Regulation of uterine and umbilical amino acid uptakes by maternal amino acid concentrations

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Thureen, Patti J., Susan M. Anderson, and William W. Hay, Jr. Regulation of uterine and umbilical amino acid uptakes by maternal amino acid concentrations. Am J Physiol Regulatory Integrative Comp Physiol 279: R849–R859, 2000.—We tested the hypothesis that decreased fetal amino acid (AA) supply, produced by maternal hypoaminoacidemia (low AA) during hyperglycemia (HG), is reversible with maternal AA infusion and regulates fetal insulin concentration ([I]). We measured net uterine and umbilical AA uptakes during maternal HG/low AA concentration ([AA]) and after maternal intravenous infusion of a mixed AA solution. After 5 days HG, all maternal [AA] except glycine were decreased >50%, particularly essential [AA] (P < 0.00005). Most fetal [AA] also were decreased, especially branched-chain AA (P < 0.001). Maternal AA infusion increased net uterine uptakes of Val, Leu, Ile, Met, and Ser and net umbilical uptakes of Val, Leu, Ile, Met, Phe, and Arg but did not change net uteroplacental uptake of any AA. Fetal [I] increased 55 ± 14%, P < 0.001, with correction of fetal [AA], despite the lack of change in fetal glucose concentration. Thus generalized maternal hypoaminoacidemia decreases uterine and umbilical uptakes of primarily the essential AA and decreases fetal branched-chain [AA]. These changes are reversed with correction of maternal [AA], which also increases fetal [I].

Sheep; fetus; glucose; hyperglycemia; placenta

A fundamental aspect of interorgan amino acid (AA) flux is the regulation provided by the plasma concentrations of the AAs. A unique example involves the transport of AAs from the maternal plasma in the uterine circulation into the uteroplacenta and into the fetal plasma of the umbilical circulation. A variety of physiological, pathophysiological, and experimental conditions have demonstrated variability in maternal plasma AA concentrations, but there has been little experimental analysis of how such variations affect placental uptake and transport of AAs or of fetal metabolic responses. For example, preliminary studies in a unique model in pregnant sheep have shown that chronic maternal hyperglycemia (HG) and associated hyperinsulinemia produce generalized maternal hyperaminoacidemia, decreased umbilical uptake of a number of essential AAs, and selective fetal hypoaminoacidemia (20). To date, however, there has been no systematic evaluation of how these transport changes are regulated in this model or how aspects of fetal metabolism, such as insulin secretion, change in response. This model also may be relevant to the human pregnant diabetic condition. For example, marked hyperglycemic-hyperinsulinemic conditions in the mother might occur with severe maternal diabetes with excessive insulin therapy and could result in a decreased supply of AAs to the fetus, possibly compromising fetal growth. Indeed, severe insulin-dependent diabetes in pregnancy has been associated with fetal growth restriction (31, 33). Furthermore, intrauterine growth restriction secondary to placental insufficiency (8, 35) is also associated with decreased supply of AAs to the fetus. Thus reversing maternal hypoaminoacidemia specifically and/or increasing fetal AA supply in general might be a potential strategy for correcting AA deficiency involved in fetal growth restriction. Whether or not increased AAs can be delivered via the mother to the fetus has received only limited attention. Furthermore, there are no data available about AA uptake by the placenta or transport to the fetus if the mother is hypoaminoacidemic. AAs are transported by active, energy-dependent transporters, and the effect of plasma AA concentrations on transport of the AAs is uncertain. Additionally, with respect to the transport characteristics of individual transporters, increased maternal plasma AA concentrations might result in competitive inhibition among individual AAs for certain transporters.

Clearly, further investigation of AA uptake by the placenta and transport to the fetus, as well as the changes in fetal metabolism in response, in such models and conditions is needed. Therefore, the present study was designed to 1) investigate the effect of chronic maternal HG on maternal and fetal AA concentrations and net uterine, uteroplacental, and fetal AA uptakes and 2) determine if infusion of a mixed AA solution into the maternal circulation could produce acute increases in maternal and fetal AA uptake, resulting in increased concentrations of those AAs that were decreased in the hyperglycemic state. A second-
ary goal was to determine whether an increase in fetal AA supply and plasma concentrations during the maternal AA infusion would augment fetal insulin secretion, as previous studies have indicated that some AAs are important for fetal pancreatic development and insulin secretion (12, 40, 42). To accomplish these goals, we made pregnant sheep chronically hyperglycemic and hyperinsulinemic by glucose-clamp technique, producing maternal panhypoaminoacidemia. We then returned the low maternal AA concentrations to baseline values (or euaminoacidemia) by maternal AA clamp using a rapid assay of lysine as an indicator AA for maintaining the AA clamp and a commercial AA mixture (Trophamine) as the clamp infusion solution. Net uterine, uteroplacental, and fetal AA uptake rates were measured in each of these periods by application of the Fick principle.

MATERIALS AND METHODS

Animal care and surgical procedure. Studies were performed in late-gestation Columbia-Rambouillet pregnant sheep obtained from a commercial breeder (Nebeker Ranch, Santa Monica, CA). Pregnancies were time dated, and all were known singleton pregnancies. After a 24-h fast, each ewe was prepared for surgery with intravenous pentobarbital sodium sedation (5 mg/kg initial dose followed by repeated bolus doses as needed for the duration of surgery) and lumbar intrathecal tetracaine hydrochloride anesthesia (6 mg in hypertonic glucose). Ampicillin (500 mg) and gentamicin (80 mg) were given intramuscularly preoperatively. At surgery, maternal polyvinyl catheters were placed via a groin incision into the femoral artery for blood sampling and into the femoral vein for infusions. After laparotomy, a catheter was placed in the uterine vein draining the pregnant uterine horn, and after hysterotomy, fetal catheters were placed into the abdominal aorta via hindlimb arteries for blood sampling and into the femoral veins via hindlimb veins for infusions. An umbilical venous catheter was placed directly at the base of the umbilical cord with the tip advanced into the common umbilical vein. Ampicillin (500 mg) was injected into the amniotic fluid just before closing the uterine incision. All catheters were tunneled subcutaneously through a maternal skin incision and kept in a plastic pouch secured to the ewe’s flank. Catheters were flushed every second day with heparinized saline (150 U heparin/ml of 0.9% wt/vol NaCl in water). All ewes were recovered and standing by 6–8 h after surgery. The ewes were kept in plastic carts in a temperature-controlled environment (18±2°C) with 18 h of variable light and 6 h of darkness. The ewes were allowed ad libitum access to alfalfa pellets, water, and a mineral block, and the carts were cleaned daily. At least two sheep were kept together at all times. All animal procedures were approved by the University of Colorado Health Sciences Center Institutional Animal Care and Use Committee. The Perinatal Research Facility where these studies were performed is accredited by the National Institutes of Health, the United States Department of Agriculture, and American Association for Accreditation of Laboratory Animal Care.

Experimental design. Studies were performed in the ewes after a postoperative recovery period of at least 5 days. Figure 1 demonstrates the overall study design. At day 0, maternal and fetal arterial blood samples were obtained to measure plasma concentrations of AAs and glucose (i.e., control period with normal maternal glucose and AA concentrations [NG,NAA]). Immediately after obtaining the control blood samples, a maternal glucose infusion was started and adjusted to achieve and maintain a maternal arterial plasma glucose concentration of ~50% above control. Prior studies have demonstrated that this degree of maternal HG produces maternal hyperinsulinemia followed by hypoaminoacidemia (1). Maternal HG was maintained from day 0 through the experimental study that was performed on approximately day 5. Blood was sampled on days 1–4 for plasma AA, glucose, and insulin concentrations. On the day of study (day 5), after a baseline blood draw at time 0 for 3H2O concentration, a primed-constant fetal intravenous infusion of 3H2O (16.8 μCi/h given in 0.9% wt/vol NaCl in H2O, with the prime equal to 80 min of infusion) was started and continued throughout the study to measure umbilical and uterine blood flows by the transplacental steady-state diffusion technique. During study period 1, blood samples were obtained at 90, 105, 120, and 135 min for fetal and maternal arterial and uterine and umbilical venous plasma concentrations of AAs,
glucose, lactate, insulin, and $^{3}$H$_{2}$O and for arterial blood hematocrit, oxygen saturation, and oxygen content. Immediately after obtaining the HG, low AA (HG,LAA) study period 1 samples, a maternal intravenous infusion of a mixed AA solution (Trophamine, McGaw, Irvine, CA) was administered. The AA infusion rate was adjusted every 15 min in response to a rapid lysine assay (2) until a steady-state lysine concentration was reached that approximated the day 0 control period maternal lysine concentration (average time to achieve steady state, $\sim$1 h). Steady-state eulysinemia was maintained by clamp procedure for the next 3 h (study period 2). At the end of this time, maternal and fetal blood samples were obtained at 15-min intervals over an hour for the same plasma and blood measurements as in study period 1. Fetal euvoelemia was maintained by transfusion of heparinized maternal blood equal to sampled volumes immediately after each sample.

At the end of the study, 12 ml of intravenous euthanasia solution (Sleepaway, Fort Dodge Laboratories, IA) were injected into the mother. At autopsy, the fetus, uterus, uterine membranes, and cotyledons were removed and weighed separately.

**Blood sampling technique and analytical methods.** Maternal and fetal arterial (2.5 ml) and venous (1.8 ml) blood samples for measurement of plasma glucose, lactate, and insulin concentrations were collected in plastic syringes lined with EDTA and in heparin-coated capillary syringes for determination of blood hemoglobin concentration and oxygen saturation. Plasma was separated within 5 min of sampling in a refrigerated centrifuge. Samples were immediately processed for plasma glucose and lactate concentrations (YSI Glucose and Lactate Analyzer, model 2700-D, Yellow Springs Instruments, Yellow Springs, OH) and blood oxygen content (OSM III Hemoximeter, Radiometer, Copenhagen, Denmark, calibrated for fetal ovine hemoglobin). Fetal arterial plasma for glucose and lysine clamp assays (0.6 ml) was collected in plastic syringes lined with EDTA. Rapid enzymatic determination of plasma lysine concentration was determined as described by Beckett et al. (2). In the presence of the enzyme saccharopine dehydrogenase (EC 1.5.1.7) and NADH, lysine combines with $\alpha$-ketoglutarate to form saccharopine. The rate of conversion of NADH to NAD in the early reaction is proportional to the lysine concentration. The rate of change in spectrophotometric absorbance at 340 nm during this reaction (Beckman DU-7 Spectrophotometer, Beckman Instruments, Fullerton, CA) was used to calculate plasma lysine concentrations from the rate of change in absorbance obtained from lysine standards.

Maternal and fetal arterial plasma samples for insulin concentration were immediately frozen and stored at $\sim$70°C until analysis. Insulin concentrations were determined with a radioimmunoassay kit (Binax, South Portland, ME) using ovine insulin standards (provided by Eli Lilly, Indianapolis, IN). For determination of $^{3}$H$_{2}$O, 0.1-ml plasma samples were solubilized in 1.0 ml of soluene-350 (quaternary ammonium hydroxide in toluene) and then mixed with 15 ml Hionic Fluor (both reagents from Packard Instrument, Meriden, CT). The $^{3}$H radioactivity was measured in a Packard Tri-Carb 460 C liquid scintillation counter.

Samples for determination of plasma AA concentrations were collected in EDTA-coated syringes, centrifuged, and stored at $\sim$70°C until analysis. Plasma concentrations were measured using a Dionex 300 model 4500 AA analyzer (Dionex, Sunnyvale, CA) after deproteinization with sulfoalicylic acid.

**Calculations.** Umbilical (PF$_{umb}$) and uterine (PF$_{ut}$) plasma flows (ml/min) were calculated from $^{3}$H$_{2}$O samples using the steady-state transplacental diffusion method with tritiated water as the flow indicator (26). Umbilical (BF$_{umb}$) and uterine (BF$_{ut}$) blood flows were calculated as follows (11):

\[
BF_{umb} = PF_{umb}/1 - \text{fractional fetal hematocrit; } BF_{ut} = PF_{ut}/1 - \text{fractional maternal hematocrit.}
\]

According to Chung et al. (11), blood arteriovenous AA concentration differences across the uterine circulation are relatively small, limiting the accuracy of AA uptake calculations. AA uptakes by the utero-placenta in pregnant sheep, therefore, are most accurately derived from plasma AA concentrations and not from red blood cells, first, because of the very slow rate of exchange of AAs between red cell cytosol and plasma and second, because AA concentration differences across the utero-placenta are $\sim$50% greater in plasma than in whole blood. Therefore, AA uptakes in the present study were determined as follows: net uterine AA uptake rate ($\mu$mol/min) = PF$_{ut}$ × $[\Delta A]_{v}$; net umbilical AA uptake rate ($\mu$mol/min) = PF$_{umb}$ × $[\Delta A]_{a}$, where $[\Delta A]$ represents the plasma AA concentration differences ($\mu$mol/ml) in the uterine venous (v), maternal arterial (A), umbilical venous (v), and fetal arterial (a) vessels, and PF$_{ut}$ and PF$_{umb}$ represent uterine and umbilical plasma flow rates (ml/min), respectively. Uterine and umbilical glucose uptakes were calculated in the same manner. Net oxygen uptakes were determined using blood flow rather than plasma flow. Uteroplacental utilization of substrates was calculated as follows: net uteroplacental substrate uptake rate ($\mu$mol/min) = uterine − umbilical net substrate uptake rate ($\mu$mol/min).

**Data analysis.** Results are expressed as means ± SE. Differences between sets of sampling periods [i.e., NG,NAA control period and HG,LAA and HG, normal AA (HG,NAA) study periods] were assessed by two-tailed paired t-test. Comparisons between days for glucose, insulin, and AA concentrations for days 1–4 were made by two-tailed unpaired t-test, because the number of observations was not equal for each day.

**RESULTS**

Gestational ages and weights of the 11 study animals are shown in Table 1. Fetal study weight was extrapolated from gestational age at study and fetal weight and gestational age at autopsy according to ovine in utero growth curves established for the breed of sheep studied in our laboratory.

Figure 2 shows the maternal and fetal arterial glucose concentrations before and during 5 days of maternal HG. The maternal hyperglycemic clamp maintained maternal and fetal arterial plasma glucose concentrations at 50–60% above the control period.

<table>
<thead>
<tr>
<th>Table 1. Fetal age and study weights</th>
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<tbody>
<tr>
<td><strong>Pregnant Columbia-Rambouillet Sheep Studied</strong></td>
</tr>
<tr>
<td>Fetal gestational age, days</td>
</tr>
<tr>
<td>Initiation of maternal hyperglycemia</td>
</tr>
<tr>
<td>Experimental study</td>
</tr>
<tr>
<td>Autopsy</td>
</tr>
<tr>
<td>Maternal weight, kg</td>
</tr>
<tr>
<td>Fetal weight at study, g</td>
</tr>
<tr>
<td>Placental weight at autopsy, g</td>
</tr>
<tr>
<td>Uterine weight at autopsy, g</td>
</tr>
</tbody>
</table>

Values expressed as means ± SE for 11 sheep.
concentration ($P < 0.0005$) for the duration of the clamp.

As shown in Fig. 3, maternal and fetal insulin concentrations increased significantly in the first 24 h after maternal and fetal HG were produced. However, the initial increase in fetal insulin concentration lasted only 1 day and then declined despite persistent, relatively constant fetal HG. By day 2, the mean fetal plasma insulin concentrations were not different from control values obtained before starting the glucose infusion. During study period 2, both maternal and fetal insulin concentrations increased acutely in response to AA infusion, despite persistent maternal HG. The maternal insulin concentration increased by $73 \pm 18\%$, and the fetal insulin concentration increased by $55 \pm 14\%$ ($P < 0.0005$ and $P < 0.001$, respectively; Fig. 3).

Figure 4 shows the effect of prolonged maternal HG on plasma leucine and lysine concentrations. Concentrations of the essential AA leucine were measured daily, because preliminary data indicated that chronic maternal HG significantly decreased maternal plasma essential AA concentrations, particularly leucine concentrations. Both maternal and fetal leucine concentrations were significantly decreased from baseline after 48 h of HG and tended to continue to decline on subsequent days. Lysine, the essential AA used for the AA clamp in this study, remained significantly de-

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Fig. 2. Means ± SE maternal and fetal arterial plasma glucose concentrations throughout the study. Daily glucose concentrations remained significantly elevated during the hyperglycemic clamp compared with the control period in both the mother and fetus ($* P < 0.0005$ by paired $t$-test).

Fig. 3. Means ± SE maternal and fetal arterial plasma insulin concentrations over 5 days. $P$ values determined by 2-tailed, paired $t$-test. Control period insulin concentrations on day 0 vs. insulin concentration on all other days: $* P < 0.01$, $** P < 0.005$; study period 1 vs. study period 2 insulin concentration: $+ P < 0.01$, $++ P < 0.0005$.

Fig. 4. Means ± SE maternal (A) and fetal (B) arterial plasma leucine and lysine concentrations over 5 days. Significance of differences was determined by 2-tailed, unpaired $t$-test: $* P < 0.05$, $** P < 0.005$, $+ P < 0.00005$, $^* P < 0.01$. 

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increased throughout this period in the maternal artery but was unchanged in the fetus.

Table 2 presents plasma glucose and lactate concentrations, blood oxygen saturation and content, and hemoglobin and hematocrit values during the two study periods (i.e., maternal HG,LAA in study period 1 and maternal HG,NAA in study period 2). Plasma arterial lactate concentration decreased during study period 2 in the mother (14%) but was unchanged in the fetus. Both fetal arterial blood oxygen saturation and content decreased in study period 2 (both by 9%). Blood flow rates and net glucose and lactate uptake rates in the two study periods are shown in Table 3. Uterine blood flow rate normalized to fetal weight was greater (13% increase) in study period 2, but umbilical blood flow per fetal weight did not change. Umbilical (fetal) glucose uptake rate was slightly greater (19% increase, P < 0.05) during the AA infusion despite no significant changes in umbilical arterial and venous arterial plasma glucose concentrations.

Maternal (Fig. 5) and fetal (Fig. 6) arterial AA concentrations are shown for control period (maternal HG,NAA) and the two study periods (day 5, maternal HG,LAA and HG,NAA for study periods 1 and 2, respectively). In the maternal circulation, there was a significant decrease (P < 0.05) in the concentrations of all AAs after 5 days of maternal HG (control period vs. study period 1) except for glycine, which was significantly increased (+40%, P < 0.005). The decrease in maternal AA concentrations was most pronounced for the essential AAs valine (−79%), leucine (−80%), isoleucine (−79%), threonine (−73%), and methionine (−57%; P < 0.00001). For the fetus, AA concentrations were significantly decreased (P < 0.05) with chronic maternal HG only for the essential AAs valine (−74%), leucine (−73%), isoleucine (−79%), threonine (−20%), and phenylalanine (−20%), plus the nonessential AA citrulline (−35%). As in the maternal circulation, fetal glycine concentration was significantly increased, but to a much greater extent (+142%, P < 0.001).

Steady-state lysine concentrations were maintained during the maternal AA infusion, and it is presumed that other AAs also were in steady state with relatively constant concentrations. Under these conditions, there were significant acute increases in the concentrations of all maternal essential and most of the nonessential AAs (Fig. 5). This resulted in significant increases in fetal plasma concentrations of all of the essential AAs except for threonine and lysine plus increases in the concentrations of the nonessential AAs arginine and ornithine (Fig. 6).

The uterine and umbilical AA uptake rates, for which there were significant changes between the two study periods, are shown in Fig. 7. These changes all
represent significant increases in uptake rates, except for a decrease in uterine glutamine uptake rate. The AAs for which uterine and umbilical uptake rates were significantly increased were not necessarily those with the greatest infusion rate into the maternal circulation (Table 4). For the AA uptakes shown in Fig. 7, there were no significant differences in net uteroplacental AA uptake (utilization) rates (data not shown).

DISCUSSION

The purpose of this study was to test how changes in maternal AA concentrations would affect uterine, umbilical (fetal), and uteroplacental net AA uptake rates, fetal AA concentrations, and fetal insulin concentrations. The results showed that chronic maternal HG of 5 days duration decreased most maternal plasma AA concentrations and uterine and umbilical uptake rates and fetal plasma concentrations of primarily the essential AAs. These changes were associated with acute increases of maternal and fetal plasma insulin concentrations followed by a persistent increase in maternal plasma insulin concentrations but a return of fetal insulin concentrations to control values by the second day of HG. Under these conditions, an acute (4 h; 1 h to reach steady state plus 3-h steady-state infusion) maternal intravenous AA infusion that was clamped to return maternal AA concentrations to control values produced significant increases in all maternal and many fetal AA concentrations. Uterine and umbilical AA uptake rates of primarily the essential AAs also increased, as did maternal and fetal plasma insulin concentrations. Thus this study shows that changes in maternal AA concentrations, at least to values that are lower than normal, directly affect the net transfer to the fetus and fetal plasma concentrations of some, but not all, AAs. The changes in fetal AA concentrations, in turn, independently affect fetal insulin concentrations.

The most likely mechanism producing maternal hypoaminoacidemia during the hyperglycemic conditions of this study was the maternal hyperinsulinemia. A variety of studies have demonstrated that insulin decreases plasma AA concentrations, primarily by suppressing protein breakdown (5, 15, 17). In contrast, data from adult humans indicate that HG can increase protein breakdown (16, 33, 43). Whether or not HG independently regulates protein breakdown in materi-
nal or fetal sheep is unclear. Liechty and colleagues (24) have shown decreased rates of protein oxidation in fetal sheep during acute HG, although leucine flux remained constant, indicating that glucose might spare leucine as an energy source during fasting. In the present study, maternal glucose concentration increased 70% between the control period and study period 1, whereas maternal insulin concentration increased nearly fourfold. This high insulin/glucose concentration ratio likely allowed antiproteolytic effects of insulin to predominate over potential hyperglycemic-induced protein degradation, resulting in net maternal hypoaminoacidemia.

The mechanisms responsible for the decreased umbilical uptake rates of selected AAs during maternal HG also remain uncertain, but the most obvious mechanism appears to be the decrease in maternal plasma AA concentrations. The particular AAs with decreased umbilical uptake rates in the present study (the branched-chain AAs) have been shown in other studies to have at least a component of direct transplacental transport (7). Jozwik et al. (21) showed a limited increase in transplacental transport of these same AAs when infused at higher than normal concentrations. As shown in Fig. 8, data from the present study and those of Jozwik et al. (21) demonstrate that transplacental transfer rates of selected AAs, notably the branched-chain essential AAs leucine, valine, and isoleucine, are directly dependent on their concentrations in the maternal plasma over the physiological range of maternal and fetal AA concentrations in pregnant sheep.

AA transport is a complex process that is dependent on a number of factors including individual AA transport affinities, AA concentrations on both sides of the membrane across which they are transported, competitive inhibition for a transporter, the hormonal milieu, and overall nutritional status (41). In general, transport across a membrane depends on the concentration of the AA at the membrane surface (10). At the same time, a decrease in the AA concentration surrounding a membrane has been shown to upregulate transporter activity (23). This is particularly true for system A transporter activity (36) but also has been demonstrated to a lesser degree for the system L transport system that is responsible for much of the transport of branched-chain AAs (37). In contrast, the present
study shows decreased transport of the branched-chain AAs at lower plasma concentrations. This observation contradicts previous in vivo and in vitro studies in which decreased AA concentrations enhanced AA transport (14, 38, 39). Clearly, kinetic studies of AA transport across the ovine placenta need further investigation to define more completely the relationships among transport rate, transport capacity, and maternal, fetal, and transplacental plasma AA concentrations.

The causes of fetal hypoaminoacidemia in response to chronic maternal and fetal HG also are not clear. Most likely, they represent decreased umbilical uptake rates, as shown in Fig. 8. It is unlikely, however, that fetal insulin concentrations at the time of study affected fetal AA concentrations, because fetal insulin concentrations at the time of study had decreased to values not different from the control period before HG. Other investigators also have demonstrated a decrease in fetal insulin concentration over time with persistent fetal HG (1, 3, 4). The independent effect of the normal fetal insulin concentrations during study period 1 affected fetal AA concentrations, because fetal insulin concentrations at the time of study had decreased to values not different from the control period before HG. Other investigators also have demonstrated a decrease in fetal insulin concentration over time with persistent fetal HG (1, 3, 4). The independent effect of the normal fetal insulin concentrations during study period 1 affected fetal AA concentrations, because fetal insulin concentrations at the time of study had decreased to values not different from the control period before HG. Other investigators also have demonstrated a decrease in fetal insulin concentration over time with persistent fetal HG (1, 3, 4).

Table 4. Amino acid concentration in mixed amino acid solution (Trophamine) and rate of maternal amino acid infusion

<table>
<thead>
<tr>
<th>Amino Acid Concentration in Trophamine, µmol/ml</th>
<th>Amino Acid Infusion Rate from Trophamine Infusion, µmol·min⁻¹·kg maternal wt⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Essential amino acids</strong></td>
<td></td>
</tr>
<tr>
<td>Val</td>
<td>73.89 ± 0.24</td>
</tr>
<tr>
<td>Leu</td>
<td>119.45 ± 0.38</td>
</tr>
<tr>
<td>Ileu</td>
<td>68.37 ± 0.22</td>
</tr>
<tr>
<td>Thr</td>
<td>41.68 ± 0.13</td>
</tr>
<tr>
<td>Phe</td>
<td>33.33 ± 0.11</td>
</tr>
<tr>
<td>Met</td>
<td>24.60 ± 0.08</td>
</tr>
<tr>
<td>Lys</td>
<td>62.55 ± 0.20</td>
</tr>
<tr>
<td>His</td>
<td>34.50 ± 0.11</td>
</tr>
<tr>
<td><strong>Nonessential amino acids</strong></td>
<td></td>
</tr>
<tr>
<td>Ser</td>
<td>39.79 ± 0.13</td>
</tr>
<tr>
<td>Gly</td>
<td>55.54 ± 0.18</td>
</tr>
<tr>
<td>Ala</td>
<td>69.27 ± 0.22</td>
</tr>
<tr>
<td>Pro</td>
<td>83.41 ± 0.22</td>
</tr>
<tr>
<td>Arg</td>
<td>91.43 ± 0.29</td>
</tr>
<tr>
<td>Tyr</td>
<td>2.77 ± 0.06</td>
</tr>
<tr>
<td>Glu</td>
<td>41.45 ± 0.13</td>
</tr>
<tr>
<td>Asp</td>
<td>27.55 ± 0.09</td>
</tr>
<tr>
<td>Tau</td>
<td>2.67 ± 0.06</td>
</tr>
</tbody>
</table>

Values are means ± SE.
metabolism of other substrates or led to decreases in fetal AA uptake rates or plasma AA concentrations. First, the study period 2 oxygen content was still higher than the control oxygen content values reported in a study that demonstrated a limitation of fetal substrate supply, including AAs, during much more marked hypoxemia (27). Also, the changes in fetal oxygen content in the present study are consistent with the results of previous studies that did not show decreased rates of uterine, fetal, or uteroplacental rates of oxygen consumption at similar fetal oxygen content values (3, 44). Furthermore, there was a brisk and relatively marked increase in fetal insulin concentrations in response to the increase in fetal branched-chain AA concentrations in the present study, in contrast to the usual quite sensitive suppression of insulin secretion by hypoxia (13).

AA infusion into the mother increased fetal AA uptake rates and plasma concentrations of several AAs, including arginine and leucine, both of which have been shown to directly stimulate fetal insulin secretion (3, 18). Indeed, fetal insulin concentrations in the present study increased over 50% during the infusion of AAs into the mother, a greater increase than that induced by infusion of arginine alone (to equivalent concentrations) into the fetus (18). Because fetal glucose concentration did not increase with maternal AA infusion, these results indicate that the suppression of insulin concentrations during the hyperglycemic period could have been due, at least in part, to the lower plasma AA concentrations, not just the HG. The capacity to reverse the suppression of fetal insulin concentrations during HG and selective hypoaminoacidemia by raising fetal AA concentrations further supports the hypothesis that fetal insulin secretion is under direct control by the plasma concentrations of selected AAs. These observations uniquely demonstrate how the supply of AAs from the placenta and mother can regulate fetal insulin secretion. Furthermore, the capacity to reverse the HG-induced suppression of fetal insulin secretion is consistent with our previous studies that showed that arginine stimulation of fetal insulin secretion was not diminished at comparable early phases (5–7 days) of HG, despite suppression of insulin secretion in response to glucose stimulation (3). Only after 2–3 wk of HG did arginine stimulation of insulin secretion also become diminished. Thus the increase in insulin secretion achieved by normalizing low concentrations of selected (branched chain) AAs in the fetus in the present study defines independent effects of glucose and AAs on insulin secretion and supports previous evidence for the time-dependent nature of hyperglycemic, hypoaminoacidemic suppression of insulin secretion.

There have been relatively few physiological studies examining the effect of maternal AA supplementation on fetal AA supply and concentrations. MacMahon et al. (25) showed that a 1-h infusion of a commercial mixed AA solution into pregnant ewes increased the concentrations of most maternal AAs but only increased the fetal concentrations of phenylalanine and alanine. Jozwik et al. (21) showed that a 12-h maternal AA infusion increased maternal concentrations of nearly all infused AAs, whereas fetal concentrations increased significantly only for phenylalanine and methionine, umbilical uptakes increased only for leucine and isoleucine, and net fetal nitrogen supply did not increase despite a significant increase in total nitrogen supply to the uterus. Jozwik et al. (21) concluded that prolonged maternal infusion of AAs into pregnant sheep that had normal maternal and fetal AA concentrations was a relatively ineffective method by which to increase fetal AA and nitrogen supply. The present study differs from the MacMahon (25) and Jozwik (21) studies in that the majority of maternal and fetal AA concentrations in the present study were significantly lower than normal, by study design, at the time of maternal AA infusion. Notably, however, the present study and that of Jozwik et al. (21) showed no significant changes in uteroplacental utilization rates of any...
AA, although there was a modest increase in uteroplacental ammonia production in Jozwik’s study.

In a review of human studies undertaken to improve fetal growth restriction with nutrient delivery to either the mother or the fetus, there was minimal evidence of any benefit of this type of nutritional intervention (19). However, a recent study of intravenous infusion of AAs into pregnant women undergoing cesarean section delivery showed increased umbilical arterial concentrations and umbilical venoarterial concentration differences relative to venoarterial oxygen content differences in clamped umbilical cord segments (34). These results demonstrated increased transfer to the fetus of most of the AAs. Clearly, a number of factors must be considered when evaluating how changes in maternal AA concentrations affect the transport of AAs across the placenta and into the fetus, including species differences in placental structure and transport capacity as well as the method of AA infusion into the mother (e.g., bolus vs. prolonged infusion).

Under normal conditions, there is a net umbilical uptake by the fetus from the placenta of all the nutritionally significant AAs with the exception of serine (11). In the present study, the maternal AA infusion rate did not correlate directly with umbilical AA uptake rate. A number of reasons may explain this lack of correlation. Recent data on the maternal-uteroplacental-fetal metabolism of individual AAs in pregnant sheep have shown that the placenta plays an important role in fetal AA supply by differential utilization and production of AAs by the placenta (6, 9, 29, 30). Additionally, there are a number of placental AA transporters with broad specificity, which might lead to transporter saturation for some AAs but competitive inhibition of others when presented with increased concentrations of AAs (22, 28). In the present study, most of the fetal AAs that demonstrated the greatest increase in umbilical uptake with maternal AA infusion are transported by the sodium-independent placental L transporter, which mediates rapid AA exchange of valine, leucine, isoleucine, and phenylalanine. The increase in transport to the fetus of these AAs in the present study argues against marked competitive inhibition among them for this transporter and supports the notion that this transporter is present in excess, has a high transport capacity, or both. Further in vivo studies of possible competition among AAs that compete for single transporters at the maternal and fetal surfaces of the placenta are necessary to resolve their individual and interactive kinetics.

In conclusion, this study confirms that sustained maternal and fetal HG produces sustained maternal hyperinsulinemia and hypoaminoacidemia, a selective decrease in fetal AA (branched chain) concentrations, and acute but transient fetal hyperinsulinemia. Under the conditions of maternal and fetal hypoaminoacidemia produced in this study, AA infusion into the mother increases fetal AA uptake of those AAs that had low umbilical uptake rates and fetal plasma concentrations during the hyperglycemic period. Normalization of these AAs in the fetal plasma also rapidly and markedly increased fetal insulin concentrations. We speculate that restoration of normal essential AA supply plus improved fetal insulin concentration may allow improved fetal growth when maternal and fetal AA concentrations are low.

**Perspectives**

Both normal and abnormal nutrient delivery to the fetus are affected by a number of factors. In most situations, substrate delivery to the fetus is dependent on maternal concentrations. AA metabolism represents a unique example of substrate delivery from mother to fetus in that most fetal plasma AA concentrations are higher than maternal plasma concentrations. In general, this transplacental AA transport involves energy-dependent transport mechanisms. Also complicating the understanding of nitrogen delivery to the fetus is the fact that the intermediary organ of fetal substrate delivery, the placenta, is not only responsible for AA transport, but also consumes, metabolizes, and produces various AAs.

Little is know about how changes in the maternal-fetal AA concentration gradient affect AA transfer to the fetus. Prior studies of maternal AA supplementation have significantly increased maternal but not fetal plasma AA concentrations (21, 25). The present study clearly demonstrates that a decrease in maternal AA concentrations reduces the umbilical delivery of AAs to the fetus and decreases their fetal plasma concentration, particularly for the essential AAs. Of note, the decreased fetal nitrogen delivery could at least partially be reversed by restoring maternal AA concentrations to normal. However, this could potentially be undesirable under conditions of fetal compromise, such as severe intrauterine growth restriction; if oxidative substrates to the fetus are restored and are oxidized in fetal tissues, then there also is the potential to increase fetal metabolic rate and oxygen consumption, which could lead to hypoxemia if uterine and umbilical blood flow are also compromised. Clearly, further studies are required to understand the effects of maternal dietary manipulation on fetal nutrition.

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**REFERENCES**

5. Castellino P, Luzi L, Simonson D, Haymond M, and Defronzo R. Effect of insulin and plasma amino acid concentra-


