uptake. Fetal umbilical arterial and venous α-ketoisocaproate concentrations increased by 100 and 90%, respectively, in response to hypoxia. Despite these concentration changes, the umbilical venoarterial difference for α-ketoisocaproate was unaffected by hypoxia.
as was the transplacental uptake of α-ketoisocaproate (Table 4). However, because α-ketoisocaproate uptake represented only 9 and 17% of total leucine carbon uptake during control and hypoxic conditions, respectively, total leucine uptake reflected the effect of hypoxia on leucine uptake and fell by 48% during hypoxia.

To measure other aspects of the leucine kinetics, [1-14C]leucine was infused into the fetus at 127,900 ± 9,000 dpm·min⁻¹·kg⁻¹. During control conditions, between 23% (29,400 ± 3,900 dpm·min⁻¹·kg⁻¹; day 1) and 21% (27,200 ± 10,300 dpm·min⁻¹·kg⁻¹; day 2) of this radioactivity was lost across the umbilical circulation to the placenta. During hypoxic conditions, this percentage increased so that on day 1, 45% (57,100 ± 9,000 dpm·min⁻¹·kg⁻¹) and on day 2, 35% (44,900 ± 10,000 dpm·min⁻¹·kg⁻¹) left the fetal compartment. This transumbilical leucine loss that occurred from the radioactivity infused before the total leucine flux was calculated. Corrected infusion rates were, therefore, 98,500 ± 8,700 dpm·min⁻¹·kg⁻¹ and 70,800 ± 11,900 dpm·min⁻¹·kg⁻¹ during control and hypoxic conditions on day 1, respectively, and 83,000 ± 8,300 dpm·min⁻¹·kg⁻¹ and 100,700 ± 8,800 dpm·min⁻¹·kg⁻¹ during hypoxic and control conditions on day 2, respectively.

The specific activity of α-ketoisocaproate, the transamination product of leucine, reached constant specific activity before blood sampling occurred (180 min) in each experiment (Fig. 4). During decreased fetal oxygen availability, α-ketoisocaproate specific activity increased while the infused radioactivity retained in the fetus decreased, total leucine flux fell by 32% during reduced fetal oxygen availability. Fetal leucine decarboxylation, an index of the flux of leucine to fatty acids other than protein synthesis, also fell by 30% (P < 0.001; Table 5).

Fetal protein metabolism. Fetal nonoxidative leucine disposal, an index of the use of leucine for fetal protein synthesis, decreased by 32% (P < 0.001) during decreased fetal oxygen availability (Table 5 and Fig. 5). Fetal protein breakdown was also significantly decreased during decreased fetal oxygen availability but to a lesser extent (22% decrease; P < 0.001). Because fetal protein synthesis decreased to a greater extent than fetal protein breakdown, fetal protein accretion (the difference between synthesis and a breakdown) fell markedly (62% decrease; P < 0.001; Table 5 and Fig. 5).

DISCUSSION

This study was performed to find whether restriction of fetal oxygen availability sufficient to constrain fetal oxygen consumption affected not only fetal protein synthesis but fetal proteolysis as well. A corollary to this purpose was to find whether fetal protein accretion, the balance between fetal protein synthesis and proteolysis, was affected by such a decrease in fetal oxygen availability. Lowering maternal inspired oxygen concentration to ~9% decreased fetal oxygen availability enough to limit fetal oxygen consumption. Under such conditions, both the uptake of leucine across the placenta and fetal oxidation of leucine decreased. In

### Table 2. Umbilical blood flow and fetal oxygenation during control and hypoxic conditions

<table>
<thead>
<tr>
<th></th>
<th>Day 1</th>
<th>Day 2</th>
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<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Hypoxia</td>
</tr>
<tr>
<td>Umbilical blood flow</td>
<td>213 ± 10</td>
<td>256 ± 22</td>
</tr>
<tr>
<td>Arterial concentration, mM</td>
<td>2.95 ± 0.19</td>
<td>0.96 ± 0.15</td>
</tr>
<tr>
<td>Umbilical v-a difference, mM</td>
<td>1.40 ± 0.04</td>
<td>0.99 ± 0.07</td>
</tr>
<tr>
<td>Availability, µmol·kg⁻¹·min⁻¹</td>
<td>925 ± 51</td>
<td>508 ± 74</td>
</tr>
<tr>
<td>Uptake, µmol·kg⁻¹·min⁻¹</td>
<td>286 ± 10</td>
<td>250 ± 20</td>
</tr>
<tr>
<td>Extraction</td>
<td>0.33 ± 0.01</td>
<td>0.54 ± 0.05</td>
</tr>
</tbody>
</table>

Values are means ± SE for 8 animals. Umbilical venoarterial (v-a) difference is concentration difference between umbilical vein and artery.

### Table 3. Fetal arterial concentrations and availability of substrate during control and hypoxic conditions

<table>
<thead>
<tr>
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<th>Day 1</th>
<th>Day 2</th>
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<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Hypoxia</td>
</tr>
<tr>
<td>Glucose</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Concentration, mM</td>
<td>1.07 ± 0.08</td>
<td>1.03 ± 0.09</td>
</tr>
<tr>
<td>Availability, µmol·kg⁻¹·min⁻¹</td>
<td>262 ± 27</td>
<td>303 ± 42</td>
</tr>
<tr>
<td>Lactate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Concentration, mM</td>
<td>1.64 ± 0.09</td>
<td>10.82 ± 1.96</td>
</tr>
<tr>
<td>Availability, µmol·kg⁻¹·min⁻¹</td>
<td>420 ± 40</td>
<td>2,430 ± 420</td>
</tr>
<tr>
<td>Amino nitrogen</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Concentration, mM</td>
<td>8.82 ± 0.47</td>
<td>10.66 ± 0.34</td>
</tr>
<tr>
<td>Availability, µmol·kg⁻¹·min⁻¹</td>
<td>1,980 ± 150</td>
<td>2,650 ± 270</td>
</tr>
</tbody>
</table>

Values are means ± SE for 8 animals. Availability is the product of umbilical blood flow and umbilical venous substrate concentration. Differences between control and hypoxic conditions (tested over both days) are significant at *P < 0.025 and †P < 0.001.
addition, not only did the use of leucine for fetal protein synthesis also diminish (32%), but the return of leucine to the intracellular pool from protein breakdown decreased as well (by 22%). Finally, because fetal protein synthesis decreased more than protein breakdown, fetal protein accretion, an important component of fetal growth, diminished.

There are both strengths and limitations to this study. First, we allowed the ewes 6 days to recover from surgery before any experiments were performed so that fetal metabolism was minimally affected by operative or experimental stress. Others (7) have shown that fetal nitrogen balance, although negative 2 h after surgery, was positive within 4 days. More importantly, the rate of leucine accretion into fetal proteins during control measurements is no different from that of normally growing fetal lambs not subjected to surgery (18). Second, we employed an experimental design (i.e., crossover) that is specifically designed to separate factors related to either the duration or order of the experiment from those related to the experimental condition itself. Thus maternal or fetal stress, diurnal variation, or blood sampling could not have accounted for the effects attributed to hypoxia. Third, we limited our sampling to <3% of fetal blood volume, a volume shown to have minimal effect on fetal homeostasis (33), and replaced sampled blood with maternal blood to maintain fetal blood volume. We did notice, however, a slight increase in fetal hematocrit during hypoxia.

The methods used in this experiment to measure fetal leucine kinetics and protein metabolism have met the conditions to appropriately make such measurements (2). First, the most fundamental assumption made in this experiment is that the specific activity of plasma α-ketoisocaproate measures the specific activity of the precursor for each of the intracellular fates of leucine. Such uniformity of specific activity through each of the intracellular leucine pools seems unlikely. Nevertheless, given that those latter amino acid pools could be sampled only if the animal were killed, an experimental design that would preclude experiments persist as the duration of oxygen deprivation is continued remains a subject for further study.

The 4% increase in fetal hematocrit that occurs during hypoxia corresponds to a decrease in fetal blood volume of 4% (4). Such a decrease has been previously reported during fetal hypoxia (4) and may be either a direct effect of hypoxia or secondary to increased fetal norepinephrine concentration (24). Finally, this experiment does not address the effects of longer-term deprivation of fetal oxygen supply. Whether the changes in fetal leucine and protein metabolism described in this report persist as the duration of oxygen deprivation is continued remains a subject for further study.

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Table 4. Fetal blood leucine and α-ketoisocaproate concentrations and transplacental uptake during control and hypoxic conditions

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Hypoxia</th>
<th></th>
<th>Day 1</th>
<th>Day 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Arterial concentration, µM</td>
<td>235 ± 18</td>
<td>329 ± 27</td>
<td>*</td>
<td>291 ± 29</td>
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<tr>
<td></td>
<td>Umbilical venous concentration, µM</td>
<td>256 ± 19</td>
<td>337 ± 26</td>
<td>*</td>
<td>301 ± 29</td>
</tr>
<tr>
<td></td>
<td>Umbilical v-a difference, mM</td>
<td>20 ± 2</td>
<td>6 ± 1</td>
<td>*</td>
<td>8 ± 2</td>
</tr>
<tr>
<td></td>
<td>Uptake, µmol·kg⁻¹·min⁻¹</td>
<td>4.23 ± 0.51</td>
<td>1.65 ± 0.41</td>
<td>*</td>
<td>2.01 ± 0.44</td>
</tr>
<tr>
<td>α-Ketoisocaproate</td>
<td>Arterial concentration, µM</td>
<td>18 ± 1</td>
<td>37 ± 3</td>
<td>*</td>
<td>34 ± 5</td>
</tr>
<tr>
<td></td>
<td>Umbilical venous concentration, µM</td>
<td>20 ± 1</td>
<td>39 ± 3</td>
<td>*</td>
<td>36 ± 5</td>
</tr>
<tr>
<td></td>
<td>Umbilical v-a difference, mM</td>
<td>2.1 ± 0.5</td>
<td>1.9 ± 0.7</td>
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<td>1.2 ± 0.4</td>
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<tr>
<td></td>
<td>Uptake, µmol·kg⁻¹·min⁻¹</td>
<td>0.45 ± 0.10</td>
<td>0.45 ± 0.16</td>
<td></td>
<td>0.30 ± 0.11</td>
</tr>
<tr>
<td></td>
<td>Total uptake, µmol·kg⁻¹·min⁻¹</td>
<td>4.68 ± 0.57</td>
<td>2.11 ± 0.33</td>
<td>*</td>
<td>2.31 ± 0.39</td>
</tr>
</tbody>
</table>

Values are means ± SE for 8 animals. Umbilical v-a difference is the difference in blood concentration between umbilical vein and artery. Differences between control and hypoxic conditions (tested over both days) are significant at *P < 0.001.

Fig. 4. Fetal plasma α-ketoisocaproate specific activity during control and hypoxic conditions on day 1 (A) and day 2 (B). Values are means ± SE. No differences were found by paired t-test analysis between 1st (1) and 2nd (2) samples on either day for either control (○) or hypoxia (●) experiments.
between control and hypoxic conditions significant at *P* \( < 0.001 \).

Table 5. Fetal leucine kinetics and protein metabolism during control and hypoxic conditions

<table>
<thead>
<tr>
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<th>Day 1</th>
<th>Day 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Hypoxia</td>
</tr>
<tr>
<td>KIC specific activity, dpm/nmol</td>
<td>8.38 ( \pm ) 0.62</td>
<td>10.32 ( \pm ) 0.59</td>
</tr>
<tr>
<td>Total leucine flux, µmol·kg(^{-1})·min(^{-1})</td>
<td>12.13 ( \pm ) 1.34</td>
<td>7.21 ( \pm ) 1.44</td>
</tr>
<tr>
<td>Leucine decarboxylation, µmol·kg(^{-1})·min(^{-1})</td>
<td>2.50 ( \pm ) 0.30</td>
<td>1.69 ( \pm ) 0.27</td>
</tr>
<tr>
<td>Protein synthesis, µmol·kg(^{-1})·min(^{-1})</td>
<td>9.62 ( \pm ) 1.12</td>
<td>5.51 ( \pm ) 1.20</td>
</tr>
<tr>
<td>Protein breakdown, µmol·kg(^{-1})·min(^{-1})</td>
<td>7.45 ( \pm ) 1.58</td>
<td>5.10 ( \pm ) 1.39</td>
</tr>
<tr>
<td>Protein accretion, µmol·kg(^{-1})·min(^{-1})</td>
<td>2.17 ( \pm ) 0.70</td>
<td>0.42 ( \pm ) 0.39</td>
</tr>
</tbody>
</table>

Values are means \( \pm \) SE for 8 animals. KIC is \( \alpha \)-ketoisocaproate, the transamination product of leucine. Differences between control and hypoxic conditions (tested over both days) are significant at *P* \( < 0.001 \).

Leucine enters fetal protein synthesis, and the flux of leucine into the placenta from the fetus (15). Thus fetal leucine disposal rate differs from the total fetal leucine flux of this report by the flux of leucine into the placenta from the fetus. Our methods for calculating quantities such as leucine uptake, decarboxylation, and use for protein synthesis are, however, identical to theirs. Finally, we assume that the loss of radioactivity from the fetus is irreversible. Actually, various measurements show that between 0 and 6% of the infused tracer leucine (17, 21, 25) returns to the fetus from the mother as labeled \( \alpha \)-ketoisocaproate. Consequently, minimal, if any, underestimation of fetal total leucine flux or the quantities derived from it, protein synthesis and protein breakdown, occurred consequent to reuptake of tracer.

A number of methods, including reduction of maternal inspired oxygen concentration (14), maternal anemia (28), and restriction of placental (3) or umbilical blood flow (30), have been used to decrease the availability of oxygen to the fetus. Unfortunately, some of these methods decrease not only oxygen availability but fetal availability of other substrates as well (30). In such cases, it is difficult to decide which variable (i.e., reduced availability of oxygen, glucose, or amino acids) is the most direct cause of the effects described. To avoid this problem, we wanted fetal oxygen availability decreased, but the availability of the other substrates important to fetal metabolism (glucose, lactate, and amino acids) either maintained constant or increased. We defined the availability of a given substrate to the fetus as that quantity transported into the fetus through the umbilical vein (in µmol·kg\(^{-1}\)·min\(^{-1}\)), a quantity that comes from two sources. A portion of each of these substrates is the net amount acquired by the umbilical circulation from the placenta. The remainder is that quantity in the umbilical artery as it comes from the fetus to the placenta. Fetal oxygen availability fell because both fetal oxygen uptake across the placenta and the quantity of oxygen remaining in the umbilical arterial circulation fell. In contrast, although fetal glucose availability was unaffected by decreased maternal inspired oxygen concentration, fetal lactate and amino acid availability rose, primarily because fetal arterial concentrations rose, and therefore more remained in the umbilical arterial circulation. Thus these experiments describe effects of decreased oxygen availability on fetal leucine kinetics in the presence of normal or increased availability of the other fetal metabolic substrates.

The fetal need for oxidative substrate is normally met by the continual transplacental uptake of three substances: glucose, lactate, and amino acids (1). Amino acid uptake in general, and tyrosine uptake in particular, are diminished during decreased fetal oxygen availability (19, 20). In the present experiment, leucine uptake also decreased during hypoxia. Transplacental transport of amino acids employs specific amino acid transporters located in both the microvillous and the basal membranes of the syncytiotrophoblast (27). Leucine transport at both membranes uses an active, carrier-mediated transporter that includes an ATPase (27). It is tempting, therefore, to postulate that decreased intracellular ATP concentrations resulting from inadequate availability of oxygen directly affect such transporters. However, it seems more likely that the process is multifactorial. For example, metabolic acidosis, noted during hypoxia, also decreases fetal leucine uptake (23). Consequently, the mechanism by which decreased leucine transport occurs in response to hypoxia remains to be defined.

Once leucine has entered the fetal compartment, it has two major fates, either decarboxylation (and further use as oxidative substrate) or use for protein synthesis. In this study, leucine decarboxylation diminished when fetal oxygen availability was decreased. In contrast, another study of amino acid oxidation during reduced fetal oxygen availability found that tyrosine decarboxylation was unaffected (19). Thus some vari-
ability exists in the response of this aspect of amino acid metabolism to reduced fetal oxygen availability. The decreased rate of leucine oxidation in the present study cannot be attributed to the effects of acidosis, because leucine oxidation is increased rather than decreased by metabolic acidosis (23). In addition, the decrease in fetal leucine oxidation is unlikely to be due to changes in leucine concentration, because the increased leucine concentrations that occur during decreased fetal oxygen availability should have increased rather than decreased leucine decarboxylation (21).

Decreased fetal oxygen availability also affected several aspects of fetal protein metabolism. First, the use of leucine for protein synthesis decreased by 32% during decreased fetal oxygen availability. In previous experiments, the use of [1-14C]tyrosine for protein synthesis decreased to 39% of the value obtained in normoxia. In adults, the depletion of intracellular ATP by hypoxia may directly diminish protein synthesis (5). This experiment neither supports nor refutes this idea. Finally, the possibility that increased concentration of leucine during reduced fetal oxygen availability affected fetal protein synthesis is unlikely, because increases in leucine concentration stimulate, rather than decrease, protein synthesis (6).

This study is the first to measure the effect of reduced fetal oxygen availability on fetal protein breakdown. Because breakdown of most proteins is a process requiring energy in the form of intracellular ATP (26), we hypothesized that fetal proteolysis would diminish if oxygen availability were decreased sufficiently to limit oxygen consumption, a condition met by the present experiment. Again, however, whether intracellular ATP depletion is the primary reason for the 22% decrease in the rate of fetal proteolysis during decreased fetal oxygen availability remains unclear. Leucine decreases proteolysis in adults (29), so the possibility that increased fetal leucine concentration contributed to the suppression of proteolysis remains a distinct possibility.

The relationship between effects on protein synthesis and breakdown becomes especially important in fetal life given the exquisite sensitivity of fetal protein accretion to changes in these two variables. In this experiment, fetal protein synthesis decreased more than breakdown. Consequently, fetal protein accretion, the difference between synthesis and breakdown, decreased by 62%. This decrease in fetal protein accretion in response to 4 h of decreased fetal oxygen availability shows, in the absence of a significant change in fetal body composition, that 4 h of reduced fetal oxygen availability affects fetal growth. Obviously, the change in fetal weight that occurs over 4 h could not be measured given the inaccessibility of the live fetus to accurate weighing. However, in other experiments, decreased fetal oxygen availability has been caused by hypobaric hypoxia (14), prolonged reduction in uterine blood flow (3), and maternal anemia (28), and, in each of these experiments, between 7 and 21 days of decreased fetal oxygen availability decreased fetal weight.

Changes in protein metabolism such as those just described have important implications for fetal oxidative metabolism. The change in leucine use for protein synthesis during hypoxia, 3.0 µmol leucine·kg⁻¹·min⁻¹ (or 45 µmol·kg⁻¹·min⁻¹ peptide bonds synthesized) would require 30 µmol·kg⁻¹·min⁻¹ less oxygen consumption during hypoxia than during normoxic conditions (9). A similar calculation for protein breakdown based on a decrease of 1.6 µmol·kg⁻¹·min⁻¹ in leucine return from proteolysis (24 µmol·kg⁻¹·min⁻¹ peptide bonds cleaved) would require 4 µmol·kg⁻¹·min⁻¹ less oxygen during hypoxia than during normoxia. The total metabolic cost of protein turnover is, therefore, 34 µmol·kg⁻¹·min⁻¹ less oxygen under hypoxia than during normoxia, a difference accounting for ~72% of the decrease in fetal oxygen consumption during hypoxia (47 µmol·kg⁻¹·min⁻¹). The effect of hypoxia on fetal protein breakdown may have resulted in even larger energy savings because, at least in turtle hepatocytes, not only does protein breakdown decrease during hypoxia but it becomes partially independent of ATP (16). Finally, there are other energy-requiring processes not measured in this experiment, such as cellular uptake of amino acids, RNA synthesis, DNA synthesis (11), and fetal breathing (12), that could have decreased sufficiently to account for the rest of the decrease of fetal oxygen needs that occurred during hypoxia.

Whether the effects of decreased fetal oxygen availability on fetal protein metabolism are directly attributable to decreased intracellular energy state or are secondary to other changes in fetal metabolism remains undefined. In this experiment, fetal pH fell while lactate and amino acid concentrations increased. Other studies make metabolic acidosis an unlikely explanation for the effects of decreased oxygen availability on fetal leucine and protein kinetics (23). However, the effects of increased lactate and amino acid concentrations on these variables remain unstudied. However, some of the changes in the fetal hormonal milieu seem unlikely modulators of the reported effects on leucine kinetics. Even though a modest decrease in fetal insulin concentration occurs during fetal hypoxemia (32), fetal insulin concentration does not affect protein synthesis (21). Although increased insulin concentration can reduce fetal proteolysis, larger concentration increases than expected during fetal hypoxia are needed for this effect. Fetal hypoxia increases fetal cortisol concentration (12), but such changes should not affect fetal protein synthesis (22) and would increase rather than decrease protein breakdown (22). However, increased fetal catecholamine concentration, which occurs during fetal hypoxemia (12), causes a constellation of effects on fetal leucine kinetics and protein metabolism similar to those of decreased oxygen availability (24). Consequently, some, or maybe even all, of the reported effects could be due to increased fetal catecholamine concentrations. One may as easily hypothesize, however, that the effects of norepinephrine are consequent to decreased peripheral blood flow in the fetus, an effect that could decrease intracellular ATP concentrations, at least in those tissues poorly
perfused (8). Thus the effects of norepinephrine could be due to tissue ATP depletion rather than the converse. In any case, whether increased fetal catecholamine concentration is required to modulate the effects of decreased fetal oxygen availability on fetal leucine kinetics and protein metabolism will require further study.

In summary, reduced availability of oxygen to the fetus decreases both transplacental uptake of leucine and fetal leucine oxidation. The use of leucine for fetal protein synthesis is also reduced, as is the return of leucine from protein breakdown, although to a lesser degree. As a result of these changes in fetal protein synthesis and breakdown, fetal protein accretion, an important component of fetal growth, is diminished by 4 h of decreased fetal oxygen availability. In addition, decreases in fetal protein synthesis and breakdown, which both require intracellular expenditure of ATP, decrease fetal energy needs sufficiently to account for most of the decrease in oxygen consumption.

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Address for reprint requests: J. R. Milley, Dept. of Pediatrics, Division of Neonatology, Univ. of Utah School of Medicine, 50 N. Medical Dr., Salt Lake City, UT 84132.

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