

# Acetylated fetal haemoglobin in neonates born to mothers with established and gestational diabetes

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## Summary

Birth weight ratios (BWRs) and cord blood C-peptide values were significantly higher in neonates born to mothers with well-controlled gestational diabetes (GD) than in those born to mothers with well-controlled established diabetes (ED) or mothers with normal results on glucose tolerance testing. The neonates born to the ED mothers had the highest cord blood acetylated fetal haemoglobin (Hb F<sub>1</sub>) values, and these values correlated with cord C-peptide values. The cord C-peptide values in the GD group correlated with BWRs, but not with Hb F<sub>1</sub> values. These results suggest differential tissue utilization of glucose in neonates born to mothers with different types of diabetes.

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The presence of acetylated fetal haemoglobin (Hb F<sub>1</sub>) in cord blood is well documented<sup>1-5</sup> and represents a particular example of the more general phenomenon of acetylation of proteins<sup>6</sup> (e.g. lens  $\alpha$ -crystallin, cytochrome C, histones, troponin, etc.). These post-translational reactions have been attributed to the action of an appropriate acetyltransferase enzyme and acetyl-co-enzyme A donor.<sup>6,7</sup> The biological implications of acetylation are not understood, although it may protect the protein from the action of exo-amino-peptidases.<sup>6</sup>

The non-enzymatic acetylation of valine (amino-terminal amino acid of  $\alpha$ - and  $\beta$ -chains), lysine and tyrosine residues of adult haemoglobin (Hb A<sub>0</sub>) has been reported,<sup>8</sup> and it has been attributed to the reaction of Hb A<sub>0</sub> with acetaldehyde. It has been suggested that this may be of use in patients who abuse alcohol, in a manner similar to the use of glycosylated haemoglobin (Hb A<sub>1</sub>) in diabetic patients.<sup>8</sup> It differs from the enzyme-mediated acetylation of proteins described above. The acetylation of fetal haemoglobin (Hb F<sub>0</sub>) has been reported to take place on the amino-terminal glycine residues of the  $\gamma$ -chain (i.e. Hb F<sub>1</sub> designated  $\alpha_2\gamma_2^{\text{acetyl}}$ ),<sup>3</sup> and the appropriate enzyme has been isolated from chicken and human cord blood erythrocytes.<sup>7</sup> The significance of this acetylation is poorly understood, although it has been suggested that it may be a useful marker for the gestational age of the neonate.<sup>9</sup>

We report on differences in Hb F<sub>1</sub> values in the cord blood of neonates born to mothers with established diabetes (ED) and those with gestational diabetes (GD), and discuss the implications of these differences.

## Patients and methods

Twenty-five pregnant diabetics and 12 pregnant non-diabetic controls were studied. Of the diabetic patients, 8 had established diabetes (ED) and 17 gestational diabetes (GD); vascular disease was carefully excluded in all (i.e. retinopathy, nephropathy and claudication were absent and cardiac function was normal). The patients and controls were predominantly (88%) of the Cape Coloured racial group.

The non-diabetic controls had normal results on glucose tolerance testing in pregnancy as judged by the criteria of O'Sullivan and Mahan.<sup>10</sup> GD was diagnosed by an abnormal result on glucose tolerance testing early in pregnancy, in which the fasting blood glucose (FBG) level was less than 5,8 mmol/l.<sup>11</sup> These patients were treated with diet only and once their condition had stabilized they were seen on a weekly basis. Their 2-hour postprandial glucose values were less than 8,3 mmol/l. Patients with ED had FBG values above 5,8 mmol/l. They were treated by appropriate administration of insulin to maintain the FBG level below 5,8 mmol/l and the 2-hour postprandial glucose level below 8,3 mmol/l. Treatment was usually carried out in hospital.

According to the criteria discussed, all patients were considered well controlled.

All patients received an intravenous infusion of 5% dextrose in water (< 10 g/h) during labour. It has been shown that this has no effect on cord blood insulin values.<sup>12</sup> Frequent determinations of maternal blood glucose levels during labour were carried out in order to keep them between 3,8 and 6,0 mmol/l.

The infants born to the mothers described were studied. No infants were delivered before 36 weeks' gestation, and there was no significant difference in gestational age at birth between the groups studied. Gestational age was determined postnatally<sup>13</sup> and the birth weight ratio (BWR) of each infant was calculated using charts appropriate to our patient population.<sup>14</sup>

A maternal venous blood specimen was taken on the day of delivery and a mixed umbilical cord blood specimen taken at delivery.<sup>15</sup> Informed consent was obtained from all mothers, and the study complied with the ethical requirements of this hospital.

Serum for the determination of C-peptide was separated and stored at -20°C until assayed. C-peptide values were determined after polyethylene glycol treatment of the specimens,<sup>16</sup> using a commercial radio-immunoassay procedure (Byk-Mallinckrodt, Dietzenbach, Germany). Plasma glucose was measured on a Beckman Astra 8 analyser.

Maternal blood was collected into ethylene-diamine tetraacetic acid (EDTA)-containing tubes, stored at 4°C and assayed within 4 days for Hb A<sub>1</sub> using a commercial microcolumn technique (Diagnostic Chemical Association, Arlington, Va, USA). Our normal range for Hb A<sub>1</sub> is 5,5-8,5%.

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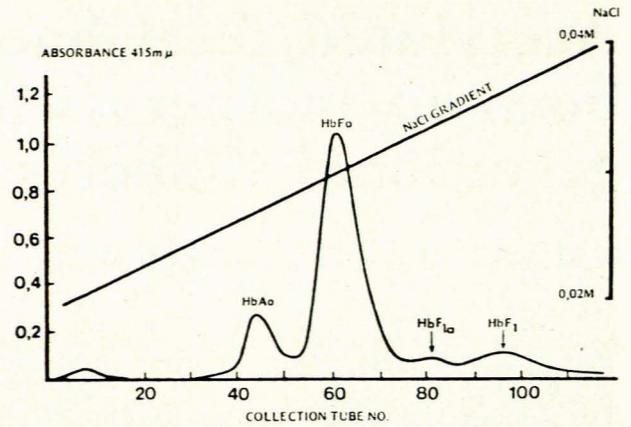
At delivery cord blood was collected into EDTA-containing tubes for the chromatographic determination<sup>5,17</sup> of Hb A<sub>0</sub>, Hb F<sub>0</sub>, Hb F<sub>1</sub> and glycosylated fetal haemoglobin (Hb F<sub>1a</sub>). The red blood cells were isolated, washed three times with 0,15M NaCl and lysed with an equal volume of buffer A containing 1% saponin. (Buffer A contained 15 g glycine + 0,1 g KCN per litre at a final pH of 7,8.) The lysate was diluted 1:1 with buffer A and dialysed overnight with stirring against 1:1 buffer A/water at 4°C. The dialysates (100-150 µl) were separated on a Whatman DE52 cellulose column using an ionic strength gradient from 0,02M NaCl (150 ml) to 0,04M NaCl (150 ml). A typical elution profile is shown in Fig. 1. The total area under all of the eluted peaks was calculated and the amount of each component peak was expressed as a percentage of the total area. Identification of the eluted peaks was performed according to previously reported work.<sup>5,17</sup>

Separate tracer experiments to identify the individual peaks were not carried out, but this gradient elution method separates acetylated and glycosylated haemoglobins, in contrast with the thin-layer electrofocusing technique used by Poon *et al.*<sup>18</sup> in cord blood or the stepwise elution procedure used by Stevens *et al.*<sup>8</sup> in adult blood. (The latter procedure may give falsely high levels of Hb A<sub>1</sub> in non-diabetic patients who abuse alcohol.)

Statistical analyses of the results was carried out with parametric statistics (Student's *t*-test and linear regression).

**Results**

There was no difference in gestational age at birth in the three groups of patients (Table I). BWRs and cord C-peptide values were significantly higher in the GD group (Table I). Cord C-peptide correlated with BWR in the GD group (Fig. 2, above), but there was no correlation in the ED (Fig. 2, below)



**Fig. 1. Chromatographic separation of fetal haemoglobin components in cord blood — a typical elution profile showing the major fetal haemoglobin, Hb F<sub>0</sub>, Hb A<sub>0</sub>, Hb F<sub>1</sub> and Hb F<sub>1a</sub>. (See Schwartz *et al.*<sup>5</sup> and Abraham *et al.*<sup>17</sup>.) (Conditions: buffer — 0,2M glycine + 0,01% KCN, pH 7,8; gradient — 150 ml buffer made 0,2M in NaCl, 150 ml buffer made 0,04M in NaCl; flow rate — 24 ml/h; fraction size — 3,0 ml; column dimensions — 25 x 1,0 cm.)**

or normal (not shown) groups. The ED group had the highest Hb F<sub>0</sub> and Hb F<sub>1</sub> values in cord blood (Table II). There was no relationship between Hb F<sub>1</sub> values and cord C-peptide values in the GD group (Fig. 3, above) or the normal group (not shown), but there was a strong positive correlation in the ED group (Fig. 3, below). There was no correlation between Hb F<sub>1</sub> values and BWRs in any of the groups studied (not shown). The trend in the neonates born to the normal mothers and to those with ED was inverse (not shown). The Hb F<sub>1</sub>

**TABLE I. GESTATIONAL AGE AT BIRTH, BWR AND CORD BLOOD C-PEPTIDE AND GLUCOSE VALUES (MEAN ± SE)**

	Normal (N = 12)	+ ED† (N = 8)	GD (N = 12)
Gestational age (wks)	39,25 ± 0,32	38,82 ± 0,53	38,92 ± 0,31
BWR	1,04 ± 0,03	1,06 ± 0,04	1,16 ± 0,04*
C-peptide (pmol/l)	412 ± 50	466 ± 88	679 ± 92** (11)
Glucose (mmol/l)	6,5 ± 0,67	6,6 ± 0,84	5,9 ± 0,69 (10)

\* P < 0,05.  
 \*\* P < 0,02.  
 † Patients with ED were classified as White's class A<sub>2</sub> or B.<sup>24</sup> Other classes were excluded from this study.  
 Numbers in brackets refer to number of patients examined for each test where these differ from the total number of patients examined in each group.  
 Within-assay and between-assay coefficients of variation for C-peptide were 6,2% and 10,4% respectively.

**TABLE II. TOTAL AND MINOR HAEMOGLOBIN VALUES IN MATERNAL AND CORD BLOOD (MEAN ± SE)**

	Normal (N = 12)	ED (N = 8)	GD (N = 17)
<b>Maternal blood</b>			
Total Hb (g/dl)	12,76 ± 0,48	12,78 ± 0,29	13,0 ± 0,20 (15)
Hb A <sub>1</sub> (%)	6,5 ± 0,21 (11)	6,57 ± 0,17 (7)	6,92 ± 0,14
<b>Cord blood</b>			
Total Hb (g/dl)	14,6 ± 0,39 (11)	15,2 ± 0,37	15,9 ± 0,48 (15)
Hb A <sub>0</sub> (%)	20,8 ± 2,9 (11)	13,4 ± 2,24*	19,12 ± 2,04 (15)
Hb F <sub>0</sub> (%)	71,4 ± 2,7 (11)	78,4 ± 2,45	74,8 ± 1,46 (15)
Hb F <sub>1</sub> (%)	7,6 ± 0,41 (11)	8,06 ± 0,53	7,6 ± 0,24 (15)
Hb F <sub>1</sub> /Hb F <sub>1</sub> + Hb A <sub>0</sub> ratio	0,29 ± 0,03 (11)	0,40 ± 0,05	0,318 ± 0,02 (15)

\* P < 0,05.  
 Numbers in brackets refer to number of patients examined for each test where these differ from the total number of patients examined in each group.  
 Within-assay and between-assay coefficients of variation for Hb A<sub>1</sub> were 2,5% and 4,6% respectively. The elution from the DE-52 cellulose column was reproducible, but coefficients of variation for Hb A, Hb F<sub>0</sub> and Hb F<sub>1</sub> were not calculated.

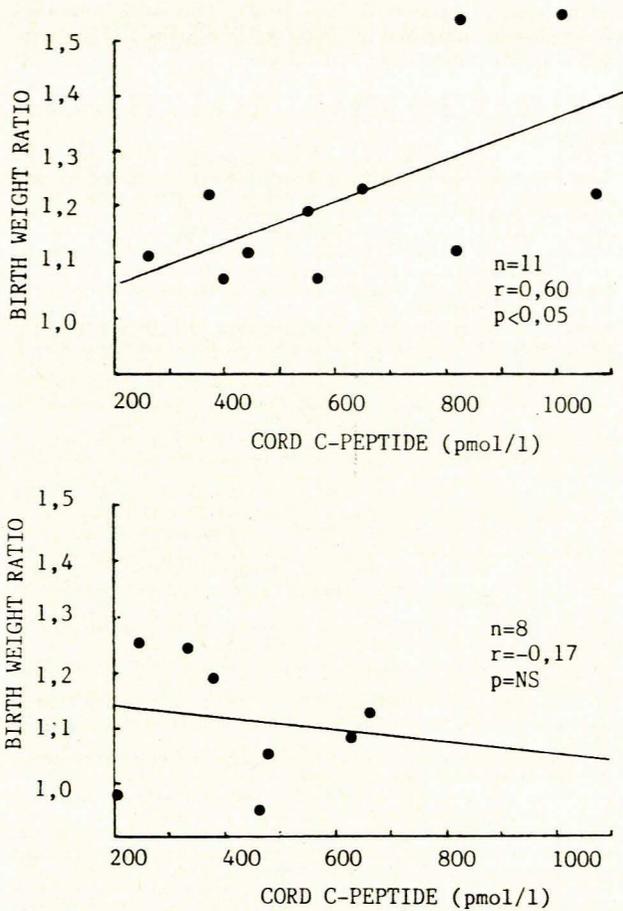


Fig. 2. BWRs v. cord C-peptide values in the GD (above) and ED (below) groups.

values correlated with maternal Hb A<sub>1</sub> values in the normal group (Fig. 4), but the trend was not significant in the two diabetic groups (not shown). There was no correlation of the Hb F<sub>1</sub>/Hb F<sub>1</sub> + Hb A<sub>0</sub> ratio of the cord blood with gestational age in any of the groups studied (not shown).

### Discussion

All diabetic patients were considered to be well controlled clinically, and this was reflected in maternal Hb A<sub>1</sub> values and cord glucose values in the normal range (Table II) in the ED and GD groups. Nevertheless, increased BWRs, and cord C-peptide values were found in the GD group (Table I), and these two measurements correlated (Fig. 2, above) in this group of patients. The size of the infant at birth may therefore depend on hyperinsulinism in the fetus, but it is to some extent independent of diabetic control in the mother. This has been discussed previously.<sup>19</sup>

The values of minor haemoglobin components in cord blood (Table II) correlate well with those found by Poon *et al.*<sup>18</sup> (Hb A<sub>0</sub> 23,0%, Hb F<sub>0</sub> 67,1% and Hb F<sub>1</sub> 7,8% in a normal group and Hb A<sub>0</sub> 18,4%, Hb F<sub>0</sub> 63,0% and Hb F<sub>1</sub> 8,6% in an ED group), although we found Hb F<sub>0</sub> values to be higher. Schwartz *et al.*<sup>5</sup> found Hb F<sub>0</sub> values between 70% and 80% in cord blood specimens from normal, GD and ED groups and Hb F<sub>1</sub> values between 10% and 20%. They found no difference in Hb F<sub>0</sub> or Hb F<sub>1</sub> values between the three groups. Our results therefore fall somewhere between those described in these two reports, and the differences may be due to the variety of methods used

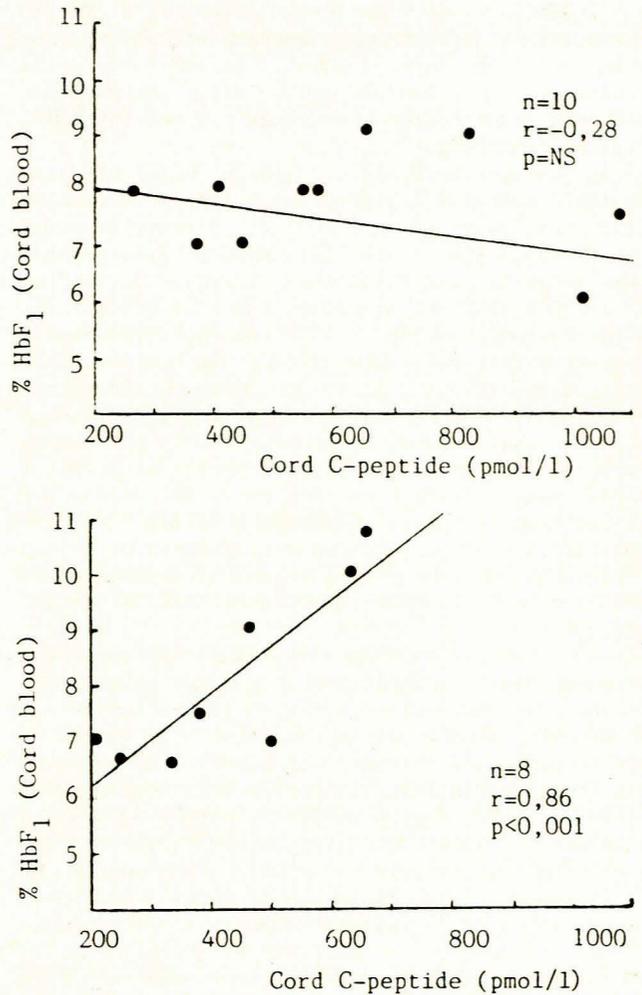


Fig. 3. Hb F<sub>1</sub> in cord blood v. cord C-peptide values in the GD (above) and ED (below) groups.

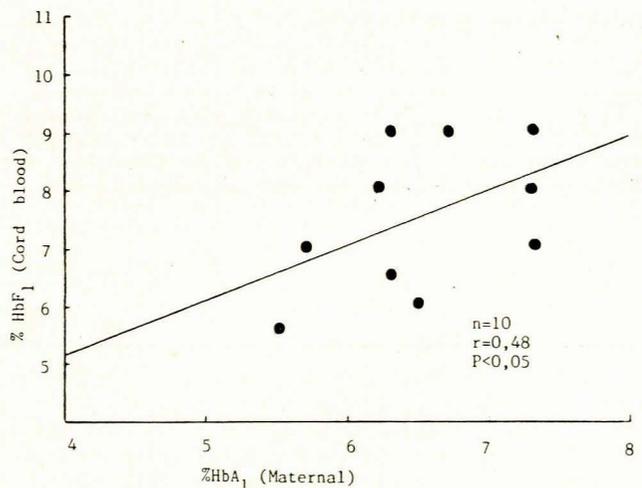


Fig. 4. Hb F<sub>1</sub> in cord blood v. Hb A<sub>1</sub> in maternal blood in the normal group.

or to differences in gestational age. It has been reported<sup>9</sup> that the ratio of Hb F<sub>1</sub>/Hb F<sub>1</sub> + Hb A<sub>0</sub> in cord blood correlates (inversely) with gestational age in normal mothers. We found that the ratio tended to fall with gestational age, but there was no significant correlation. Poon *et al.*<sup>18</sup> reported that Hb F<sub>1</sub> correlates with maternal Hb A<sub>1</sub>. This was confirmed by our

own results, but only in our normal group (Fig. 4). We feel that additional factors (e.g. oxygenation of the fetus) may make the Hb F<sub>1</sub>/Hb F<sub>1</sub> + Hb A<sub>0</sub> ratio unsuitable for the determination of gestational age in diabetic pregnancy and may preclude a correlation of maternal Hb A<sub>1</sub> with cord Hb F<sub>1</sub> in diabetic pregnancy.

The increased Hb F<sub>1</sub> values (Table II) in our ED group correlated with cord C-peptide values (Fig. 3, above), but there was no correlation in the GD (Fig. 3, below) or normal (not shown) patients. It has been suggested<sup>18</sup> that non-enzymatic glycosylation of Hb F<sub>0</sub> and co-elution of the resultant Hb F<sub>1a</sub> with Hb F<sub>1</sub> may give falsely high levels of Hb F<sub>1</sub>. We found low values of Hb F<sub>1a</sub> (<2%) in cord blood from 3 diabetic mothers and a value of 4% in the cord blood of a single normal infant. Hb F<sub>1a</sub> was not detected in the majority of samples. This corresponds with the findings of Schwartz *et al.*,<sup>15</sup> who could detect Hb F<sub>1a</sub> in only 41% of the patients they examined. (Also the cord C-peptide values were greatest in our GD patients (Table I) and there was no difference in cord glucose values between our patient groups (Table I).) We feel rather that the column procedure used separated Hb F<sub>1</sub> from Hb F<sub>1a</sub> (Fig. 1) and that our results indicate some fundamental difference between neonates born to ED and GD mothers (Fig. 3).

The C-peptide values indicate pancreatic insulin response to prevailing blood glucose, but it is generally accepted that insulin is not required for uptake of glucose into mature erythrocytes. Nevertheless, insulin receptors are present on erythrocytes<sup>20</sup> and, compared with neonates born to normal mothers and to those with GD, neonates born to mothers with ED have significantly enhanced glucose tolerance 3 hours after birth and a tendency to hypoglycaemia.<sup>21</sup> Presuming that glucose forms the precursor for the acetyl moiety necessary for the formation of Hb F<sub>1</sub> in the fetal erythrocytes, our results suggest to us a differential tissue utilization of glucose in our ED and GD patients. C-peptide does not affect formation of Hb F<sub>1</sub> (Fig. 3, above) but was found to correlate with BWR (Fig. 2, above) in our GD group, while Hb F<sub>1</sub> was found to correlate with C-peptide (Fig. 3, below) in our ED group. Differential tissue utilization of glucose has been demonstrated in alloxan-diabetic rats<sup>22</sup> (adipose tissue v. liver) and in obese adults<sup>23</sup> (adipose tissue v. muscle).

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