A-V Differences of Free Fatty Acids and Glycerol in the Ovine Umbilical Circulation[1] (35999)

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The nature of the metabolic fuels used by the mammalian fetus *in utero* has long been of interest. Recent investigations (1) have shown that glucose is a significant metabolite of the sheep fetus but does not account for the total oxygen consumption. It would, therefore, be of interest to determine what roles various other substances play in fetal nutrition.

The present investigations were carried out to answer several questions: (i) What are the normal blood concentrations of free fatty acids and glycerol in the unstressed sheep fetus? (ii) Are there significant venous-arterial concentration differences across the maternal uterine or fetal umbilical circulations? and (iii) Can fetal lipolysis be stimulated by administration of lipolytic agents to the fetus *in utero* or by starvation of the mother?

Previous studies on the roles of free fatty acids and glycerol in fetal metabolism have been performed only in the acute animal preparation (2–6). Because of possible metabolic alterations caused by surgical stress, all studies in the present investigation were carried out on unstressed, unanesthetized sheep at least 24 hr after surgery had been performed.

Materials and Methods. Surgical preparation. Seven pregnant Dorset and Western ewes were studied. The gestational ages of the fetuses ranged from 125 to 146 days. Surgery was performed under intravenous pentobarbital sodium sedation and spinal anesthesia. Polyvinyl catheters were placed in the maternal uterine vein (V), maternal femoral artery (A), and fetal umbilical vein (v) according to techniques previously described (7), and in the fetal femoral artery (a) and fetal femoral vein. After isolation and catheterization of the appropriate pedal vessels of the fetus, the catheters were advanced proximally and secured with suture and tissue adhesive as previously described (7). The foot was then replaced in the uterus and the uterine incision was closed. The free ends of all catheters were closed with metal pins and the catheters were brought through a subcutaneous tunnel to the flank of the ewe. All animals were given a 24-hr recovery period before studies were begun. For the group as a whole, the studies were carried out between postoperative days 2 and 14.

Sampling. All sheep were allowed food and water in a restraining pen for at least 3 hr before sampling was begun. (Initial studies had shown high blood concentrations of free fatty acids in the ewe immediately on arrival in the laboratory, with stable blood concentrations established only after 1 to 2 hr in the pen.) Catheters were flushed with heparin solution (1000 units/ml) only at the termination of the day's sampling, with no heparin being infused during the 20-hr period before sampling. Samples were drawn into dry plastic syringes from the fetal artery, umbilical vein, maternal femoral artery, and uterine vein at 2-hr intervals. Two to four samples were collected per day. All samples were obtained in duplicate, and two determinations were done on each sample.

Fasted animals were treated in the same manner, except that food was withheld from 1 to 7 days before sampling. Water was allowed *ad libitum*.

Norepinephrine-treated fetuses received 50

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TABLE I. Blood Free Fatty Acid and Glycerol Concentrations in Six Fed Pregnant Ewes and Their Fetuses.

Vessel	Conc of FFA (μEq/liter; mean ± SEM)	No. of observations	Conc of glycerol (μmoles/liter; mean ± SEM)	No. of observations
Umbilical artery	41 ± 3	37	34 ± 4	37
vein	42 ± 3	38	39 ± 4	39
Maternal artery	328 ± 45	35	41 ± 4	35
vein	326 ± 39	36	40 ± 4	36
Umbilical v-a	2 ± 2	36	6 ± 1	37
Maternal V-A	10 ± 8	32	2 ± 1	32

μg of norepinephrine (Levophed, Winthrop) (equiv to 25 μg of the base)/kg of estimated fetal weight as an intravenous infusion via the fetal femoral vein catheter. In retrospect, by extrapolation from the birth weight to obtain the expected weight on the day of infusion [the extrapolation was based upon data about the growth of fetal sheep which are available in the literature (8)] the dose of norepinephrine was calculated to average 46 μg/kg. The infusion was given in a total volume of 10 ml of 0.9% saline solution over a 5-min period. Samples were obtained 1 to 2 hr before and 2 min before the beginning of infusion, and 10 and 30 min after the beginning of infusion. Five such infusions were performed in two fetuses.

Analyses. Free fatty acid concentrations in whole blood were determined according to the method of Dalton and Kowalski (9), using the Technicon AutoAnalyzer. Short chain acids (*i.e.*, acetic through valeric acids) are not detected by this method. Whole blood, 0.4 ml, was used rather than serum, since fatty acid added to whole blood was found, both in our laboratory and by Itaya and Ui (10), to be recovered as quantitatively as in the case of fatty acid added to serum. Extractions were carried out immediately after the sample was drawn.

Glycerol concentrations in whole blood were determined on 0.1-ml samples according to the enzymatic fluorometric method of Laurell and Tibbling (11). Deproteinization with 0.087 *M* zinc sulfate and 0.042 *M* barium hydroxide was done immediately and the supernatants were frozen.

Results. Tables I, II, and III show the data obtained on free fatty acids and glycerol in fed, fasted, and norepinephrine-treated animals. No significant venous–arterial differences were found for free fatty acids in the maternal uterine or fetal umbilical circulations. However, the umbilical vein–artery difference of glycerol concentration in the fetuses of fed animals was significant at $p < .001$.

Fasted animals, when compared with fed animals, showed higher concentrations of free fatty acids in the fetal artery, umbilical vein, maternal artery, and uterine vein.

TABLE II. Blood Free Fatty Acid and Glycerol Concentrations in Three Fasted Pregnant Ewes and Their Fetuses.

Vessel	No. of observations	Conc of FFA (μEq/liter; mean ± SEM)	Difference from fed animals (Table I) p value	Conc of glycerol (μmoles/liter; mean ± SEM)	Difference from fed animals (Table I) p value
Umbilical artery	5	73 ± 9	$p < .001$	46 ± 3	$.02 < p < .05$
vein	4	74 ± 13	$.02 < p < .05$	55 ± 9	$.1 < p < .2$
Maternal artery	5	577 ± 100	$.02 < p < .05$	79 ± 12	$.001 < p < .01$
vein	5	584 ± 94	$.01 < p < .02$	88 ± 21	$.02 < p < .05$

Venous–arterial differences did not change significantly. Glycerol concentrations were also elevated in the fetal artery and in the maternal artery and vein (see Table II for p values).

The mean change in blood free fatty acid and glycerol concentrations in the fetal artery of norepinephrine-treated fetuses between 2 min before and 10 min after the beginning of infusion was significant, with p between .001 and .01 for free fatty acid and between .02 and .05 for glycerol (Table III). By 30 min, no significant differences were noted. Significant differences in the maternal circulation were not observed. Also, venous–arterial differences did not change significantly. Fetal mean arterial blood pressure was measured in one experiment during a norepinephrine injection. The blood pressure rose from a base line of 46 to 90 mm Hg beginning 15 sec after the start of the infusion, with maximum pressure attained by 2.5 min.

Discussion. No significant venous–arterial difference of free fatty acid was found in fetuses of fed, unstressed animals, and the mean venous–arterial difference of glycerol was found to be 6 ± 1 μmoles/liter (mean ± SEM).

The mean umbilical vein–artery difference of oxygen in the type of preparation used in this study is 1.8 mmoles/liter (1). Using this datum, a ratio of the glycerol to oxygen venous–arterial difference can be calculated to determine what proportion of the fetal oxygen consumption would be accounted for if all the umbilical glycerol uptake were completely metabolized to carbon dioxide and water.

The quantity of glycerol transferred to the fetus is equal to the umbilical venous–arterial difference multiplied by the umbilical blood flow, or

$$\dot{Q}_{\text{Glycerol}} = (\text{v–a})_{\text{Glycerol}} \times f = 6\ \mu\text{moles/liter} \times f. \quad (1)$$

Similarly,

$$\dot{Q}_{\text{Oxygen}} = (\text{v–a})_{\text{Oxygen}} \times f = 1800\ \mu\text{moles/liter} \times f. \quad (2)$$

If glycerol is completely metabolized, according to the equation,

$$2CH_2OHCHOHCH_2OH + 7O_2 \rightarrow 6CO_2 + 8H_2O, \quad (3)$$

1 mole of glycerol requires 3.5 moles of oxygen for conversion to carbon dioxide and water. Six micromoles of glycerol would therefore require 6 × 3.5 or 21 μmoles of oxygen.

The proportion of the fetal oxygen uptake which would be used for complete oxidation of the glycerol transferred may be represented by a glycerol–oxygen ratio, in this case, 21:1800 = 0.012.

From a similar consideration of glucose venous–arterial differences, the glucose–oxygen ratio has been found to equal 0.47 ± 0.03 (1). A maximum of half of the fetal oxygen uptake is therefore accounted for by glucose metabolism, and only a small proportion (1.2%) can be accounted for by glycerol metabolism. If the placenta were to supply a quantitatively important amount of free fatty

TABLE III. Change in Blood Free Fatty Acid and Glycerol Concentrations with Fetal Norepinephrine Administration.[a]

Vessel	FFA (μEq/liter; mean change ± 1 SEM)	Glycerol (μmoles/liter; mean change ± 1 SEM)
Umbilical artery	↑ 10 ± 2	↑ 14 ± 4
vein	↑ 8 ± 4	↑ 16 ± 5
Maternal artery	↓ 19 ± 18	↓ 7 ± 6
vein	↓ 12 ± 19	↓ 3 ± 6
Umbilical v-a	↓ 2 ± 3	↑ 2 ± 1
Maternal V-A	↑ 8 ± 7	↑ 4 ± 4

[a] Ten minutes after beginning of injection.

acids to the fetus, this amount could have been easily detected by the present method. Assume, for example, a supply rate of palmitic acid sufficient to account for 10% of the fetal oxygen consumption. In such a case, the umbilical vein concentration would have to be 20% higher than the umbilical artery concentration to account for this amount of transfer. Therefore, long-chain free fatty acids and glycerol do not appear to constitute a significant proportion of the metabolic fuel of the sheep fetus *in utero*.

Previous studies have shown pregnant ewes to have free fatty acid concentrations of between 400 and 1000 μEq/liter of plasma (4, 5). These high values may be due to the fact that the samples were obtained from the animal on the operating table. Studies on unanesthetized ewes in which blood samples were obtained through an indwelling jugular catheter gave glycerol concentrations similar to ours (12, 13). Our data show that fasting significantly elevates the maternal free fatty acid and glycerol concentrations and the concentrations in the fetus; however, venous–arterial differences are not significantly changed, indicating that no increase in transfer occurred. Thus, it appears that maternal starvation can influence fetal lipolysis, perhaps as a consequence of decreased availability of glucose from the maternal circulation.

While considerably higher concentrations of free fatty acid and glycerol may have obtained within the first 10 min following the norepinephrine infusion, it is clear that, in these studies on unanesthetized, unstressed pregnant sheep, norepinephrine infusion significantly increased plasma free fatty acid and glycerol concentrations. This result is in contrast to the findings of Van Duyne *et al.* (6) on sheep fetuses studied under acute operative stress.

Conclusions. 1. Measurement of venous–arterial differences across the umbilical circulation of the sheep fetus *in utero* and across the maternal uterine circulation in the chronic animal preparation shows no significant passage of free fatty acids to the fetus. This is true despite relatively high maternal blood free fatty acid concentrations. There is a small venous–arterial difference of glycerol across the umbilical circulation; this is, however, sufficient to account for only a small portion of the fetal oxygen consumption due to substances other than glucose. Free fatty acids and glycerol therefore do not appear to constitute a significant part of the metabolic fuel supplied by the mother to the sheep fetus *in utero*.

2. The fetuses of fasted ewes show an elevation of blood free fatty acid and glycerol concentrations. Measurements of venous–arterial differences across the umbilical circulation indicate that this free fatty acid and glycerol rise is of fetal origin.

3. Fetal infusion of norepinephrine results in lipolysis in the fetus, as evidenced by elevation of fetal blood free fatty acid and glycerol concentrations.

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