Diabetologia (1984) 26: 389–391

Diabetologia

Short communication

Erythrocyte 2,3-bisphosphoglycerate concentrations and haemoglobin glycosylation in normoxic Type 1 (insulin-dependent) diabetes mellitus

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Summary. Increased erythrocyte 2,3-bisphosphoglycerate concentrations are associated with increased haemoglobin glycosylation in patients with Type 1 diabetes mellitus who have no cause or symptoms of hypoxic stress. This change in 2,3-bisphosphoglycerate metabolism is additional to its response to changes in circulating haemoglobin concentration. Statistical analysis of data showed that erythrocyte 2,3-bisphosphoglycerate concentration did not correlate with the absolute concentration of circulating haemoglobin A_{1c}, but with the proportion of haemoglobin glycosylated. This finding is consistent with the hypothesis that it is changes in the position of the erythrocyte oxygen dissociation curve which modulate the increase in 2,3-bisphosphoglycerate synthesis upon haemoglobin A_{1c} formation.

Key words: Haemoglobin A_{1c}, Type 1 diabetes, oxygen dissociation curve, 2,3-bisphosphoglycerate, P_{50}.

The suggestion that excessive haemoglobin glycosylation may contribute to tissue hypoxia [1] has been challenged on the grounds that any uncompensated left-shift in the erythrocyte oxygen dissociation curve brought about by haemoglobin A_{1c} (HbA_{1c}) formation would only be of the order of 2 mmHg at the P_{50} value (the partial pressure of oxygen at which half the available heme groups are oxygenated), and therefore physiologically insignificant [2]. While practical [3] and theoretical [4] investigations agree with this figure, its clinical importance remains uncertain.

Erythrocytes from non-acidotic diabetic patients have increased 2,3-bisphosphoglycerate (2,3-DPG) content, but without corresponding right-shifts in oxygen dissociation curve [3, 5]. The interpretation has been offered that the left-shift in oxygen dissociation curve brought about by increased haemoglobin glycosylation is compensated by a right-shift resulting from increased 2,3-DPG concentrations acting on remaining unmodified haemoglobin [3]. This hypothesis has been difficult to prove since intra-erythrocytic concentrations of 2,3-DPG are altered by many conditions frequently encountered among diabetic subjects [6–8]. While most authors have applied some exclusion criteria in order to select preferentially normoxic subjects when studying the relationship between 2,3-DPG synthesis and HbA_{1c} formation [5, 9], these criteria have not been exhaustive. In the present work particular care was taken to exclude from study any diabetic patients who had cause, symptoms or history of tissue hypoxia.

Subjects and methods

Patient selection

Selected for study were ambulatory, Type 1 (insulin-dependent) diabetic subjects in stable, though not necessarily good, metabolic control, as judged by clinical history and routine measurements of blood glucose and HbA_{1c} performed serially over several, approximately 6-weekly, clinic visits. Verbal consent was obtained from each patient before taking blood for study.

Subjects were selected for study before proceeding with any laboratory measurements relating to erythrocyte oxygen delivery function. The following criteria were applied in patient selection.

History. Patients were excluded from study if they had any history or symptoms of cardiovascular or pulmonary disease, or impaired renal function. They were excluded if they were pregnant or lactating, or had received any medication besides insulin in the 4-week period before initial study, or smoked tobacco or other materials.

Physical examination. If their clinical history was acceptable, patients were given a physical examination. They were included in the study if (a) they were aged between 18 and 50 years at the time of initial study; (b) they had normal blood pressure and pulse rate, measured upright and prone; (c) they had no discernable irregularity in pulse when measured over a 1 min period; (d) they had no abnormality in the presence and strength of peripheral pulses; (e) they had no abnormality of reflexes and peripheral sensation; (f) they were not greater than 140% of expected body weight [10]; (g) fundoscopy showed no retinal abnormality; (h) they had no ketonuria detectable by 'Ketostix' or proteinuria detectable with 'Multistix' (Ames Laboratories, Elkhart, Indiana, USA).

Laboratory examination. A blood sample was taken from each subject not excluded by the results of physical examination and analysed for haemoglobin type and concentration, and plasma creatinine and phosphate concentrations. Patients were excluded from the study if (a) their haemoglobin concentrations was less than 12 g/dl if female or 13 g/dl if male; (b) they had a haemoglobin variant; (c) their plas-

Table 1. Statistical correlations observed between haemoglobin, 2,3-bisphosphoglycerate and haemoglobin A_{1c} concentrations and P_{50} value in normoxic Type 1 diabetic subjects

x	y		Correlation coefficient	*p*	Number of observations
Simple correlations		Equation			
HbA_{1c}	2,3-DPG	y= 0.126x+ 3.70	0.444	0.012	26
HbA_{1c}	2,3-DPG/haemoglobin	y= 0.298x+ 6.69	0.486	0.006	26
Haemoglobin	2,3-DPG	y=−0.20x + 7.73	−0.486	0.006	26
Haemoglobin	2,3-DPG/haemoglobin	y=−0.50x +16.81	−0.545	0.002	26
Haemoglobin	HbA_{1c}	y=−0.25x +12.93	−0.233	0.126	26
HbA_{1c} concentration	2,3-DPG	y= 0.34x + 4.41	0.179	0.191	26
HbA_{1c} concentration	2,3-DPG/haemoglobin	y= 0.69x + 8.53	0.204	0.158	26
HbA_{1c}	P_{50}	y=−0.246x+26.62	−0.291	0.114	19
Partial correlations		Parameters controlled			
P_{50}	HbA_{1c}	2,3-DPG	−0.842	<0.001	19
P_{50}	HbA_{1c}	Haemoglobin	−0.458	0.028	19
P_{50}	HbA_{1c}	Haemoglobin+2,3-DPG	−0.842	<0.001	19
P_{50}	HbA_{1c} concentration	2,3-DPG	−0.745	<0.001	19

Units used: HbA_{1c}, %; HbA_{1c} concentration, g/dl; 2,3-DPG, mmol/l; Haemoglobin, g/dl; P_{50}, mmHg; 2,3-DPG/haemoglobin, molar ratio

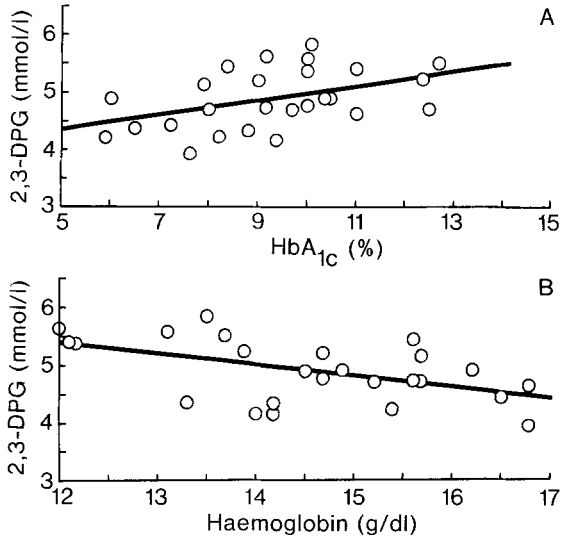

Fig. 1. A and B. Correlations between erythrocyte 2,3-bisphosphoglycerate concentration and **A** percentage HbA_{1c}, and **B** circulating haemoglobin concentration in clinically normoxic, Type 1 diabetic subjects. Statistical data are given in Table 1

ma phosphate concentration was less than 0.9 or more than 1.4 mmol/l; (d) their plasma creatinine was greater than 0.12 mmol/l. *Follow up.* Data from a subject were not used in analysis if the subject could not still meet the inclusion criteria 2 years after initial study.

Rigorous application of these criteria resulted in selection of eight female and 18 male subjects who had no cause, symptom or history of tissue hypoxia. They were aged 19–46 years, weighed 48–111 kg with body weights being 87% to 134% of expected weights, and were taking 14–102 units of insulin daily. The duration of their diabetes extended from 6 months to 12 years. Venous blood pH values at the time of initial study were between 7.301 and 7.426 (mean 7.361).

Laboratory measurements

Erythrocyte 2,3-DPG concentrations were measured on cell lysates [11]. HbA_{1c} was quantified by cation exchange chromatography [12]. Erythrocyte oxygen dissociation curves were measured (Hem-O-Scan, Aminco Laboratories Silver Spring, Maryland, USA) following equilibration of cells to pH 7.4, carbon dioxide tension 38 mmHg [13].

Statistical analyses

Data were analysed using the statistics program SPSS [14] on the PRIME-850 computer of Flinders University. Non-parametric statistics were used throughout.

Results

A significant, positive association was found between erythrocyte 2,3-DPG concentration and the proportion of haemoglobin glycosylated (percentage HbA_{1c}) in the selected group of patients (Fig. 1). An expected negative association between 2,3-DPG concentration and circulating haemoglobin concentration was also demonstrated. However, no significant association between percentage HbA_{1c} and circulating haemoglobin concentration could be shown. Erythrocyte 2,3-DPG concentrations did not correlate significantly with absolute circulating HbA_{1c} concentrations, but only with the proportion of circulating haemoglobin glycosylated (Table 1).

P_{50} values recorded under standardised conditions [13] for 19 of the subjects ranged from 22.0–26.4 mmHg (mean 24.3 mmHg). Direct comparison between observed P_{50} and percentage HbA_{1c} values showed there was no significant association between them. Using partial correlation calculations and controlling for 2,3-DPG concentrations, however, produced a very strong, negative association between these two parameters. When both circulating haemoglobin and 2,3-DPG concentrations were controlled no improvement in strength of the association occurred. Controlling the calculations for circulating haemoglobin concentration alone produced a much weaker association. Consistent

with other findings, the partial correlation between P_{50} value and the absolute rather than proportional concentration of HbA_{1c} showed a weaker association.

Discussion

Strict exclusion criteria were rigorously applied to select only normoxic diabetic subjects in the present study of the possible response of erythrocyte 2,3-DPG metabolism to increased haemoglobin glycosylation. In the group of subjects finally studied, a positive association between erythrocyte 2,3-DPG concentration and the proportion of haemoglobin glycosylated was clearly demonstrable. Not unexpectedly, 2,3-DPG concentrations also correlated inversely with circulating haemoglobin concentrations in these subjects. Since no significant association between percentage HbA_{1c} and circulating haemoglobin concentration was found it may be argued that the change in 2,3-DPG concentrations associated with haemoglobin glycosylation is additional to a response to changes in circulating haemoglobin concentrations.

The change in 2,3-DPG concentrations associated with HbA_{1c} formation correlated with the proportion of circulating HbA_{1c} rather than with its absolute concentration. Glycosylation of a fixed proportion of the circulating haemoglobin thus appears to elicit the same response from 2,3-DPG metabolism irrespective of the total haemoglobin concentration. This observation suggests that the increase in 2,3-DPG concentrations associated with HbA_{1c} formation is modulated by shifts in the position of the erythrocyte oxygen dissociation curve. When simple correlations were sought, however, increased extents of haemoglobin glycosylation were found not to be associated with left-shifts in the oxygen dissociation curve in the subjects studied. This lack of association between erythrocyte P_{50} value and percentage HbA_{1c} is not surprising if it is hypothesised that the expected left-shift in oxygen dissociation curve resulting from increased haemoglobin glycosylation [15] is masked by increased 2,3-DPG synthesis. In support of this idea, partial correlation analysis showed a very strong negative association between the P_{50} value and percentage HbA_{1c}, provided that allowance was made for changes in 2,3-DPG concentration. The strength of this association was not increased by allowing for changes in other influences on the oxygen dissociation curve. These findings are thus totally consistent with the hypothesis that increased haemoglobin glycosylation would lead to left-shifts in the oxygen dissociation curve of erythrocytes if left uncompensated, and that the major compensatory influence is an increase in 2,3-DPG synthesis [3, 5].

Two inferences follow from this analysis. Firstly, a response of 2,3-DPG metabolism to increased haemoglobin glycosylation implies a physiological significance in the effect of HbA_{1c} formation on the position of the oxygen dissociation curve, even though the magnitude of this latter effect is numerically small. Secondly, since the studied subjects were able to maintain clinical normoxia over a 2-year period from study, any response of 2,3-DPG metabolism to HbA_{1c} formation is probably totally compensatory.

Acknowledgement. The authors wish to thank Drs. S. J. Judd, P. J. Phillips and G. D. Calvert of the Endocrinology Unit, Flinders Medical Centre, for their assistance with the clinical assessments of the patients in this study.

References

1. Ditzel J, Standl E (1975) The problem of tissue oxygenation in diabetes mellitus I. Its relation to the early functional changes in the microcirculation of diabetic subjects. Acta Med Scand Suppl 578: 49–58
2. Bunn HF, Gabbay KH, Gallop PM (1978) The glycosylation of hemoglobin: relevance to diabetes mellitus. Science 200: 21–27
3. Arturson G, Garby L, Robert M, Zaar B (1974) Oxygen affinity of whole blood in vivo and under standard conditions in subjects with diabetes mellitus. Scand J Clin Lab Invest 34: 19–22
4. Ryall RG, Story CJ (1981) The effect of haemoglobin glycosylation on the red cell oxygen dissociation curve in diabetic subjects. In: Garlick D (ed) Progress in microcirculation research, University of New South Wales, Sydney, pp 620–628
5. Ditzel J, Jaeger P, Kjaergaard JJ (1979) Hemoglobin A_{1c} and red cell oxygen-releasing capacity in relation to early microvascular responses in ambulatory diabetics. Adv Microcirc 8: 1–13
6. Alberti KGMM, Darley JH, Emerson PM, Hockaday TDR (1972) Red cell 2,3-diphosphoglycerate and diabetes. Lancet 1: 843–844
7. Gibby OM, Veale KEA, Hayes TM, Jones JG, Wardrop CAJ (1978) Oxygen availability from the blood and the effect of phosphate replacement on erythrocyte 2,3-diphosphoglycerate and haemoglobin-oxygen affinity in diabetic ketoacidosis. Diabetologia 15: 381–385
8. Kanter Y, Bessman SP, Bessman AN (1975) Red cell 2,3-diphosphoglycerate levels among diabetic patients with and without vascular complications. Diabetes 24: 724–729
9. Samaja M, Melotti D, Carenini A, Pozza G (1982) Glycosylated haemoglobins and the oxygen affinity of whole blood. Diabetologia 23: 399–402
10. Desirable weights of adults (1970) In: Diem K, Lentner C (eds) Documenta Geigy, 7th edn, JR Geigy SA, Basle, Switzerland, p 712
11. Nygaard SF, Rorth M (1969) An enzymatic assay of 2,3-diphosphoglycerate in blood. Scand J Clin Lab Invest 24: 399–403
12. Trivelli LA, Ranney HM, Lai H-T (1971) Hemoglobin components in patients with diabetes mellitus. New Engl J Med 284: 353–357
13. Ryall RG, Story CJ (1983) An equilibrium model of the oxygen association curve of normal human erythrocytes under standardized conditions. Clin Chem 29: 1819–1822
14. Nie NH, Hull CH, Jenkins JG, Steinbrenner K, Bent DH (1975) Statistical package for the social sciences, 2nd ed, McGraw-Hill, New York
15. McDonald MJ, Bleichman M, Bunn HF, Noble RW (1979) Functional properties of the glycosylated minor components of human adult hemoglobins. J Biol Chem 254: 702–707

Received: 9 August 1983
and in revised form: 20 February 1984

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