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Umbilical V-A Differences of Acetoacetate and β-Hydroxybutyrate in Fed and Starved Ewes[1] (37915)

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Recent investigations have shown that, although glucose is important in fetal sheep metabolism, the rate of its provision from the placenta is inadequate to meet all of the substrate requirements of oxidative metabolism in near-term fetuses of well-nourished ewes (1–3). Similarly, fructose (1), glycerol, and medium- and long-chain fatty acids (4) have been shown not to be supplied in significant amounts to such fetuses. The present report describes the results of an investigation of the umbilical uptake of acetoacetate and β-hydroxybutyrate during adequate maternal nutrition and during starvation.

Materials and Methods. Surgical preparation. Six Dorset and Western ewes were studied at gestational ages of 113–147 days. They were prepared for study by placement of indwelling polyvinyl catheters into an umbilical vein, fetal pedal artery, and maternal femoral artery as previously described (2, 3). All catheters were exteriorized to a flank pouch. Ampicillin (500 mg) was instilled into the amniotic cavity at surgery, and procaine penicillin (300,000 U) and streptomycin sulfate (0.5 g) were given intramuscularly to the ewes once daily for 3 postoperative days.

Experimental Procedure. Ewes were fasted for 2 preoperative days. Postoperatively, they were offered dehydrated alfalfa pellets containing 17% crude protein (Farmers Marketing Association, Denver, Colorado) *ad lib.* Following 4–7 days of the alfalfa diet, ewes were begun on a starvation regimen that continued for as long as 13 days.

Studies were performed at 1- to 3-day intervals from the first postoperative day until the ewe delivered or the catheters failed. During each study period, 1.6-ml blood samples were withdrawn simultaneously from the three catheters into dry plastic syringes. Of each sample, 0.5 ml was used for glucose assay and 0.8 ml for determination of the ketones.

Analyses. Blood for plasma glucose determination was immediately centrifuged and the plasma analyzed in triplicate by a Beckman Glucose Analyzer. Blood for ketone assay was immediately deproteinized with ice-cold perchloric acid and analyzed by the enzymatic fluorometric micromethod of Olsen (5).

Linear regression equations were calculated by the least-squares method and tested for significance by the *F*-ratio test.

Results. Twenty-eight studies were performed on the six animals. Table I presents the mean maternal and fetal arterial concentrations of glucose and keto acids. Maternal arterial keto acid concentrations increased with the development of starvation-induced hypoglycemia. Figure 1 illustrates the inverse relationship between maternal arterial plasma glucose concentrations and maternal arterial blood acetoacetate and β-hydroxybutyrate concentrations which occurred over the range of

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TABLE I. Maternal and Fetal Arterial Concentration of Glucose, Acetoacetate, and β-Hydroxybutyrate For Fed and Fasted Ewes.

	Maternal arterial conc. Mean ± SEM		Fetal arterial conc. Mean ± SEM	
	Fed $N = 12$	Fasted $N = 6$	Fed $N = 12$	Fasted $N = 16$
Glucose in plasma (mg/100 ml)	63.1 ± 2.0	40.6 ± 2.2	21.7 ± 1.2	17.3 ± 0.6
Acetoacetate in whole blood (mM)	0.0903 ± 0.025	0.328 ± 0.029	0.035 ± 0.004	0.048 ± 0.005
β-Hydroxybutyrate in whole blood (mM)	0.602 ± 0.072	1.208 ± 0.123	0.082 ± 0.003	0.118 ± 0.008

maternal nutrition studied. Despite elevated maternal concentrations of acetoacetate during starvation, the umbilical venous − umbilical arterial concentration differences of acetoacetate (ΔAcAc, mM) did not change significantly. The mean ΔAcAc for all studies was 0.0065 mM (95% C.L. 0.005, 0.008). However, the umbilical

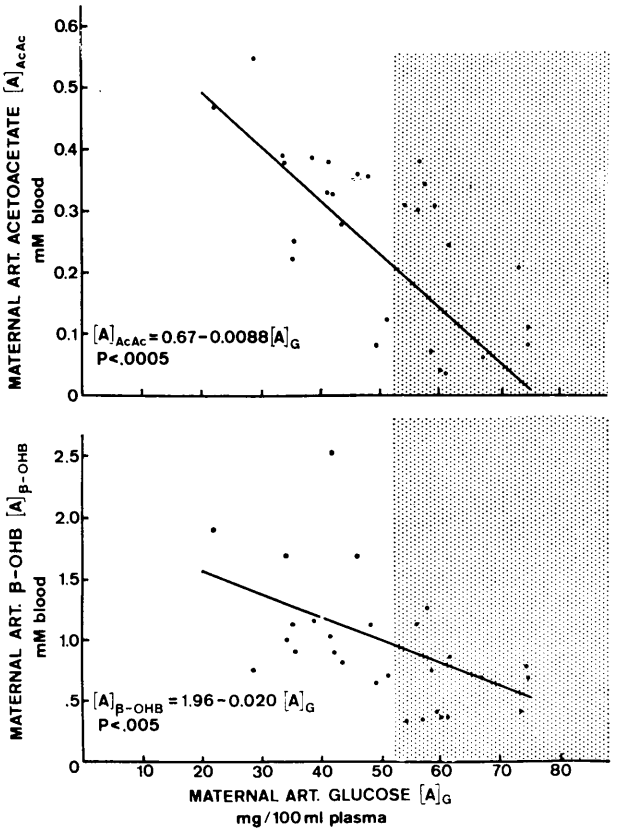

FIG. 1. Maternal arterial whole-blood acetoacetate (top) and β-hydroxybutyrate (bottom) concentrations versus material arterial plasma glucose concentrations observed during periods of starvation and adequate nutritional intake. The shaded regions indicate the plasma glucose concentration (mean ± 2 SD) for 78 observations in ewes fed the same diet *ad lib.* (12).

venous − umbilical arterial concentration differences of β-hydroxybutyrate (Δβ − OHB, m*M*) increased significantly as maternal concentrations of β-hydroxybutyrate ($[A]_{\beta\text{-OHB}}$, m*M*) rose, according to the equation:

$$\Delta\beta - \text{OHB} = -0.00090 + 0.0039\ [A]_{\beta\text{-OHB}}, \qquad P < 0.025.$$

This relationship is illustrated in Fig. 2.

Discussion. Previously reported investigations in our laboratory have shown that the extent to which glucose can serve as a fuel for aerobic metabolism in the fetus depends upon the maternal blood glucose concentration (1–3). During the maternal fed state, glucose can supply approximately 50% of the substrate for aerobic fetal metabolism. When the ewe is starved and becomes hypoglycemic, glucose can contribute only about 20% of the fuel required to account for the umbilical uptake of oxygen. To determine the nature of the balance of the substrate oxidized, we have examined umbilical venous − umbilical arterial differences of fructose (1), glycerol, and free fatty acids of carbon-chain-length six or greater (4) in the fed and/or fasting state and found that the umbilical uptake of these potential fuels does not contribute significantly to the metabolic needs of the fetus. This report expands the list of substrates which are not taken up in appreciable quantities by the umbilical circulation during either adequate maternal nutrition or starvation.

Blood concentrations of ketones have been shown to rise during acute fasting in mammals, birds, and insects (6–8). Reports of the effect of prolonged fasting on blood ketone concentrations, however, are available only for man, in whom whole-blood acetoacetate and β-hydroxybutyrate concentrations rise rapidly during the first several days of fasting and thereafter rise more slowly throughout the 6 weeks of starvation observed (9–11). In man, the ratio of blood concentrations of acetoacetate:β-hydroxybutyrate increased during starvation. The reported effects of acute fasting on unstressed sheep are similar to those observed in man: (a) both acetoacetate and β-hydroxybutyrate concentrations increase during the first 72 hr of fasting, and (6) the ratio acetoacetate: β-hydroxybutyrate is also increased (7). In the present study, arterial blood ketone concentrations were observed to increase

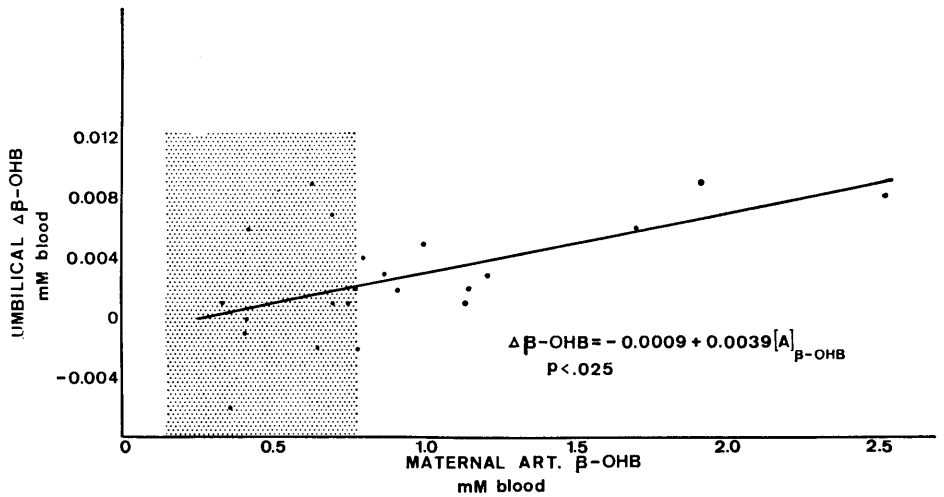

FIG. 2. Umbilical venous − umbilical arterial whole-blood differences in β-hydroxybutyrate concentration versus maternal arterial whole-blood β-hydroxybutyrate concentrations that occur over the range of maternal nutritional intake studied. The shaded region indicates the maternal arterial β-hydroxybutyrate concentration (mean ± 2 SD) for well-nourished ewes observed in this study.

throughout the first 5–7 days of fasting and then to remain stable at an elevated level. Moreover, the ratio of acetoacetate:β-hydroxybutyrate remained elevated throughout 13 days of fasting. The values for sheep whole-blood acetoacetate concentration obtained in the present study are similar to values reported by Katz and Bergman (7) in unstressed pregnant ewes during the fed state (0.07 ± 0.02 m*M*) and after 72 hr of fasting (0.38 ± 0.16 m*M*).

Despite the development of elevated maternal blood levels of acetoacetate during fasting, the umbilical ΔAcAc remains small. The mean umbilical venous-arterial O_2 difference for a comparable group of animals was 1.53 m*M* (3). Using these data, a stoichiometric ratio of the acetoacetate-to-O_2 differences can be used to calculate what ΔAcAc would be needed if acetoacetate were to account for 10% of the umbilical O_2 uptake. If acetoacetate is completely oxidized according to the equation:

$$1\ CH_3COCH_2COOH + 4\ O_2 \rightarrow 4\ CO_2 + 3\ H_2O,$$

the AcAc that would be required for acetoacetate to account for 10% of the umbilical O_2 uptake can be calculated as:

$$\text{AcAc} = \frac{0.1 \times 1.53\ \text{m}M}{4} = 0.038\ \text{m}M.$$

This ΔAcAc is approximately five times larger than the value observed in the present study. Thus, umbilical uptake of acetoacetate may supply about 2% of the sheep fetal oxidative substrate.

Beta-hydroxybutyrate concentration also rose in maternal blood during fasting. Associated with the higher maternal β − OHB levels was an increase in the concentration difference across the umbilical circulation of β − OHB during maternal fasting. Nevertheless, the amount of β − OHB taken up by the umbilical circulation can account for only a small fraction of the umbilical O_2 uptake even in starvation.

Calculations similar to those above reveal that a Δβ − OHB of 0.034 m*M* would be required for β − OHB to account for 10% of the umbilical O_2 uptake. The Δβ − OHB values obtained in the present study were smaller than 0.034 m*M* by a factor of 30 during the fed state and by a factor of 3 during starvation. Thus, even in starvation, the umbilical uptake of β-hydroxybutyrate might provide only 3% of the total fuel for oxidative metabolism.

Simmons *et al.* (12) have reported that maternal starvation is associated with two phases of fetal metabolic adjustment. The first phase, occurring during the first week of maternal starvation, is characterized by a fetal glucose concentration which is stable, after an initial fall, probably because of gluconeogenesis from protein. Continuing stable fetal glucose concentrations during the second phase (after 7 days) were not due to increased protein catabolism. The observations of umbilical ΔAcAc and Δβ − OHB made in the present study during the second week of maternal starvation indicate that together the umbilical uptake of these ketones does not contribute more than 5% of the fetal fuels during the second phase of maternal starvation.

In the present investigation, the Δβ − OHB, but not the ΔAcAc, was observed to increase with increasing maternal blood concentrations of the respective ketone. A similar dependence of the arteriovenous difference of ketones across an organ upon the arterial concentration has been observed for dog kidney (13) and rat brain (6). Moreover, Šabata *et al.* (14) reported that total blood ketones in the human umbilical vein are proportional to the maternal venous ketone concentration and that the umbilical artery blood ketone concentration was correlated with the umbilical vein concentration, expressed by a linear regression equation with slope less than 1.0. This implies that the human umbilical venous-arterial concentration difference for total ketones increased with increasing maternal concentrations.

Thus, glucose and amino acids (15) remain the major metabolic fuels of the sheep fetus so far identified, together accounting for approximately 80 of the fetal O_2 consumption.

Conclusions. (a) Starvation in the preg-

nant ewe is associated with an increase in maternal blood concentrations of acetoacetate and β-hydroxybutyrate.

(b) Despite elevated maternal concentrations of acetoacetate, the umbilical venous-arterial blood acetoacetate difference does not change during maternal starvation. However, rising maternal levels of β-hydroxybutyrate are associated with increasing umbilical venous-arterial β-hydroxybutyrate concentration differences.

(c) Neither acetoacetate nor β-hydroxybutyrate umbilical uptake is used by the sheep fetus as a major fuel of aerobic metabolism, even during maternal starvation.

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