Effect of hyperinsulinemia on amino acid utilization and oxidation independent of glucose metabolism in the ovine fetus

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Submitted 7 June 2006; accepted in final form 29 June 2006

Brown, Laura D., and William W. Hay, Jr. Effect of hyperinsulinemia on amino acid (AA) utilization and oxidation rates independent of insulin-enhanced glucose metabolism in fetal sheep. Metabolic studies were conducted in each fetus (n = 11) under three experimental periods. After control period (C) study, a fetal hyperinsulinemic-euglycemic-euaminoacidemic (HI-euG-euAA) clamp was established, followed by a hyperinsulinemic-hypoglycemic-euaminoacidic (HI-hypoG-euAA) clamp to decrease glucose metabolic rates toward C values. Infusions of $^3$H$_2$O, $l$-[1-$^{13}$C]$l$leucine, and $[^{14}$C(U)]glucose were administered to measure blood flow, leucine oxidation, and fetal glucose uptake, utilization, and oxidation in each period. Fetal glucose utilization rate increased 1.7-fold with hyperinsulinemia (C 0.2 mg kg$^{-1}$ min$^{-1}$, HI-euG-euAA 1.3 mg kg$^{-1}$ min$^{-1}$, $P < 0.0001$), returning to rates not different from C (C 0.2 mg kg$^{-1}$ min$^{-1}$ vs. C value, $P = 0.15$). Fetal glucose oxidation rate increased 1.7-fold with hyperinsulinemia (C 3.1 ± 0.2 mg kg$^{-1}$ min$^{-1}$, HI-euG-euAA 5.4 ± 0.4 mg kg$^{-1}$ min$^{-1}$, $P < 0.0001$) and decreased to near control rates with hypoglycemia (4.0 ± 0.3 HI-hypoG-euAA vs. C value, $P = 0.006$). AA utilization rates increased with hyperinsulinemia for all essential and most nonessential AAs ($P < 0.001$) and did not change when insulin-induced increases in glucose utilization returned to control rates. Leucine oxidation rate increased 1.7-fold with hyperinsulinemia (C 1.0 ± 0.3 µmol min$^{-1}$ kg$^{-1}$, HI-euG-euAA 1.7 ± 0.3 µmol min$^{-1}$ kg$^{-1}$, $P < 0.002$) and did not change when glucose oxidation rate was decreased with hypoglycemia. These results demonstrate that, in fetal sheep, insulin promotes AA utilization and oxidation independent of its simultaneous effects on glucose metabolism. In acute hyperinsulinemic conditions, AA oxidation does not change when insulin-induced glucose utilization is prevented.

insulin; leucine; fetal sheep

Fetal growth is regulated by a complex interaction of hormonal signals and substrate availability. Insulin, a major fetal growth hormone, has potent effects on glucose metabolism and anabolic effects on protein metabolism during fetal growth. The availability of glucose and amino acids (AAs) to the fetus is also critically important to maintain optimal fetal growth during gestation and has marked effects on protein synthesis. Previous studies in fetal sheep have demonstrated anabolic effects of insulin on protein metabolism. Insulin increases whole fetal amino-nitrogen uptake (27), fetal hindlimb uptake of select AAs (34), and whole fetal AA and nitrogen utilization rates (31). Insulin has been shown to increase nonoxidative AA disposal, reflecting net protein accretion (20, 26, 29). Fetal protein metabolism is also affected by fetal glucose and AA concentrations, as demonstrated by several studies comparing fasted and fed maternal ewes (18, 22, 30) and studies that have varied fetal glucose and AA concentrations by direct infusion of these substrates into the fetus (17, 19, 21, 25). Insulin produces a 20–40% decrease in fetal plasma AA concentrations (26, 28, 29, 31, 34), which confounds the ability to determine the independent effect of insulin on AA metabolism. Studies that have maintained euglycemic, euaminoacidic conditions during experimentally produced hyperinsulinemia to eliminate the simultaneous effects of plasma glucose and AA concentrations on insulin action (26, 29, 31) continue to demonstrate an independent anabolic role of insulin in fetal protein metabolism.

In all of these previous studies, however, experimental designs prevented the ability to determine whether or not effects of increased glucose metabolism (both oxidative and nonoxidative), which occur in response to hyperinsulinemia when euglycemia is maintained by simultaneous glucose (dextrose) infusion into the fetus, contribute to the effect of insulin on AA metabolism. It is possible, for example, that the mechanism by which insulin promotes net fetal protein accretion is by simultaneously increasing glucose utilization and oxidation, thereby sparing AA oxidation. Our hypothesis, however, is that insulin directly promotes AA accretion into protein independent of its effects on glucose metabolism. The purpose of this study, therefore, was to test whether the specific effect of acute fetal hyperinsulinemia on fetal whole body AA utilization is independent of the effect of insulin to promote whole body glucose utilization and oxidation rates.

MATERIALS AND METHODS

Animal Care and Surgical Procedure

Studies were performed in 11 late-gestation Columbia-Rambouillet sheep obtained from a commercial breeder (Neheber Ranch, Santa Monica, CA). All were time-dated singleton pregnancies. Sheep were fasted 24 h prior to surgery. Prior to surgical preparation, a maternal jugular catheter was placed for administration of Diazepam (10 mg), Ketofen (33 mg into 1 ml of 0.9% wt/vol sodium chloride in water), and Ketamine (800 mg) to induce anesthesia. The ewes were maintained on 2–4% isoflurane inhalation anesthesia for the remainder of the surgical procedures. Hysterotomy was performed, and fetal catheters for blood sampling and infusions were placed into the abdominal aorta via hindlimb arteries and femoral veins via hindlimb veins. A fetal catheter was inserted into the umbilical vein at the base of the cord and advanced into the common umbilical vein for the purpose of measuring umbilical blood flow. For maternal blood sampling and infusion.

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infusion, catheters were placed into the femoral artery and vein through a small incision in the groin of the ewe. All catheters were filled with heparinized saline (50 units/ml sodium heparin in 0.9% wt/vol NaCl in water), tunneled subcutaneously through a maternal skin incision, and stored in a plastic pouch sutured to the ewe’s flank. Ampicillin (500 mg) was administered into the amniotic fluid just prior to closure of the uterus, and procaine penicillin (6,000,000 U) was given intramuscularly to the ewe. The ewes were allowed to recover for 5 to 7 days prior to study. Buprinex (0.6 mg) was given every 12 h for 2 days postoperatively for pain management. During postoperative recovery, ewes were housed in a temperature-controlled environment (18 ± 2°C) in standard carts. Sheep were fed alfalfa pellets ad libitum during recovery and during the study. Maternal and fetal catheters were flushed every other day. All in vivo procedures and studies were performed at the University of Colorado Health Sciences Center Perinatal Research Center. The Perinatal Research Center is accredited by the US Department of Agriculture, the National Institutes of Health, and the American Association for the Accreditation of Laboratory Animal Care.

Experimental Design

Studies were performed at 126 ± 4 days gestation. Figure 1 shows the overall study design. A primed constant infusion of 3H2O (to measure umbilical blood flow), [14C(U)]glucose (to measure net fetal glucose uptake, glucose utilization, and glucose oxidation rates), and L-[1-13C]leucine (to measure fetal leucine oxidation on the basis of α-isoketocaprate enrichment) was started in a fetal femoral vein at time 0. After 70 min of equilibration, four consecutive control period (C) blood draws 10 min apart were obtained at euglycemic, euaminoacidemic, and euinsulinemic baseline conditions (period 1). Directly following C draws, a fetal hyperinsulinemic-euglycemic-euaminoacidemic clamp (HI-euG-euAA) was established (period 2). Recombinant insulin (Humulin R; Eli Lilly, Indianapolis, IN) was prepared with 0.9% wt/vol NaCl in water to provide a 180 mU/kg estimated fetal weight bolus followed by a constant infusion of 3 mU·min⁻¹·kg⁻¹ estimated fetal weight. Fetal euaminoacidemia was maintained with a maternal glucose infusion (50% wt/vol dextrose in water; Abbott Laboratories, North Chicago, IL) adjusted according to frequent fetal arterial glucose concentration ([G]) measurements (11, 12) from the technique introduced by DeFronzo et al. (6). Euaminoacidemia was maintained with a commercial-mixed AA infusion (Trophamine; Central Admixture Pharmacy Services, Denver, CO) into the fetus adjusted according to rapid spectrophotometric branched-chain AA assay (2). On average, 4 h were required to produce steady-state glucose and AA concentrations at C concentrations. Four draws 10 min apart were obtained at period 2 steady state. Immediately following period 2, a hyperinsulinemic-hypoglycemic-euaminoacidemic clamp (HI-hypoG-euAA) was established to return glucose utilization rate to C values (period 3). To create these conditions, the maternal glucose infusion was stopped to allow fetal glucose concentrations to fall ~50% below baseline [G]. If needed, a maternal insulin infusion (100 mU/kg bolus followed by 0.2–1.0 mU·min⁻¹·kg⁻¹) was started to achieve reduced fetal [G]. On average, 2 h was required to reduce fetal [G] and maintain AA concentrations at C concentrations. Four draws 10 min apart were obtained at period 3 steady state.

Isovolemic transfusions of maternal blood were administered to the fetus at a constant rate during all three draw periods. Arterial and umbilical venous blood was sampled simultaneously during draw periods and analyzed for hematocrit, blood oxygen content, oxygen saturation, and concentrations of plasma glucose, lactate, insulin, AAAs, and 3H2O. Isotopic enrichments of α-isoketocaprate (KIC) and 13CO2 were measured during baseline and experimental steady-state periods for calculation of leucine oxidation rates. Whole blood 14CO2 concentrations were obtained for calculation of glucose oxidation rates. At the conclusion of the study, the ewe and fetus were euthanized with intravenously administered Sleepaway pentobarbital sodium (Fort Dodge Laboratories, Fort Dodge, IA). Fetal weight was obtained at autopsy.

Analytical Methods

Fetal plasma glucose and lactate concentrations were measured rapidly using the YSI Glucose and Lactate model 2700 analyzer (Yellow Springs Instruments, Yellow Springs, OH). Plasma branched-chain AA concentrations (leucine, isoleucine, and valine) were measured with a spectrophotometric assay described by Beckett el al (2) using an Ultrospec 4300 pro UV/visible spectrophotometer (Amerham Pharmacia Biotech, Cambridge Science Park, Cambridge, UK). Blood gas determinations, oxygen content, and hematocrit were measured on whole fetal blood (ABL 520 Hemoximeters, Copenhagen, Denmark). A portion of fetal plasma was collected by centrifugation for 3 min at 4°C and stored at −70°C for analysis of insulin and AA concentrations as well as isotopic enrichment analysis of KIC. Fetal plasma insulin concentrations were measured using ALPCO Ovine Insulin ELISA (Windham, NH). Individual plasma AA concentrations were determined using a Dionex HPLC AA Analyzer (Dionex, Sunnyvale, CA).

Fig. 1. Study design. Each animal was studied under control, hyperinsulinemic-euglycemic-euaminoacidemic (HI-euG-euAA), and hyperinsulinemic-hypoglycemic-euaminoacidemic (HI-hypoG-euAA) periods.
To determine umbilical blood flow, $^3$H$_2$O (PerkinElmer Life Sciences, Boston, MA) was infused into the fetal femoral vein, and after a 70-min equilibration period whole fetal blood samples were obtained during each of the study period draws. Plasma (0.1 ml) was solubilized in 1.0 ml of Soluene-350 (quaternary ammonium hydroxide in toluene; Packard) and then mixed with 15 ml of Hionic Fluor (Packard). Counts were measured in a Packard Tri-Carb 460 C liquid scintillation counter. To determine fetal glucose utilization and oxidation rates, universally labeled $^{[14]}$Cglucose tracer (PerkinElmer Life Sciences) was infused with $^3$H$_2$O, and glucose tracer methodology was used as previously described (14).

To determine whole blood $^{13}$CO$_2$ enrichment, CO$_2$ was isolated from the blood using 0.1 ml of saturated citric acid solution and collected in an Exetainer storage tube. The tubes were processed through an autosampler, and the captured gas was passed through a gas chromatograph column and introduced to the Europa Scientific 20/20 gas isotope ratio mass spectrometer. The ratio of $^{13}$CO$_2$ to $^{12}$CO$_2$ (mass 45 to 44) was measured in the sample and compared with a reference gas (5% CO$_2$, balance 75% N$_2$, 20% O$_2$). This reference gas has been calibrated with respect to its isotope ratio against the international standard Pee Dee Belemate limestone. Atoms percent excess (APE) was calculated as sample atoms percent minus baseline atoms percent.

For stable isotope-labeled KIC analysis, plasma samples were prepared with the OPDA (O-phenylenediamine) derivative of the ketoacid function followed by a $t$-butyl trimethylsilyl derivative on the carboxylic acid. A Hewlett-Packard 5890 gas chromatograph coupled to a 5989A mass spectrometer was autotuned in Electron Impact mode according to the manufacturer’s specifications. A standard curve was prepared and analyzed. Ions representing natural or “unlabeled” KIC ($m/z = 259$) and $[1^{13}$C]KIC ($m/z = 260$) were monitored. Total area counts were compared with the standard curve and used to calculate mole fractions.

Calculations

Blood flows and fetal glucose and AA uptake rates. Umbilical plasma flows were determined by the transplacental diffusion method, with tritiated water as the flow indicator (24). Umbilical blood flows were then calculated using the formula

$\text{umbilical blood flow} = \frac{\text{plasma blood flow}}{(1 - \text{fractional fetal hematocrit})}$

Umbilical venous-fetal arterial differences in glucose and individual AAs are multiplied by umbilical blood or plasma flow (Fick principle) to calculate nutrient uptake across the fetus:

$\text{fetal glucose uptake} = \text{umbilical blood flow} \times (\Delta G)_{v-a}$

$\text{umbilical AA uptake} = \text{umbilical plasma flow} \times (\Delta AA)_{v-a}$

where $(\Delta G)_{v-a}$ is the whole blood glucose concentration difference between the umbilical venous and fetal arterial vessels and $(\Delta AA)_{v-a}$ is the difference between the umbilical venous and fetal arterial vessels calculated for each AA.

Fetal glucose utilization and oxidation rates and AA utilization rates. All equations used to calculate fetal glucose utilization and oxidation rates are described in detail by Hay et al. (14) using $[1^{14}$C]glucose tracer methodology. Individual AA utilization rates were determined by adding the AA umbilical uptake rate to the steady-state infusion rate of each AA in Trophamine that was required to maintain baseline AA concentrations.

Fetal leucine oxidation rate. The plasma enrichment of KIC has been shown to be a representative tracer of the intracellular leucine enrichment (16). Leucine oxidation was calculated as

$$\text{Leucine oxidation} = \frac{([a^{13}CO_2 \times aCO_2] - [uv^{13}CO_2 \times uvCO_2])}{\text{MPE}_{KIC}} \times \text{umbilical blood flow}$$

where $a^{13}CO_2$ = fetal arterial CO$_2$ enrichment (APE), $aCO_2$ = fetal arterial CO$_2$ content, $uv^{13}CO_2$ = umbilical venous CO$_2$ enrichment (APE), $uvCO_2$ = umbilical venous CO$_2$ content, and MPE$_{KIC}$ = KIC moles percent excess.

Statistical Analysis

Results are expressed as means ± SE. For all measurements, differences between control and experimental periods were analyzed by a two-way analysis of variance (3 periods by 11 sheep). A priori contrasts were utilized to compare period 2 with periods 1 and 3. P values <0.05 were considered significant.

RESULTS

Fetal gestational age at study was 128.6 ± 1.2 days. Fetal weights were similar among all animals (2.99 ± 0.18 kg). Measurements of fetal hematocrit, oxygen saturation, oxygen content, umbilical oxygen uptake, arterial pH, lactate, umbilical lactate uptake, and umbilical whole blood and plasma flows are listed in Table 1. There was a minor, progressive decrease in hemoglobin concentration observed, possibly because the maternal blood transfusion into the fetus was not quite isovolumetric with the blood sampled, but also because of dilution by continued infusion of fluids (tracers, AAs, insulin) into the fetal circulation. We also observed minor changes in oxygen saturation during the study, most likely the result of a right shift in the oxyhemoglobin dissociation curve from adult hemoglobin in the maternal blood used for blood replacement, which would have lowered the oxygen saturation of hemoglobin in the umbilical vein and thus the umbilical artery. The experimental periods also had slightly lower values of fetal blood oxygen content and pH than in the control period, although the experimental periods did not differ from each other. Oxygen and lactate uptake rates were not different between periods. On the basis of previous studies of this nature (19, 25, 31), it is unlikely that the overall physiological status of the fetus and the fetal metabolic rates measured were affected by these minimal changes in experimental conditions. Umbilical plasma

Table 1. Hematological, blood gas, and blood flow results in the 3 study periods

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>HI-euG-euAA</th>
<th>HI-hypoG-euAA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin, g/dl</td>
<td>6.4±0.1</td>
<td>6.2±0.1†</td>
<td>6.0±0.1‡</td>
</tr>
<tr>
<td>Oxygen saturation, %</td>
<td>48.8±2.4</td>
<td>37.2±2.5‡</td>
<td>32.6±2.8‡</td>
</tr>
<tr>
<td>Oxygen content, mmol/l</td>
<td>3.0±0.1</td>
<td>2.1±0.2*</td>
<td>2.1±0.2*</td>
</tr>
<tr>
<td>Umbilical oxygen uptake, μmol/min·kg⁻¹</td>
<td>363±19</td>
<td>378±26</td>
<td>365±26</td>
</tr>
<tr>
<td>Arterial pH</td>
<td>7.36±0.01</td>
<td>7.33±0.01*</td>
<td>7.33±0.01*</td>
</tr>
<tr>
<td>Arterial lactate, mmol/l</td>
<td>1.9±0.2</td>
<td>2.1±0.3</td>
<td>1.9±0.3</td>
</tr>
<tr>
<td>Umbilical lactate uptake, μmol/min·kg⁻¹</td>
<td>16.9±2.1</td>
<td>17.6±2.0</td>
<td>13.6±2.1</td>
</tr>
<tr>
<td>Umbilical plasma flow, ml/min·kg⁻¹</td>
<td>135.8±11.1</td>
<td>129.3±9.5</td>
<td>137±7.6</td>
</tr>
<tr>
<td>Umbilical blood flow, ml/min·kg⁻¹</td>
<td>198.3±15.3</td>
<td>186.9±14.1</td>
<td>198±9.2</td>
</tr>
</tbody>
</table>

Values are means ± SE. HI-euG-euAA, hyperinsulinemic-euglycemic-euaminocaidemic; HI-hypoG-euAA, hyperinsulinemic-hypoglycemic-euaminocaidemic. Different symbols denote group differences (*differences from control; †differences from HI-hypoG-euAA; ‡differences from HI-euG-euAA).
and whole blood flows per kilogram fetal body weight were unchanged with different experimental conditions (Table 1).

Fetal plasma glucose, insulin, and branched-chain AA concentrations are shown in Fig. 2. Fetal plasma insulin concentrations increased 23-fold from 5.5 ± 0.6 μU/ml in period 1 (C) to 126.5 ± 15.2 μU/ml in period 2 (HI-euG-euAA) and 130.3 ± 17.0 μU/ml in period 3 (HI-hypoG-euAA). Fetal arterial plasma glucose concentrations did not change between period 1 and period 2 and were effectively reduced to 50% of baseline in period 3 (C 19.6 ± 0.6 mg/dl, HI-euG-euAA 18.8 ± 0.6 mg/dl, and HI-hypoG-euAA 10.0 ± 0.5 mg/dl). By adjusting a fetal infusion of Trophamine, branched-chain AA concentrations, measured with a rapid spectrophotometric assay, were maintained at baseline concentrations (C 0.53 ± 0.05 μmol/ml, HI-euG-euAA 0.50 ± 0.05 μmol/ml, HI-hypoG-euAA 0.54 ± 0.05 μmol/ml). Because the rapid spectrophotometric assay measures only the three branched-chain AAs, HPLC technique analyzing all 21 AA concentrations was also performed. There were small but significant differences in all of the essential AA concentrations, except lysine, and in some of the nonessential AAs (Fig. 3). However, the insulin-stimulated decrease in plasma AA concentrations was clearly prevented with Trophamine infusion.

By decreasing fetal glucose entry rate and plasma glucose concentration in period 3, fetal glucose metabolism returned from an increased rate with hyperinsulinemia toward the mean control rates. As shown in Fig. 4, net fetal glucose uptake rate was significantly increased from the mean control value during period 2 under conditions of hyperinsulinemia at baseline concentrations of glucose and AAs (C 6.3 ± 0.4 mg·kg⁻¹·min⁻¹, HI-euG-euAA 12.7 ± 1.1 mg·kg⁻¹·min⁻¹, P < 0.0001) and decreased toward control rates with hypoglycemia in period 3 (HI-hypoG-euAA 9.0 ± 1.0 mg·kg⁻¹·min⁻¹, P = 0.01 vs. C value). Fetal glucose utilization rate increased significantly from the C value with HI in period 2 (C 5.8 ± 0.8 mg·kg⁻¹·min⁻¹, HI-euG-euAA 10 ± 1.3 mg·kg⁻¹·min⁻¹, P < 0.0001) and was not significantly different from the mean control period rate with hypoglycemia in period 3 (7.1 ± 0.9 mg·kg⁻¹·min⁻¹, P = 0.15 vs. C). Similarly, fetal glucose oxidation rate increased with hyperinsulinemia (C 3.1 ± 0.2 mg·kg⁻¹·min⁻¹, HI-euG-euAA 5.4 ± 0.4 mg·kg⁻¹·min⁻¹, P < 0.0001), and when glucose utilization was returned to C values, fetal glucose oxidation rate also significantly decreased almost to C values (HI-hypoG-euAA 4.0 ± 0.3 mg·kg⁻¹·min⁻¹ vs. C 3.1 ± 0.2 mg·kg⁻¹·min⁻¹, P = 0.006).

Net total fetal umbilical AA uptake rate was unchanged between experimental periods (data not shown), although net uptake rates for leucine, isoleucine, and valine were decreased by ~20% in the experimental periods compared with control. As shown in Fig. 5, fetal AA utilization rates increased with hyperinsulinemia for all essential AAs and most nonessential AAs (except taurine, asparagine, citrulline, cystine, tyrosine, and ornithine; P < 0.001). These rates did not change significantly when insulin-induced increases in glucose utilization rate returned to control values.

The isotopic enrichments achieved in this study are shown in Fig. 6. Fetal plasma leucine oxidation rate was increased from control with hyperinsulinemia. When glucose oxidation was returned toward C values, leucine oxidation rate did not significantly change (Fig. 7).

DISCUSSION

This is the first experimental demonstration of the effect of acute fetal hyperinsulinemia on fetal AA metabolism that is independent of both plasma glucose and AA concentrations and simultaneous insulin-induced changes in fetal glucose metabolic rate. Our novel study design allowed the comparison of two fetal hyperinsulinemic periods, one with increased fetal glucose metabolism and one with decreased fetal glucose metabolism, to determine the independent effect of acute fetal hyperinsulinemia on whole body AA utilization and oxidation by the fetus. In period 2 of our study, fetal hyperinsulinemia produced a doubling of fetal whole body glucose uptake, utilization, and oxidation rates. This was accomplished by infusing glucose (dextrose) into the mother, producing an increased rate of transplacental glucose transport to the fetus to maintain the control period fetal plasma glucose concentration. In period 3, fetal glucose supply was reduced by stopping the maternal glucose (dextrose) infusion while maintaining fetal hyperinsulinemia by fetal insulin infusion, allowing fetal plasma glucose concentrations to decrease by ~50%. This resulted in a 30% decrease in fetal glucose utilization rate and a 25% decrease in glucose oxidation rate, whereas insulin concentrations remained elevated. Total fetal AA utilization rates continued at increased rates ~42% above control period rates under these conditions, similar to AA utilization rates that

![Fig. 2. Fetal plasma glucose, insulin, and branched-chain amino acid (BCAA) concentrations under control (hatched bars), HI-euG-euAA (gray bars), and HI-hypoG-euAA (black bars) conditions. *P < 0.01 from control, †P < 0.01 from HI-euG-euAA period.](http://ajpendo.physiology.org/)

*AJP-Endocrinol Metab • VOL 291 • DECEMBER 2006 • www.ajpendo.org*
occurred in period 2 with concurrently increased rates of glucose utilization and oxidation. This independent effect of insulin on AA utilization rates was observed with all of the essential AAs and most of the nonessential AAs. Therefore, because acute insulin-induced increase in fetal AA utilization did not change when the concurrent insulin-induced increase in fetal glucose utilization was prevented, these results demonstrate that insulin-induced fetal AA metabolism is independent of fetal glucose utilization.

To determine whether part of the insulin-induced increase in AA utilization with restricted glucose supply was due to increased AA oxidation, fetal leucine oxidation was measured by infusion of [1-13C]leucine. The plasma KIC enrichment, which is in rapid equilibrium with the intracellular pool of leucine, was used to estimate intracellular leucine oxidation. Our results demonstrated a 1.7-fold increase in leucine oxidation under hyperinsulinemic, euglycemic, euaminoacidemic conditions compared with baseline. There was no significant change in leucine oxidation in response to hyperinsulinemia when glucose oxidation was decreased significantly with fetal hypoglycemia, demonstrating that the effects of insulin on AA oxidation were independent of glucose metabolism in the fetus. Other investigations have indicated that insulin promotes growth partly by increasing glucose oxidation, sparing AA oxidation so that the AAs can be used more exclusively for protein synthesis. Our data indicate otherwise, since leucine oxidation was increased in response to insulin when glucose oxidation was both concurrently increased and then significantly decreased.

Our results also indicate that under acute hyperinsulinemic conditions, leucine is not preferentially oxidized to support fetal energy requirements when glucose is restricted as an energy source. Other carbon compounds must have been oxidized by the fetus in response to insulin to maintain energy balance, since oxygen consumption rate was unchanged. When glucose was restricted as an energy source in our study, glucose oxidation decreased significantly, but not to the desired control period rates we had hoped to achieve. This could account for why we did not see a further increase in leucine oxidation in response to hyperinsulinemia and hypoglycemia and is a limitation of the study. Previous work from our laboratory (10, 13), however, has demonstrated that, in the fetal lamb, glucose oxidation is directly related to the additive effects of fetal plasma glucose and insulin concentrations. In fact, fetal glucose concentrations produce a larger change in glucose oxidation than does the accompanying change in fetal insulin concentration (10). Therefore, we expected that the decrease in glucose concentration during period 3 would remove its effect on glucose oxidation and return glucose oxidation back to control period rates. This was not achieved completely, likely due to several factors. We might not have had a large enough decrease in fetal glucose concentration, a long enough period of hypoglycemia, or a long enough duration of hyperinsulinemia. More likely, however, glucose oxi-
Dissation rate in period 3 might have actually returned to or even been below control period values and was simply overestimated due to the contribution of $^{14}\text{CO}_2$ derived from labeled products of glucose metabolism, such as lactate, fructose, or glycogen, that accumulated during periods 1 and 2. Furthermore, studies performed after prolonged maternal fasting indicate that, over time, leucine oxidation continues to increase as carbon sources for energy are conserved, and phenylalanine hydroxylation increases in response to hyperinsulinemia under maternal fasting conditions (18, 30). In our own previous studies (32), the ratio of leucine oxidation to disposal in fetal sheep was significantly increased after 5 or more days of maternal fasting-induced hypoglycemia. In the present study, however, plasma glucose concentrations and glucose utilization and oxidation rates were at normal values, not reduced as in the fasting studies. Thus AA oxidation in the ovine fetus possibly increases only when fetal glucose utilization rate is reduced below normal values for extended periods.

We found that insulin at pharmacological concentrations increased leucine oxidation. Previous studies in the ovine fetus have shown that pharmacological hyperinsulinemia has either a suppressive effect or no effect on AA oxidation. Liechty et al. (18) demonstrated a suppressive effect of insulin on leucine oxidation, but circulating AA concentrations were not maintained in the study. This proves to be a significant variable because there is a positive correlation between leucine concentration and leucine oxidation rates (21, 26). Furthermore, a study by Milley (26) found that acute fetal hyperinsulinemia suppressed leucine oxidation only when AA concentrations were allowed to decrease and had no effect on leucine oxidation when AA concentrations were maintained. Even with euaminoacidemic conditions, however, Shen et al. (29) demonstrated a suppressive effect of insulin on phenylalanine hydroxylation under conditions of fetal hyperinsulinemia, eu-glycemia, and euaminoacidemia. The variability in findings among these studies could be attributed to subtle differences in study design, such as duration and concentration of pharma-
cologal insulin used as well as which AA is measured to represent overall AA kinetics, since differences in the metabolism of phenylalanine and leucine have been described (3). Though fetal studies have demonstrated variable results, several postnatal animal and human studies have shown consistently that simultaneous intravenous infusion of insulin and AAs promotes AA oxidation, particularly of leucine. Leucine oxidation is increased two-fold in pigs treated acutely with low and high insulin concentrations under euglycemic and euamniocidic conditions (33). Human studies have demonstrated that hyperinsulinemia suppresses leucine oxidation when AA concentrations are not maintained (1, 8) and increases leucine oxidation when AAs are simultaneously infused (3–5, 9, 15, 23, 35). One possible mechanism for this finding could be that insulin has been shown to activate the enzyme α-keto acid dehydrogenase, which oxidizes KIC to CO2 in adipocytes (7).

W. W. Hay, Jr.); Trainee L. D. Brown) and National Institute of Diabetes and

GRANTS

performed in this study.

ACKNOWLEDGMENTS

We thank Dr. Gary Zerbe for assistance with the statistical analyses performed in this study.

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