Effect of Diabetes in Pregnancy on Rat Maternal Liver Δ^4-3-Ketone-Steroid Reductase Activity in vitro

Increased concentrations of certain corticosteroids and estrogens have been observed in maternal plasma in pregnancy complicated by diabetes[1–5]. The present study investigates the possibility that this situation may be due at least in part to a disturbance in the maternal liver capacity to metabolize steroids as a result of the diabetes.

The Δ^4-3-ketone-steroid reductase activity of liver slices of diabetic pregnant rats was compared to that of normal pregnant rats, using corticosterone as the steroid substrate. This enzyme activity is normally the predominant pathway of corticosteroid metabolism in adult liver tissue.

Methods. Virgin Wistar rats were mated, rendered diabetic by a single i.v. injection of streptozotocin (55 to 60 mg/kg) on days 6, 7, or 8 of pregnancy, and maternal liver obtained from rats at day 22 of gestation, by methods previously described[6].

The Δ^4-3-ketone-steroid reductase activity of liver slices was determined using the in vitro incubation technique and method of assay of URQUHART et al.[7] with slight modification; quadruplicate samples of 200 mg of tissue were incubated with 150 µg of corticosterone in 5 ml of Krebs-Ringer phosphate buffer under 100% oxygen for 30 min. The results are expressed in µg of corticosterone reduced per g liver slices per 30 min of incubation. Specimens of fresh maternal liver were also immediately fixed in 10% formalin and after processing stained with haematoxylin and eosin or periodic acid Schiff reagent for histological studies.

Results. As shown in the Table, the maternal and fetal blood glucose levels were significantly increased in the streptozotocin-injected rats. These rats also gained significantly less weight during pregnancy, had significantly fewer live-born fetuses, and had significantly more apparent resorptions per litter than did controls. Fetal weights were significantly reduced in the diabetic group but the average placental weight was significantly greater than in controls.

Average maternal liver weights were also significantly greater (by 22%) in the diabetic group. The enlargement of the liver was apparently dependent upon the presence of live fetuses in utero; the liver weights in one diabetic rat with no sign of pregnancy at day 22 and in another one with 15 resorptions but no live fetuses at this time were only 9.2 and 10.8 g, respectively.

The Δ^4-3-ketone-steroid reductase activity of maternal liver was significantly less in the diabetic rats, with the average activity being 35% less than in controls; the individual values are shown in the Figure. Values for all 5 diabetic rats with manifest ketonuria were less than that of the mean; these rats also had markedly growth-retarded fetuses, with the average fetal weight being only 2.78 g at day 22 of pregnancy.

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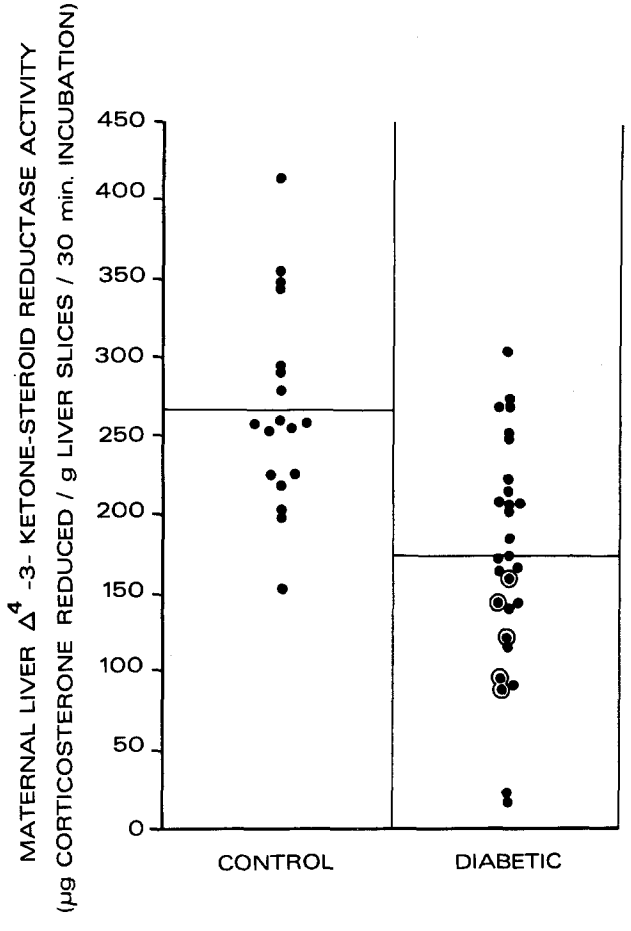
Comparison of findings in diabetic and non-diabetic control rats at day 22 of pregnancy

	Control	Diabetic (injected with Streptozotocin)[a]
Maternal weight gain (g)	117 ± 25 (20)	86 ± 30 (31) t = 3.83 (P < 0.001)
Maternal blood sugar (mg/100 ml)	78 ± 13 (15)	275 ± 28 (31) (P < 0.001)
Fetal blood sugar (mg/100 ml)	61 ± 13 (15)	254 ± 31 (28) (P < 0.001)
Number of live-born fetuses per litter	13.09 ± 2.26 (22)	11.26 ± 3.16 (30) t = 2.42 (P < 0.02)
Number of apparent resorptions per litter	0.55 ± 0.83 (20)	2.00 ± 2.85 (31) t = 22.1 (P < 0.001)
Average placental weight (g)	0.45 ± 0.07 (20)	0.53 ± 0.05 (29) t = 7.24 (P < 0.001)
Average weight of live-born fetuses (g)	4.98 ± 0.39 (20)	3.73 ± 0.81 (30) t = 6.41 (P < 0.001)
Maternal liver weight (g)	12.70 ± 1.50 (20)	15.50 ± 1.98 (31) t = 5.44 (P < 0.001)
Maternal liver Δ^4-steroid reductase activity (µg corticosterone reduced per g liver slices per 30 min incubation)	268 ± 65 (18)	174 ± 74 (28) t = 4.46 (P < 0.001)

Values shown are the mean ± standard deviation. Figures in parentheses are the number of samples. [a] Single injection into tail vein (55–60 mg/kg) on days 6, 7, or 8 of pregnancy.

Light microscopic examination of histological specimens consistently revealed a homogeneous cytoplasm which lacked sites of glycogen deposits in the liver tissue of diabetic rats. Apart from this abnormality which is characteristic of the diabetic condition, no evidence for a direct toxic effect of streptozotocin on the maternal liver tissue was apparent.

Discussion. The liver is the major site of the metabolic processes which inactivate adrenocortical and progestational steroids. A hepatic deficiency such as the decrease in the Δ^4-3-ketone-steroid reductase activity in the maternal diabetic liver observed in the present study could result in the concentrations of the most biologically active forms of steroids being increased not only in the maternal circulation but in the fetal circulation as well, since these steroids pass readily across the placenta[8,9]. Apart from the reduced maternal liver steroid reductase activtiy, the other abnormalities of pregnancy which were observed in the diabetic rats and which were especially pronounced in those with manifest ketonuria, (i.e., decreased number of live fetuses, increased number of fetal resorptions, and marked fetal growth retardation) have been observed by others in non-diabetic pregnant animals treated with exogenous corticosteroids[10–14].



Comparison of Δ^4-3-ketone-steroid reductase activity of maternal liver obtained from day 22 of normal rat pregnancies and from pregnancies in which diabetes had been induced by streptozotocin injection. The horizontal lines indicate the mean values. ⊙, Values for diabetic rats with manifest ketonuria.

It is obviously impossible to determine whether a deficiency in hepatic Δ^4-3-ketone-steroid reductase activity, such as that observed in the pregnant diabetic rats, occurs in the pregnant human diabetic as well. Possibly, hepatic enzymes regulating other aspects of steroid metabolism (e.g., glucuronyl-conjugating capacity) may also be adversely affected by diabetes[5]

It would also be difficult to establish whether there is any relationship between the increased maternal blood levels of corticosteroids and estrogens observed in pregnancy complicated by diabetes[1–5] and the increased incidence of abortion, congenital defects of the fetus and the fetal growth retardation characteristic of this condition[15–20]. It is of interest that apart from diabetes, there is another complication of human pregnancy, intrahepatic cholestasis, with manifest symptoms of liver disfunction (hyperbilirubinemia and pruritus) in which there is coexistence of elevated steroid levels in maternal plasma and increased risk of premature labor and fetal mortality [21–25].

Résumé. La production d'un diabète par injection i.v. de streptozotocine au début de la grossesse chez les rates de Wistar, amène au niveau du foie maternel une diminution du métabolisme et de la corticostérone par l'entremise de la réductase Δ^4-3-cétone-stéroïde, in vitro.

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