Feto-Placental Steroid Metabolism in Growth Retarded Human Fetuses[1]

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ABSTRACT. The goal of the study was the determination of the relative roles of the placenta and the fetus in causing low serum estriol (E_3) levels in women bearing fetuses with intrauterine growth retardation (IUGR). Umbilical venous levels of E_3 and dehydroepiandrosterone sulfate (DHAS) were measured in 31 samples from fetuses with IUGR, 21 of whom were vaginally delivered and 10 who were delivered by cesarean section. In addition, estrone (E_1) and estradiol (E_2) were measured in 11 of the samples. The results were compared with 11 samples from cesarean section delivered control term infants and 54 samples from vaginally delivered control infants. The vaginally delivered IUGR group had a significantly lower mean umbilical venous DHAS level than did their control group (2128 ± 158 ng/ml SEM *versus* 2645 ± 130, $p < 0.05$). Both the vaginally delivered and cesarean section delivered IUGR infants had umbilical venous E_3 levels significantly lower than in their control groups (70 ± 10 ng/ml SEM *versus* 144 ± 10, $p < 0.001$, and 46 ± 11 ng/ml SEM *versus* 136 ± 23, $p < .01$, respectively). Umbilical venous E_1 and E_2 levels were not different from the control values. E_1, E_2, E_3, and DHAS were measured in eight maternal venous samples obtained from mothers bearing fetuses with IUGR. In comparison with 11 control mothers, only E_3 was significantly different (10.7 ± 3.0 ng/ml SEM in mothers with IUGR fetuses *versus* 25.0 ± 4.9 in control mothers $p < 0.01$). The study provides evidence for reduced DHAS secretion in one group of the fetuses with IUGR, and no evidence for decreased placental conversion of DHAS to the estrogens E_1 and E_2. The significantly low E_3 values in both umbilical and maternal samples are postulated to result not only from the reduced fetal adrenal DHAS secretion, but also underactive 16α-hydroxylase activity in fetal liver or low efficiency of 16α-OH-DHAS, relative to DHAS, as a substrate for placental conversion to an estrogen. (*Pediatr Res* 20: 166–168, 1986)

Abbreviations

IUGR, intrauterine growth retardation
DHAS, dehydroepiandrosterone sulfate
E_1, estrone
E_2, estradiol
E_3, estriol

Women bearing fetuses with IUGR frequently have low serum E_3 levels in late gestation (1, 2). One origin of the low E_3 production in these pregnancies may be reduced secretion of fetal adrenal cortex-derived neutral steroid precursors of estriol synthesis in the placenta (3). However, there is evidence that placental conversion of the neutral precursors to estrogen may be low in association with a fetus with IUGR (4). In order to investigate the relative importance of these two possible causes of low E_3 production, umbilical venous, and maternal venous neutral steroid and estrogen levels were assessed in a series of pregnancies in which the fetus had IUGR.

MATERIALS AND METHODS

Patients. All pregnant women studied in this investigation were hospitalized in the Oregon Health Sciences University Hospital. Fetuses with IUGR were diagnosed when their birth weights were more than 2 SD below the mean for gestational age, as defined by the Babson and Benda (5) fetal growth curves. No fetuses with congenital infections or major anomalies were included in the study.

The maternal diagnoses included pregnancy-induced hypertension, chronic hypertension, chronic abruptio placenta, placenta previa, bifid uterus, and chronic leakage of amniotic fluid. A number of cases had fibrotic placentas with no obvious associated maternal pathologic process. The number of patients in each subcategory were insufficient to allow comparisons of patients in the subgroups.

Umbilical venous blood samples were obtained from 31 fetuses with IUGR, 21 of whom were delivered vaginally and 10 by cesarean section. All but two were 37 wk gestation or older. DHAS and unconjugated E_3 were determined in all samples, and in 11 samples, E_1 and E_2 17β were measured in addition. Six of these 11 samples were from vaginally delivered infants and five from infants delivered by cesarean section. Maternal blood samples were collected a few hours prepartum from eight of the mothers of the latter group of 11 infants for E_1, E_2, E_3, and DHAS concentration measurements. All of these eight pregnancies were 38 wk gestation or more.

Control samples for steroids in umbilical venous serum and maternal venous serum from cesarean section deliveries were obtained from 11 women at term with uncomplicated pregnancies. Each had a weight gain of 20–35 lb, normal blood pressure, did not smoke, and took no medications chronically. Each woman was delivered via elective cesarean section prior to labor. The maternal venous samples were obtained within 12 h of delivery. Control values for umbilical venous steroids of fetuses from women with vaginal deliveries were obtained from 54 samples collected from women with normal pregnancies at term previously reported by Barnhart *et al.* (6).

Steroid assay procedures. The procedure for the DHAS-radioimmunoassay was described by Turnipseed *et al.* (7) except that dextran coated charcoal was used to separate bound from free

steroid. The method for unconjugated E_3 assay was described by Barnhart *et al.* (6), and cortisol was measured as described by Barnhart *et al.* (6).

Unconjugated E_1 was extracted from serum samples with benzene. An antiestrone-6-(CMO) bovine serum albumin antibody E-001 from Steranti Research Ltd. was used for the radioimmunoassay system in a 1:1000 dilution, and dextran-coated charcoal was used for bound and free steroid separation. The cross-reactivities of the E_1 antibody were (E_1—100%): E_2 17β—0.1%, E_3—0.01%, DHA—0.01%, progesterone—<0.01%. Intraassay variability was 2.1% and interassay variability was 13.2%.

Unconjugated E_2 was assayed in serum samples extracted with benzene. A rabbit anti-E_2 antibody (R-14) provided by Dr. L. Don Keith, Veteran's Administration Hospital, Portland, OR was used at a 1:400,000 dilution, and dextran-coated charcoal was used for bound and free steroid separation. The cross-reactivities of the E_2 antibody were (E_2—100%): E_1—2.0%, E_3—0.2%, DHA—0.01%, testosterone—0.06%, cortisol—0.03%. Intraassay variability was 7.0% and interassay variability was 13.0%.

The significance of differences between means was determined by use of Student's *t* test.

RESULTS

Umbilical venous serum levels of DHAS and E_3 in 21 samples from vaginally delivered infants with IUGR and 10 from infants with IUGR delivered by cesarean section are listed in Table 1. In addition, E_1 and E_2 were assayed in six of the vaginally delivered and five of those delivered by cesarean section (Table 1). Values of E_1, E_2, E_3, and DHAS in 11 control umbilical venous samples from infants delivered by cesarean section and values of E_3 and DHAS in 54 samples from vaginally delivered control infants are included in Table 1 for comparison. The umbilical venous E_1 and E_2 values in the IUGR infants are similar to those of the control infants. The umbilical E_3 values in both subgroups of IUGR infants are significantly less than E_3 values in the control groups: IUGR (vaginal) *versus* control (vaginal)—70 ± 10 ng/ml (SEM) *versus* 144 ± 10 ($p < 0.001$); IUGR (cesarean section) *versus* control (cesarean section)—46 ± 11 ng/ml (SEM) *versus* 136 ± 23 ($p < 0.01$). The mean umbilical venous DHAS value in the vaginally delivered subgroup of IUGR infants is significantly less than that in the control vaginally delivered group: 2128 ± 158 ng/ml (SEM) *versus* 2645 ± 130, respectively ($p < 0.05$).

Table 2 shows the maternal venous levels of E_1, E_2, E_3, and DHAS in mothers of eight of the 11 fetuses with IUGR listed in Table 1 who had the same serum steroids assayed. The steroid values in control prepartum samples from the 11 control women

Table 1. *Steroid concentrations in umbilical venous serum samples of IUGR and control infants**

	n	E_1	E_2	*n*	E_3	DHAS
Control						
Vaginal				54	144 ± 10†	2645 ± 130‡
Cesarean section	11	32.1 ± 6.4	13.7 ± 1.7	11	136 ± 23§	2034 ± 220
IUGR						
Vaginal	6	31.6 ± 6.2	14.8 ± 2.8	21	70 ± 10†	2128 ± 158‡
Cesarean section	5	25.7 ± 7.6	10.6 ± 2.1	10	46 ± 11§	1508 ± 213

* Values recorded as ng/ml ± SEM.

Significance of differences between control and IUGR groups: † $p < 0.001$; ‡ $p < 0.05$; § $p < 0.01$.

Table 2. *Steroid concentrations in maternal venous blood samples from pregnancies with IUGR and normal wt newborn infants**

	n	E_1	E_2	E_3	DHAS
Control	11	15.2 ± 2.6	26.5 ± 2.9	25.0 ± 4.9†	594.7 ± 119
IUGR	8	8.6 ± 1.6	21.1 ± 3.0	10.7 ± 3.0†	911.7 ± 227

* Values recorded as ng/ml ± SEM.

† IUGR *vs* control $p < 0.01$.

delivered by cesarean section are listed for comparison. Only the E_3 level differed in the two groups. The mean value of 10.7 ± 3 ng/ml is significantly lower ($p < 0.01$) in the women carrying fetuses with IUGR than the value of 25.0 ± 4.9 ng/ml in the control women.

DISCUSSION

Adrenocortical function of growth retarded fetuses has long been known to be potentially abnormal. Naeye (8) described the adrenal cortex of the IUGR newborn infant as relatively smaller than other organs, with the fetal zone even more retarded than the permanent zone. In a study by Reynolds and Mirkin (3), newborns with IUGR were shown to have reduced urinary excretion of 16α-hydroxy DHAS, a metabolic product of DHAS which is secreted mainly by the fetal zone of the adrenal cortex. However, these infants had normal urinary levels of the metabolic products of cortisol, 6β-hydroxy cortisol and tetrahydrocortisone, which is secreted mainly by the permanent zone. In another group of IUGR newborns 36 wk gestation or greater studied on the 1st day of life, the serum DHAS levels were found to be significantly lower than in normally grown newborn infants of the same gestational age (7).

Further evidence of disturbed adrenocortical function in the IUGR group comes from reports that the mean maternal serum E_3 levels (1, 2) and maternal urinary excretion of E_3 (9) are lower than expected for the duration of gestation and fetal size in women bearing a fetus with IUGR. As more than 90% of E_3 in a woman in late pregnancy is derived from fetal adrenal-derived neutral steroid precursors, principally DHAS (10), diminished levels of maternal E_3 generally are considered to indicate abnormally low fetal adrenocortical steroid secretion.

In the sequence of biosynthetic steps leading from DHAS to E_3, the DHAS first undergoes 16α-hydroxylation in the fetal liver, then in the placenta undergoes conversion to E_3. The placental enzymes necessary for the biosynthesis of E_3 from 16α-OH-DHAS include sulfatase, 3β-hydroxysteroid dehydrogenase, and aromatizing enzymes. Potentially, a diminished activity of any one of these enzymatic steps could lead to low E_3 production in the fetoplacental unit in the face of normal adrenal DHAS secretion. Hepatic 16α-hydroxylase has been reported to be low, in association with normal fetal DHAS production, in only one instance (11). The fetus had cirrhosis, and the mother had increased E_1 and E_2 excretions, and abnormally low E_3 excretion. In normal pregnancies, the placental enzymes are considered to have a large capacity, and not to be rate limiting in estrogen biosynthesis (12). Their activities show no correlation with urinary estrogen levels over a wide range of estrogen excretions (12).

In abnormal placentas, steroid metabolizing enzymes assessed *in vitro* have been variously reported to be low (4, 13) or increased (14) in mothers with toxemia, or in mothers bearing IUGR fetuses. There are recent studies indicating that *in vivo* placental conversion of DHAS to E_1 and E_2 is depressed in women with fetuses showing IUGR (15–17). In addition, diminished clearance of DHAS by these patients is shown by a prolonged half-life of administered DHAS (18, 19). The placental clearance of DHAS by conversion to E_2 appears to be a direct reflection of

uteroplacental blood flow (20), and the reduced clearance in women bearing fetuses with IUGR is most probably related to the high frequency of vascular pathology (21) and the reduced blood flow (22) found in the placentas of these pregnancies. Thus, in pregnancies where uteroplacental blood flow is reduced as part of a pathologic process, the placental role in estrogen production may be limiting in proportion to the decrease in placental blood perfusion, as well as limiting because of the pathologic damage to the placenta itself.

The results of the present study indicate that in the IUGR groups studied there was some, although inconsistent, evidence that fetal adrenocortical function was disturbed. There were significantly lower umbilical venous DHAS levels in the vaginally delivered IUGR infants than in the normally grown control group of vaginally delivered infants, suggesting a reduced fetal adrenocortical secretion of DHAS in the subgroup with IUGR. The mean umbilical serum DHAS level in the IUGR subgroup delivered by cesarean section was somewhat lower than in their control group of normally grown cesarean section delivered infants, but the difference did not reach the level of significance.

The reductions in umbilical venous E_3 levels in the IUGR groups, as compared to the control groups, were more consistent and were of greater magnitude than were the reductions in DHAS levels. This difference in the patterns of DHAS and E_3 reductions suggested that there was not only a fetal factor, but also a placental factor in the causation of the low umbilical venous and maternal venous E_3 levels. To investigate the role of a general placental aromatizing enzyme underactivity as a cause of the low umbilical venous E_3 levels, umbilical venous E_1 and E_2 levels were measured in a subset of 11 cord blood samples, and E_1, E_2, E_3, and DHAS were measured in maternal venous samples obtained from the mothers of eight of these 11 infants. The umbilical venous E_1 and E_2 levels were in the normal range. The maternal E_3 levels were found to be significantly low. The maternal venous E_1 and E_2 levels were lower and the DHAS levels were higher in the IUGR group, but the differences from the control group did not reach significant levels. However, the differences suggest an underactivity of placental aromatizing enzymes in mothers of IUGR fetuses.

The normal umbilical venous E_1 and E_2 levels would indicate that there was not a general reduction of the placental enzymes responsible for conversion of neutral fetal steroids to estrogens. It is possible that the neutral steroid precursor of E_3, 16α-OH-DHAS, is a less efficient substrate for the placental enzymes than is DHAS, the neutral steroid precursor of E_1 and E_2. There is no *in vitro* evidence for this possibility, although it has been suggested by *in vivo* studies in women in late pregnancy (23).

Another possible explanation for the discrepancy in umbilical DHAS and E_3 levels is a reduced fetal hepatic 16α-hydroxylase activity, as well as reduced DHAS secretion, in fetuses with IUGR. Nothing is known about the control of this enzyme in the fetus. The activity of this enzyme in women in late pregnancy is increased, probably as a result of the high circulating estrogen levels (24). However, in the human fetus, placental sulfatase deficiency, leading to very low circulating fetal E_1 and E_2 levels, is not associated with low hepatic 16α-hydroxylase activities (25). In addition, the fetuses we studied had normal serum E_1 and E_2 levels. Thus, there is no evident hormonal basis for reduced fetal hepatic 16α-hydroxylase activity. However, the liver in fetuses with IUGR is known to be particularly retarded in its growth (8), and certain hepatic enzymes, *e.g.* the gluconeogenic system, may be underactive in IUGR (26, 27). Definitive proof for the hypothesis that hepatic 16α-hydroxylase activity is reduced in the IUGR group is not available and would require the demon-stration of low fetal circulating 16α-OH-DHAS levels and low E_3 levels, in relation to the serum DHAS concentrations.

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