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DELAYED EFFECTS OF EXPERIMENTAL MATERNAL DIABETES ON PLASMA CHOLESTEROL LEVEL AND VASCULAR PROSTACYCLIN SYNTHESIS IN THE OFFSPRING*

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The effect of streptozotocin-induced diabetes during pregnancy in rats on prostacyclin synthesis in aorta and heart of offsprings was investigated. Although the aortic and heart syntheses of 6-keto-$PGF1_{\alpha}$ in the offsprings of diabetic rats were not altered at birth, a significant rise in aorta and a decrease in the heart were evident at weaning. At weaning, offsprings of diabetic rats also show a significant rise in plasma cholesterol. These studies show that maternal diabetes might cause effects in the offspring which might become evident in later life.

The metabolic effects of maternal diabetes on the fetus and the neonate have been extensively investigated (1). Recent studies in streptozotocin-induced diabetes in rats showed a decrease in vascular PGI_2 synthesis (2-4). This finding was also confirmed in spontaneously diabetic BB rats (a model of type I of human diabetes) (5), indicating that this was not due to a direct effect of streptozotocin. The decrease in vascular PGI_2 synthesis was observed in humans with adult and juvenile onset diabetes (6,7). However, the effect of maternal diabetes on the vascular prostacylin synthesis in the neonate has not been investigated. Since vascular prostacyclin synthesis will be important in the regulation of blood flow in the fetus and in the neonate (8), it was decided to investigate the effect of streptozotocin induced diabetes in pregnant rats on the vascular prostacyclin synthesis and plasma lipid levels in the offsprings at birth and at weaning. The studies reveal that offsprings of diabetic rats show delayed abnormalities in plasma cholesterol levels and vascular PGI_2 synthesis which become evident only at weaning.

Pregnant Sprague-Dawley rats were obtained from Charles Rivers Laboratories Inc. (Wilmington, Mass.). Animals were fed Purina chow stock diet and water ad libitum. At 1 week of pregnancy rats were randomly separated into control and streptozotocin treatment groups. Rats in the latter group were made diabetic (9,10) by intravenous (tail vein) injection of 50 mg/kg body weight streptozotocin (Sigma Chemical Co, St. Louis, MO) in citrate buffer (0.1 M citric acid and 0.145 M NaCl, pH 4.5). Control rats were injected with an equivalent volume of citrate buffer. The induction of diabetes was confirmed two days later by elevated levels of plasma glucose, total cholesterol and triglycerides. Plasma glucose was measured by the glucose oxidase technique, using a Beckman glucose analizer. Plasma total cholesterol and triglycerides were

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TABLE I: Plasma cholesterol, triglycerides and glucose in pregnant rats after streptozotocin administration

GROUP	CHOLESTEROL (mg/dl)	TRIGLYCERIDES (mg/dl)	GLUCOSE (mg/dl)
CONTROL (4)†	83.25 ± 7.03	218.00 ± 41.80	134.75 ± 3.75
DIABETIC (4)	164.00 ± 16.89*	1090.50 ± 77.28*	531.75 ± 59.70*

†number of animals

Results are presented as $\bar{X} \pm$ SEM

*$p < 0.01$

measured by the methods of the Lipid Research Clinics (11).

At the 20th day of pregnancy, the rats were submitted to a Caesarean section. Blood was collected from the neonates by decapitation (9). Aorta and heart tissues were dissected rapidly and used for prostaglandin studies. Some of the pregnant rats were allowed to reach term and the neonates were kept with their respective mothers until weaning (3 weeks). In the three-week-old offsprings blood was collected through cardiac puncture, and aortic and heart tissues were used for prostaglandin synthesis studies. Blood plasma was used for total cholesterol and triglyceride determinations (11).

In the animals of both groups, the thoracic aorta was quickly dissected, cleared of connective and fatty tissue, and cut into small rings 3-5 mm in length. The hearts were also removed, cleared of blood, and cut into small pieces. Aorta rings and heart pieces were placed individually in 0.5 ml of phosphate-buffered saline (NaCl, 8 g/liter; KCl, 0.2 g/liter; $CaCl_2$, 0.1 g/liter; $MgCl_2\ 2H_2O$, 0.1 g/liter; $Na_2HPO_4.2H_2O$, 1.15 g/liter; KH_2PO_4, 0.2 g/liter; glucose, 1 g/liter, pH 7.2) and incubated at 37°C for 10 min. At the end of the incubation period the solution was removed with a pipette and frozen at -20°C. The concentration of 6-keto-$PGF1_\alpha$ was then measured using radioimmunoassay with anti-sera obtained from Kew Scientific, Inc. (Columbus, OH).

For aortic assays the amount of 6-keto-$PGF1_\alpha$ formed was normalized by the surface area of the aortic ring (10). Surface area was measured after cutting the ring open and laying it flat. Two measurements of length and width were made using a Bausch and Lomb measuring magnifier equipped with a metric scale (one division = 0.1mm). Surface area was calculated by taking the product of the average length and width. For heart assays the amount of 6-keto-$PGF1_\alpha$ formed was normalized by the tissue weight.

Statistical analysis was carried out with the Student's t test, with p-values ≤ 0.05 being considered as statistically significant.

Table I shows the plasma glucose and lipid levels in control and diabetic pregnant rats. In accordance with previous observations (4,5), diabetes caused a significant increase in the level of all these components.

Table II shows the body weight and plasma lipid levels at birth and at weaning of offsprings from control and diabetic rats. The neonates from diabetic rats weighed significantly less ($p < 0.01$) than control neonates. The plasma lipid levels at birth were not different between the two groups, but weaning offsprings of diabetic rats showed a significant rise in plasma cholesterol.

Table III shows the synthesis of 6-keto-PGF1 in hearts and aortas of offsprings from the two groups of animals. The values between the two groups were not different at birth.

TABLE II: Plasma cholesterol and triglycerides and body weight of offsprings of rats treated with streptozotocin during pregancy

	CHOLESTEROL (mg/dl)		TRIGLYCERIDES (mg/dl)		BODY WEIGHT (g)	
GROUP	Newborn	3 weeks	Newborn	3 weeks	Newborn	3weeks
CONTROL	70.15 ± 6.30 (5)†	139.00 ± 37 (4)	54.21 ± 3.72 (5)	64.25 ± 6.39 (4)	5.80 ± 0.19 (13)	31.64 ± 0.88 (4)
DIABETIC	67.61 ± 4.21 (5)	225.83 ± 19.09* (6)	47.89 ± 6.89 (5)	68.50 ± 7.74 (6)	4.30 ± 0.11** (17)	20.08 ± 0.93** (6)

†number of animals

Results are presented as $\bar{X} \pm$ SEM

*$p < 0.02$
**$p < 0.01$

TABLE III: 6-Keto-PGF1_α synthesis in aorta and heart of offsprings of rats treated with streptozotocin during pregnancy

GROUP		Basal 6-Keto-PGF1_α Synthesis	
		AORTA (pg/mm^2/10min)	HEART (pg/mg/10 min)
CONTROL	Newborn	166.25 ± 44.97 (7)†	328.89 ± 47.19 (13)
	3 weeks	210.22 ± 21.61 (4)	92.26 ± 7.68** (4)
DIABETIC	Newborn	183.38 ± 57.56 (8)	289.55 ± 46.92 (17)
	3 weeks	735.08 ± 29.23* (5)	63.28 ± 9.90**# (5)

†number of animals

Results presented as $\bar{X} \pm$ SEM

*$p < 0.01$ in comparison to newborn of diabetic group and to 3 week old rats from control group.
**$p < 0.02$ in comparison to newborns of the same group.
#$p < 0.05$ in comparison to 3 week old rats from control group.

But, at weaning, offsprings of diabetic rats showed a significant ($p < 0.05$) rise in aortic and a decrease in the cardiac synthesis of 6-keto-$PGF1_\alpha$.

The results of this study show that although maternal diabetes does not alter plasma lipid levels and vascular PGI_2 synthesis at birth, it causes some delayed effects which become evident at weaning. The most striking change is the marked increase in plasma cholesterol levels in offsprings of diabetic rats. Since rats are known to be highly resistant to hypercholesterolemia (12), this marked rise in plasma cholesterol indicates that maternal diabetes causes a change in some key parameter of cholesterol metabolism.

Surprisingly, changes in the prostacyclin syntheses in the aorta and heart were in opposite directions. While the heart protacyclin synthesis at weaning in the offsprings of diabetic rats decreased significantly, levels in the aorta increased. In adult rats, streptozotocin-induced diabetes is known to cause opposite changes in vascular prostacyclin synthesis, with increases in heart (13) and decreases in aorta (4,5). The mechanism responsible for these diverse changes is not clear. One possibility to be considered is that the neonates of diabetic mothers are hyperinsulinemic (14). This may be responsible for the increase in aortic PGI_2 synthesis unlike in the adult whose diabetes causes hypoinsulinemia. This might also explain why the heart in the neonate of diabetic rats responds in the opposite direction to that noted in adults (13). Why these effects did not appear in the newborns immediately after birth is not known.

In this connection it is interesting to note that Stuart et al. (15) have shown that PGI_2 synthesis is decreased in umbilical cords of infants from diabetic mothers. Our studies in rat aortas do not confirm their observations.

In our study it is not possible to delineate whether or not the noted changes in many of the parameters are due to the effect of diabetes: a) during the fetal period or b) during suckling. Further studies during different stages of development are needed to determine the exact site and nature of the effect of maternal diabetes. Nevertheless, our studies show that maternal diabetes causes effects in the offspring which may become evident in later life.

REFERENCES

1. Merkatz IR, Adam PAJ. Diabetic pregnancy, a perinatal perspective. New York: Grunne and Stratton, 1979.
2. Harrison HE, Reece AH, Johnson M. Decreased vascular prostacyclin in experimental diabetes. Life Sci 23:351-356, 1978.
3. Gerrard JM, Stuart MJ, Rao GHR, Steffes MW, Mauer SM, Brown DM, White JG. Alteration in the balance of prostaglandin and thromboxane synthesis in diabetic rats. J Lab Clin Med 95:950-958, 1980.
4. Wey H, Subbiah MTR. 6-keto-PGF_1 synthesis in diabetic rat aorta: effect of substrate concentration and cholesterol feeding. Proc Soc Exp Biol Med 171:251-257, 1982.
5. Subbiah MTR, Dietemeyer D. Altered synthesis of prostaglandins in platelet and aorta from spontaneously diabetic Wistar rats. Biochem Med 23:231-235, 1980.
6. Johnson M, Harrison HE, Raferty AT, Elder JB. Vascular prostacyclin may be reduced in diabetes in man. Lancet 1:325-326, 1979.
7. Silberbauer K, Schernthaner G, Siuzinger H, Piza-Katzer H, Winter M. Decreased vascular prostacyclin in juvenile-onset diabetes. N Engl J Med 300:366-367, 1979.
8. Cassin S. Role of prostaglandins and thromboxanes in the control of the pulmonary circulation in the fetus and newborn. Semin Perinatol 4:101-107, 1980.
9. Hassan AS, Subbiah MTR. Effect of diabetes during pregnancy on maternal and neonatal bile acid metabolism in the rat. Proc Soc Exp Biol Med 165:490-495, 1980.
10. Wey H, Subbiah MTR. Altered aortic prostaglandin synthesis in a mild form of diabetes and the influence of dietary cholesterol. J Lab Clin Med 104:312-320, 1984.
11. Lipid and Lipoprotein Analysis. In: LRC Manual of Labs Operation. Washington, DC: NHLI-DHEW publication no. 75-028, U.S. Govt. Printing Office, Vol. 1, 1979.

12. Mathe' D, Lutton C. Le cholesterol. Aspects of dynamiques et metaboliques. J Physiol (Paris) 79:41-97, 1984.
13. Rosen P, Schror K. Increased prostacyclin release from perfused hearts of acutely diabetic rats. Diabetologia 18:391-394, 1980.
14. Oakley NW, Beard RW, Turner RC. Effects of sustained maternal hyperglycemia on the foetus in normal and diabetic pregnancies. Brit Med J 1:466, 1972.
15. Stuart MJ, Sunderji SG, Allen JB. Decreased prostacyclin production in the infant of the diabetic mother. J Lab Clin Med 98:412-416, 1981.

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