and consistent effect. It seems reasonable therefore, in view of the evidence we have presented, to advocate that women should reduce their cigarette consumption or stop smoking not only during pregnancy but also when they are attempting to become pregnant.

We thank Patricia Brown, Claire Henry, Melanie Lawless, and the research assistants, doctors, nurses, and administrative staff working in the clinics for their hard work and loyal support. We received helpful advice and comments from Dr W H James and Dr D Coleman. The Oxford Family Planning Association contraceptive study is funded by the Medical Research Council. During this project Professor Howe was in receipt of an Eleanor Roosevelt International Cancer Fellowship of the American Cancer Society and Dr Westhoff was supported by the Milbank Scholar Program.

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(Accepted 28 February 1985)

Abnormalities in vascular arachidonic acid metabolism in the infant of the diabetic mother

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Abstract

The infant of the diabetic mother has an increased incidence of thromboses in utero and in the neonatal period. In the adult with diabetes a decrease in prostacyclin formation has been suggested as a cause for the atherothrombotic tendency. We therefore evaluated arachidonic acid metabolism in infants of diabetic mothers. Endogenous radioimmunoassayable 6-keto prostaglandin $F_{1\alpha}$ ($PGF_{1\alpha}$) was normal in umbilical vessels obtained from the infants of diabetic mothers whose glucose homeostasis was maintained when compared with control values. Nevertheless, a significant inhibition of vascular production of 6-keto $PGF_{1\alpha}$ was observed in infants born to mothers with raised HbA_{1c} concentrations. A decrease in the concentration of plasma 6-keto $PGF_{1\alpha}$ was also seen in the infants of diabetic mothers when compared with control neonates.

The correlation observed between plasma 6-keto $PGF_{1\alpha}$ concentrations and endogenous vascular prostacyclin formation in the infants of diabetic mothers indicates that the in vitro deficiency of prostacyclin formation reflects a concomitant in vivo abnormality.

Moreover, since prostacyclin functions as an endogenous pulmonary vasodilator, a decrease in plasma concentrations might contribute to the transient respiratory distress observed in infants of diabetic mothers.

Introduction

Abnormalities in the production of platelet thromboxane and vascular prostacyclin have been implicated in the atherothrombotic tendency observed in diabetes mellitus in both humans and animals.¹⁻⁴ The infant of the diabetic mother is recognised as showing an increased incidence of thromboses and thromboembolic phenomena both in utero and in the immediate neonatal period.⁵ The pathogenesis of this complication in infants of diabetic mothers, however, has not been extensively investigated. Our study was designed to evaluate vascular arachidonic acid metabolism in the infant of the diabetic mother. Methods included both radioimmunoassays for 6-keto prostaglandin $F_{1\alpha}$ ($PGF_{1\alpha}$) (the stable end product of prostacyclin), and the use of a radioactive precursor to assess possible changes in the pattern of vascular arachidonate metabolism.

Subjects and methods

The study was performed in two parts. Initially, for the assessment of vascular arachidonic acid metabolism, umbilical cords were obtained from 15 control infants and from 17 infants of comparable gestational age born to diabetic mothers at the time of delivery by caesarean section. Classification of maternal diabetes was established according to standard criteria and included class A ($n=4$), class B ($n=8$), class C ($n=3$), class D ($n=1$), class R ($n=1$). No control or diabetic mother smoked or had taken aspirin within two weeks of delivery. Maternal blood samples were evaluated for fasting blood glucose concentrations and for glycosylated haemoglobin (HbA_{1c}) before delivery.⁶ The umbilical cords were dissected, the umbilical

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arteries isolated, cut into 3-5 mm segments, and opened longitudinally before use. Histological sections of the vessels were evaluated concomitantly, revealing an intact endothelial layer in all arteries studied.

METABOLISM OF ARACHIDONIC ACID IN UMBILICAL VESSELS

Vascular segments from both the controls and the infants of diabetic mothers were incubated in TRIS Hanks buffered salt solution pH 7.4 with calcium chloride (5 mmol/l) and magnesium chloride (1 mmol/l) for 30 minutes at 37°C to allow for maximum production of endogenous vascular prostacyclin. Aliquots were removed for radioimmunoassay of 6-keto PGF_{1α} using antibody obtained from Seragen (Boston, Mass). For the evaluation of exogenous ¹⁴C 6-keto PGF_{1α} production by the vasculature of controls and infants of diabetic mothers, concomitant with the above studies, arterial segments were homogenised in 50 mmol/l TRIS HCl buffer, pH 8.0 containing 2 mmol/l reduced glutathione (TRIS-GSH), microsomal fractions prepared, and protein contents determined.⁷ The microsomal fraction was incubated with 20 μmol/l ¹⁴C arachidonic acid (50-60 Ci/mol) at 37°C for 10 minutes in TRIS-GSH buffer with hydroquinone (250 μmol/l) and haemin (1 μmol/l). Samples were acidified, extracted, dried over anhydrous MgSO₄, and evaporated to dryness under a stream of nitrogen. Products were analysed by thin layer chromatography in a solvent system of ethyl acetate-acetic acid (99/1 by vol)⁸ with appropriate standards including arachidonic acid and the prostaglandin metabolites. Prostanoid production was measured by radioautography and liquid scintillation counting.

PLASMA 6-KETO PGF_{1α} IN INFANTS OF DIABETIC MOTHERS

In the second phase of our study placental blood for evaluation of plasma 6-keto PGF_{1α} concentrations was obtained after caesarean section from 12 control neonates and from 12 infants of comparable gestational age born to diabetic mothers (class A=3, class B=4, class C=3, class D=1, class R=1). Five controls and nine infants of diabetic mothers were common to both parts of our study—that is, concomitant studies on vascular arachidonate metabolism and plasma prostanoid values were performed in these 14 infants. Blood was drawn by a two syringe technique into ice cold anticoagulated tubes containing indomethacin (30 μmol/l final concentration). Platelet poor plasmas were separated by centrifugation and stored at -70°C before analysis. The test samples were acidified to pH 3.0, applied to washed octadecyl silica cartridges (Waters Associates, Milford, Ma) by a modification of the method of Powell⁹ and the acetonitrile-water (80%:20%) fraction evaporated and assayed for 6-keto PGF_{1α} by radioimmunoassay¹⁰ using antisera purchased from Seragen (Boston, Ma). Interassay variation was less than 8%. To ensure that the material eluting from the Sep-paks in the acetonitrile-water (80:20) fraction was in fact the indicated prostanoid in some samples the latter fraction was also subjected to reverse phase high pressure liquid chromatography separation. In all cases only one peak of cross reacting material was seen, and that eluted identically with authentic 6-keto PGF_{1α} directly injected.

Statistical analysis was performed by Student's *t* test for unpaired samples.

Results

ARACHIDONIC ACID METABOLISM IN UMBILICAL ARTERIES

Vascular prostacyclin production (measured as radioimmunoassayable 6-keto PGF_{1α} formed by intact umbilical arterial segments) increased linearly for the first 15 minutes with maximum production occurring by 30 minutes. When compared with control neonates (whose mean (SD) value was 6.5 (1.5) nmol/g) neonates born to diabetic mothers in poor glucose homeostasis (as evinced by raised HbA_{1c} values (>9.5%)) had a significantly decreased ability to produce vascular prostacyclin (4.3 (1.1); *p*<0.02) (fig 1a). Vascular prostacyclin production in the infants born to mothers in glucose homeostasis was not significantly different from that in normal neonates (6.4 (1.8) nmol/g). When ¹⁴C arachidonic acid was used as substrate by microsomes from these same vessels ¹⁴C 6-keto PGF_{1α} production increased linearly for the first 10 minutes. As seen in fig 1b, no statistically significant differences were observed between ¹⁴C 6-keto PGF_{1α} production in controls (975 (478) nmol/g microsomal protein), infants born to diabetic mothers in metabolic homeostasis (1199 (479) nmol/g), and infants born to diabetic mothers with

FIG 1—(a) Vascular production of endogenous radioimmunoassayable 6-keto PGF_{1α} and (b) microsomal production of ¹⁴C 6-keto PGF_{1α} were assayed in umbilical arteries from control neonates, infants born to diabetic mothers with normal HbA_{1c} concentrations, and infants born to diabetic mothers in abnormal glucose homeostasis. Mean (SD) birth weights for each group were 3244 (294), 3704 (413), and 3536 (734) g respectively. Gestational ages were not significantly different at 38.2 (0.8), 37.8 (1.0), and 37.2 (0.6) weeks respectively. Results are expressed as mean (SD) nmol/g.

FIG 2—Measurement of radioimmunoassayable plasma 6-keto PGF_{1α} in control neonates and in infants of diabetic mothers. Results expressed as mean (SD) nmol/l.

significantly raised HbA_{1c} values (1092 (577) nmol/g). Similarly, when total prostanoid production was assessed (sum of 6-keto PGF_{1α}, PGF_{2α}, and PGE₂) as a measure of cyclo-oxygenase activity no significant differences were observed between control neonates or either of the groups of infants of diabetic mothers (1613 (621) v 2090 (920) v 1830 (930) nmol/g microsomal protein). No significant correlation was observed in the infants of diabetic mothers between endogenous prostacyclin production by vascular segments and microsomes isolated from these same vessels (*r*=0.45; *p*>0.05).

PLASMA 6-KETO PGF_{1α} CONCENTRATIONS IN CONTROL NEONATES AND IN INFANTS OF DIABETIC MOTHERS

A significant decrease in the plasma concentration of 6-keto PGF_{1α} was observed at birth in the infants of diabetic mothers (0.75 (0.24) nmol/l) when compared with control neonates (1.65 (1.2) nmol/l; *p*<0.02) (fig 2). A significant correlation (*r*=0.68; *p*<0.05) was observed between endogenous vascular 6-keto PGF_{1α} production by umbilical arteries in the infants of diabetic mothers and plasma concentrations of 6-keto PGF_{1α} in these infants (fig 3). Values for 6-keto PGF_{1α} in plasma from normal adults assayed by this procedure are threefold to fourfold less than those observed in the neonate at birth (0.44 (0.15) nmol/l; *n*=17).

FIG 3—Correlation between endogenous vascular 6-keto PGF_{1α} production by umbilical arteries in infants of diabetic mothers and their plasma levels of 6-keto PGF_{1α}.

Discussion

Maternal diabetes is an important causal factor in the pathogenesis of both the arterial and the venous thromboses that can occur in utero and in the neonatal period in their infants.⁵ Dehydration, polycythaemia, or disseminated intravascular coagulation have been suggested as causative factors to explain this predisposition towards thromboses in these infants. Studies of coagulation factors on cord blood and during the early neonatal period in infants of diabetic mothers have, however, failed to show significant alterations in the fluid phase of coagulation.^{11 12}

In the adult with diabetes mellitus a decrease in vascular production of the potent antiaggregatory metabolite prostacyclin has been suggested in some studies to be related to the development of diabetic angiopathy.^{3 13} This finding, however, has not been documented by others. Moreover, the relevance of an in vitro assay for vascular prostacyclin production to in vivo production has been called into question by reports showing that circulating plasma concentrations of 6-keto PGF_{1α} may be normal in diabetes mellitus.^{14 15} The umbilical vessels provide us with the unique opportunity of assessing human vasculature obtained from the diabetic milieu and subjecting this tissue to various techniques (including both radioimmunoassay and the use of radioactive precursor substrate) to assess possible changes in the pattern of vascular arachidonate metabolism. Using such methods we have shown normal endogenous production of prostacyclin by vasculature obtained from a diabetic milieu where glucose homeostasis was maintained. In contrast, a significant diminution in the endogenous production of vascular prostacyclin was observed in those infants born to diabetic mothers with raised concentrations of glycosylated haemoglobin. Further support for the presence of an important abnormality in vascular arachidonate metabolism in the infants of diabetic mothers was provided by the finding of lower concentrations of plasma 6-keto PGF_{1α} in cord blood obtained from these infants than in that obtained from control neonates. Moreover, the correlation we have observed between circulating plasma 6-keto PGF_{1α} levels and endogenous umbilical vascular prostacyclin formation gives credence to the view that in infants of diabetic mothers the deficiency in vascular prostacyclin production reflects a concomitant in vivo abnormality.

Abnormalities in the formation of prostacyclin may result either from an abnormality in the arachidonic acid content of membrane phospholipids, abnormal release of this fatty acid substrate, or defective conversion via cyclo-oxygenase or prostacyclin synthetase, or both. Our finding of abnormal endogenous production of vascular prostacyclin in infants of

diabetic mothers in the absence of a concomitant decrease in ¹⁴C 6-keto PGF_{1α} formation when radiolabelled arachidonic acid was provided as substrate to vascular microsomes suggests that the abnormality in vascular prostacyclin production in these infants might be due to a decrease in the availability of substrate arachidonate. A previous preliminary report, however, showed a decrease in ¹⁴C 6-keto PGF_{1α} formation in the infants of diabetic mothers when radiolabelled precursor arachidonic acid was provided to intact vascular segments.¹⁶ This latter finding coupled with our present observations suggests the possibility that an inhibitor of vascular cyclo-oxygenase was operating in the studies evaluating intact vascular segments but that this inhibitor is no longer present in the isolated microsomal preparations.

The clinical importance of a decrease in vascular prostacyclin synthesis and in the concentration of plasma 6-keto PGF_{1α} in the infants of diabetic mothers remains speculative. An underlying imbalance in platelet-vascular prostaglandin synthesis which favours a prothrombotic tendency might not only result in the vascular thrombus formation observed with increased frequency in the infants of diabetic mothers but may also favour intrauterine growth retardation and congenital caudal malformations (two complications in the infants of diabetic mothers that appear to occur on a vascular basis). Moreover, since prostacyclin functions as an endogenous pulmonary vasodilator in the neonate, a decrease in the in vivo production of this compound may contribute to pulmonary vasoconstriction and to the transient respiratory distress observed in infants of diabetic mothers in the absence of radiological evidence of hyaline membrane disease.¹⁷ No clinical evidence of thrombosis was observed among our patient group, and no significant correlation was observed between prostacyclin production and neonatal birth weight. Nevertheless, of potential interest is the observation that the two infants who showed the greatest decreases in vascular and plasma 6-keto PGF_{1α} concentrations had deliveries complicated by placental obliterative endarteritis and transient respiratory distress. Our study also shows the relevance of the in vitro assay for vascular prostacyclin formation as an index of in vivo plasma levels in a pathological state—that is, in tissues obtained from the diabetic milieu.

Carolyn Ganley and Sherry Boone provided technical help and Nancy Bovalino secretarial support.

Supported by PHS, grant No 14405 from the National Institute of Child Health and Human Development, and by a Clinical Research Grant from the National Foundation March of Dimes to MJS.

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(Accepted 13 February 1985)