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SHORT COMMUNICATIONS

Fetal platelet size and glycoprotein Ib and IIIa expression in diabetic pregnancies

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In postnatal life, diabetes mellitus is associated with altered platelet function as a result of increased platelet adhesiveness and aggregation (Colwell & Halushka 1980). The aim of this study is to investigate fetal platelet function in diabetic pregnancies by measuring platelet size and glycoprotein Ib and IIIa expression both in the resting state and after stimulation with ADP.

Subjects and methods

Umbilical venous cord blood was obtained at elective caesarean section at 37 to 40 weeks of gestation from seven pregnancies complicated by maternal diabetes mellitus (four established and three gestational) treated with insulin. Umbilical venous cord blood also was obtained from 35 normal pregnancies undergoing elective caesarean section at term for reasons such as previous caesarean section, placenta praevia and breech presentation. In all cases the infants were normal and their birthweights were above the 5th centile for gestational age. All infants survived and were anatomically normal.

Blood was collected into sodium citrate for measurement of platelet size and glycoprotein expression, using a flow cytometer (FACscan, Becton Dickinson, Oxford) after staining with the appropriate monoclonal antibodies (CD42b and CD61) (Dako Ltd, High Wycombe) in the absence or presence of 1 mmol/l adenosine diphosphate. The Mann-Whitney *U* test was applied to determine the significance of any difference between the two groups.

Results

In pregnancies complicated by maternal diabetes mellitus, the platelet size and glycoprotein expression were increased both before and after stimulation with adenosine diphosphate (Table 1 and Fig. 1).

Discussion

Compared with normal pregnancies the data demonstrate that in pregnancies complicated by gestational or established maternal diabetes mellitus, fetal platelets are larger and express more surface glycoproteins both in the resting state and after stimulation by ADP. Similar findings of increased platelet reactivity have been reported in adult diabetics in whom no relation was found to either duration of the disease or degree of glycaemic control (Tschoepe *et al.* 1990). The mechanism for these changes in platelets is unknown but is presumably the same for both the diabetic mother and her fetus.

The observed increase in platelet reactivity, combined with the reported polycythaemia (Salvesen *et al.* 1992) in fetuses of mothers with diabetes mellitus, may explain their increased susceptibility to intravascular thromboses during the perinatal period (Oppenheimer & Esterly 1965) and may be one of the underlying mechanisms for unexplained stillbirth.

Appendix 1. Supplementary flow cytometry protocol

Blood (500 µl) was collected into sodium citrate (50 µl, 3·8% w/v) for analysis by flow cytometry, which was performed on the same day of sampling. Platelet-rich plasma was prepared by centrifugation (225 *g*, 10 min) and incubated with a saturating volume (5 µl) of fluorescent antibody in the absence or presence of 1 mmol/l ADP (Sigma Chemical Company Ltd, Poole, UK). Fluorescein-isothiocyanate (FITC) conjugated monoclonal mouse anti-human antibodies were used (CD42b and CD61) (Dako Ltd, High Wycombe, UK). In order to prevent platelet aggregation, the reaction mixture was diluted with phosphate buffered saline (Sigma Chemical Company Ltd, Poole, UK) to give a final volume of 100 µl. The tubes were incubated for 30 min in the dark at room temperature; 500 µl of phosphate buffered saline was then added to each tube prior to analysis by flow cytometry, which was carried out using a fluorescence-activated cell sorter (FACscan) and Consort 32 software (Becton Dickinson, Oxford, UK). The flow cytometer was calibrated for size and fluorescence before each analysis using a flow cytometry fluorescence intensity standardization kit (Coulter Ltd., Luton, UK). Samples were gated using forward angle and 90° light-scattering properties to exclude leucocytes. Control staining of platelet-rich plasma with anti-mouse monoclonal IgG_{2a}-PE/IgG_1-FITC was performed on each sample, and background readings of less than 1% were obtained. A minimum of 5000 cells were acquired in the platelet fraction and analysed to calculate the percentages and the mean fluorescence intensity (MFI) of each sample. The

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Table 1. The median (range) platelet size (RPS), percentage expression (%) and surface density (RFI) of GPIb and GPIIIa in fetal blood obtained from diabetic and normal pregnancies, both before and after stimulation with adenosine diphosphate. CS = caesarean section.

	Diabetic pregnancies		Elective CS		*z*	*P*
Platelet size RPS	1·72	(1·66–1·76)	1·35	(1·14–2·44)	3·9	$P < 0{\cdot}001$
GPIb %	90	(56–97)	77	(46–98)	1·69	—
GPIb RFI	1·93	(1·65–2·05)	1·32	(1·09–1·88)	3·97	$P < 0{\cdot}001$
GPIIIa %	95	(70–98)	86	(64–98)	1·62	—
GPIIIa RFI	3·92	(3·62–4·69)	2·16	(1·23–4·45)	3·56	$P < 0{\cdot}001$
Stimulated GPIb %	95	(57–98)	80	(48–98)	1·6	—
Stimulated GPIb RFI	1·57	(1·47–2·57)	1·41	(1·09–2·0)	2·94	$P < 0{\cdot}01$
Stimulated GPIIIa %	94	(72–98)	88	(58–98)	1·5	—
Stimulated GPIIIa RFI	3·51	(2·74–4·57)	2·28	(1·20–4·33)	2·23	$P < 0{\cdot}05$

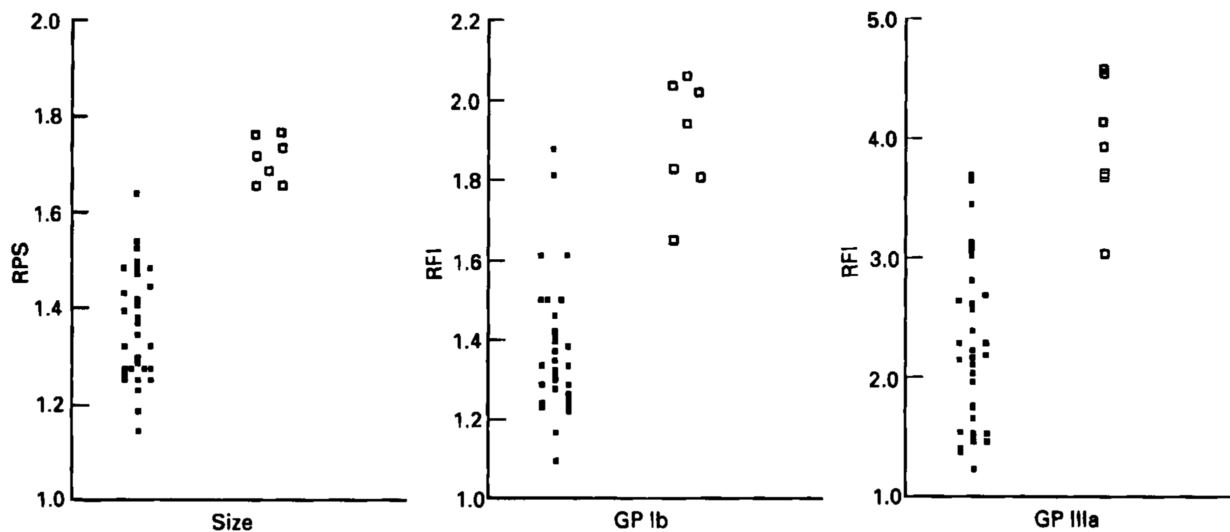

Fig. 1. Fetal platelet size (RPS) and glycoprotein Ib and IIIa surface density (RFI) following elective caesarean section in diabetic (□) and nondiabetic (■) pregnancies.

density of surface glycoproteins was measured by calculating the relative fluorescence intensity (RFI) by using the formula RFI = antilog(MFI/number of channels per decade). The platelet size was estimated by calculating the relative platelet size (RPS) by using the formula RPS = antilog(median foward scatter/number of channels per decade).

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