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ORIGINAL ARTICLE

Altered Erythrocyte Glycolytic Enzyme Activities in Type-II Diabetes

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Abstract The activity of enzymes of glycolysis has been studied in erythrocytes from type-II diabetic patients in comparison with control. RBC lysate was the source of enzymes. In the diabetics the hexokinase (HK) activity increased 50 % while activities of phosphoglucoisomerase (PGI), phosphofructokinase (PFK) and aldolase (ALD) decreased by 37, 75 and 64 % respectively but were still several folds higher than that of HK. Hence, it is possible that in the diabetic erythrocytes the process of glycolysis could proceed in an unimpaired or in fact may be augmented due to increased levels of G6P. The lactate dehydrogenase (LDH) activity was comparatively high in both the groups; the diabetic group showed 85 % increase. In control group the HK, PFK and ALD activities showed strong positive correlation with blood sugar level while PGI activity did not show any correlation. In the diabetic group only PFK activity showed positive correlation. The LDH activity only in the control group showed positive correlation with marginal increase with increasing concentrations of glucose.

Keywords Diabetic erythrocytes · Hexokinase · Phosphoglucoisomerase · Phosphofructokinase · Aldolase · Lactate dehydrogenase

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Introduction

Diabetes mellitus (DM) is a metabolic disorder characterized by varying or persistent hyperglycemia ascribed to the decreased synthesis of insulin or improper utilization of glucose [1]. Non-enzymatic glycosylation of proteins is the major cause of diabetic complications [2]. In normal human red blood cells (RBCs) about 5 % of hemoglobin (Hb) designated as Hb A_{1c} is glycosylated which increases at least twofold in diabetic patients [3–5]. It has been reported that under in vitro conditions the rate of glycosylation with glucose 6-phosphate (G6P) is 20 times higher than that with glucose: the reaction is less readily reversible due to glycosylation at the N-terminus of the β chain. The G6P-Hb may be an intermediate in the conversion of Hb A to Hb A_{1c} [6].

The concentration of G6P has been found to be increased in the RBCs from diabetic patients [7–9] as also in the erythrocytes from normal individuals incubated with high concentrations of glucose [10]. Based on these in vitro studies it was suggested that higher concentrations of glucose may partially relieve inhibition of hexokinase by G6P. However, in these experiments abnormally high concentrations of glucose (40–100 mM) were used despite which the effect was only marginal [10]. However, as has been demonstrated earlier, increase in erythrocyte hexokinase activity by itself seems to be responsible for increased G6P content [7–9]. While G6P is several folds more effective than glucose in glycosylating Hb, it is possible that increased G6P content in the diabetic state might also influence the downstream enzymes in the glycolytic pathway which in turn could also be responsible for accumulation of G6P in the erythrocytes. We tested this possibility by determining the activities of major enzymes of glycolytic pathway in erythrocytes from type II diabetic

patients in comparison with normal healthy subjects. The results of these investigations are summarized in the present article.

Materials and Methods

Chemicals

ATP, NADP$^+$, NADH, glucose-6-phosphate dehydrogenase (G6PDH), aldolase, α glycerophosphate dehydrogenase, triosephosphate isomerase, fructose 1,6 bisphosphate (FBP), Fructose-6-phosphate (F6P), cysteine, glucose, sodium pyruvate, imidazole, dithioerythritol (DET), glycylglycine, tris (tris hydroxymethyl aminomethane) were purchased from Sigma Aldrich, St. Louis, MO. USA. All other chemicals were of analytical reagent grade and were purchased locally.

Blood Samples

5.0 mL Blood samples were collected in vials containing EDTA after written informed consent from six Type II diabetic patients and six normal healthy individuals; protocols approved by the Institutional Ethics Committee were followed. The mean age of the diabetic patients was 41.86 ± 6.27 years (ranging from 33 to 71 years); the mean blood sugar levels was 230.83 ± 35.30 mg/dL (ranging from 160 to 343 mg/dL). For the normal male volunteers the mean age was 38.5 ± 2.24 years (ranging from 31 to 45 years) and the mean blood sugar levels 93.33 ± 4.72 mg/dL (ranging from 78 to 104 mg/dL). The individual values are shown in Fig. 1.

Erythrocytes were separated by centrifugation at 400xg for 10 min followed by repeated washing with normal saline (0.9 % NaCl solution). The packed cells were resuspended to original volume with 0.9 % NaCl solution and the cell count was recorded.

Enzyme Activities

The resuspended RBCs were lysed (1:10) in 10 mM sodium-phosphate buffer, pH 7.4 and the lysate was used as the source of enzyme. The enzyme activities: hexokinase (HK), phosphoglucoisomerase (PGI), phosphofructokinase (PFK), Aldolase (ALD) and lactate dehydrogenase (LDH, P → L) were determined according to the methods described earlier, with some modifications [11–15]. The activity measurements were carried out at 25° C in duplicate. The activities are expressed as n mole/min/unit number of RBCs as indicated in Table 1.

Statistical Analysis

Statistical analysis of the data and regression analysis were carried out using GraphPad Prism version 5. The p values <0.05 were considered statistically significant.

Results

The results on the glycolytic enzyme activities are given in Table 1 from which it can be noted that in the control subjects the activity of HK was the lowest whereas the activities of PGI, PFK and ALD were significantly high and comparable. It would thus appear that in the erythrocytes from normal healthy individuals HK is the rate limiting enzyme. In the diabetic group the HK activity increased by 50 % while that of the remaining three enzymes decreased by 37, 75 and 64 % respectively. Nonetheless, these activities were still several folds higher than that of HK. The activity of LDH was comparatively high in both the groups; the diabetic group showed 85 % increase above the control value.

We wanted to determine as to whether the changes in the enzyme profiles were related with or were influenced by plasma glucose concentration. Hence we carried out regression analysis to find the correlation between the observed enzyme activities and plasma glucose concentration in both control as well as diabetic groups. These results are shown in Fig. 1. It can be noted that in control group the HK, PFK and ALD activities showed strong positive correlation while PGI activity did not show any correlation. HK activity was influenced maximally whereas the influence on PFK and ALD was marginal. In the diabetic group only PFK activity showed positive correlation. The LDH activity only in the control group showed positive correlation with marginal increase with increasing concentrations of glucose.

Discussion

It is apparent from the data presented that in the diabetic group only HK and LDH activities increased by 50 and 85 % while the activities of PGI, PFK and ALD decrease from 37 to 75 %. It is also clear that in the control subjects activity of HK is the lowest which would suggest that in the controls HK is the rate limiting step. In the diabetic subjects also HK seems to be rate limiting although the activity is greater than that in the control subjects. Our observations on increased HK activity in the diabetic group are consistent with observations of other investigators [7–

Fig. 1 Regression analysis of enzyme activities versus blood sugar levels in control and diabetic erythrocytes samples. *Note* that scales are different for control and diabetic groups
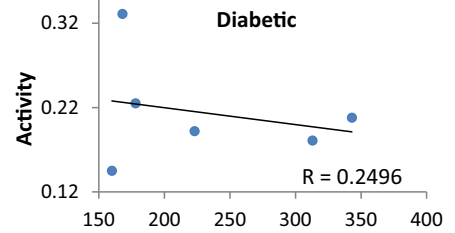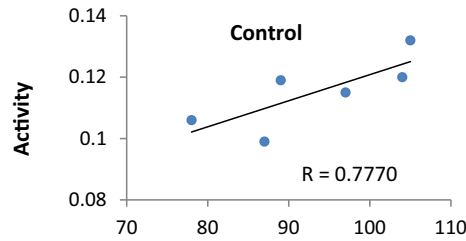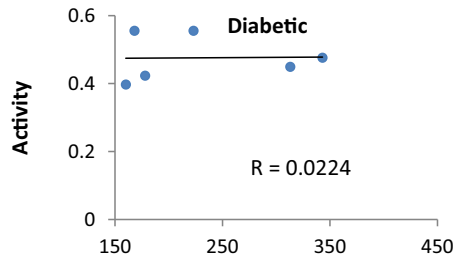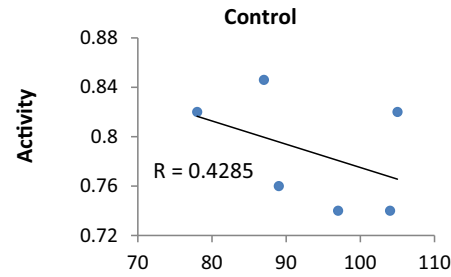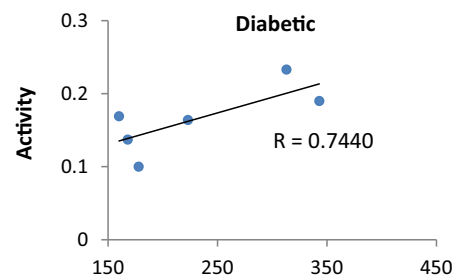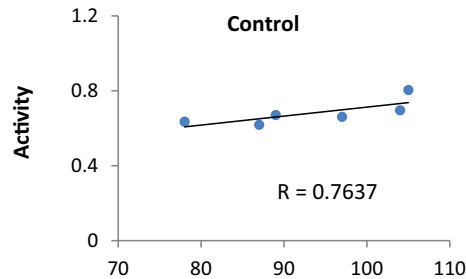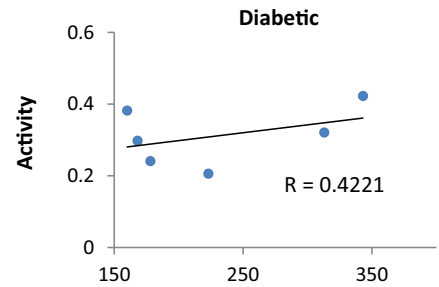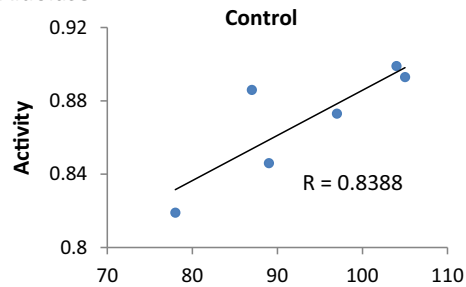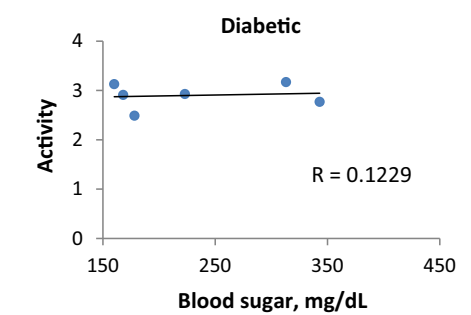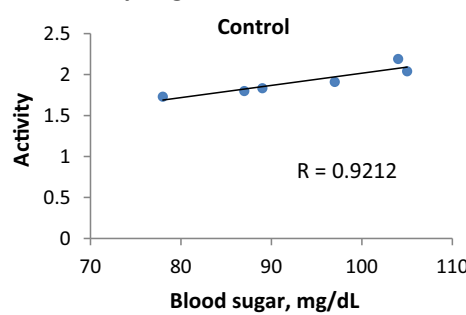
Table 1 glycolytic enzyme activities in erythrocytes from type 2 diabetic patients

Enzyme	Subject	Activity	Change (%)
Hexokinase	Control	0.012 ± 0.004	–
	Diabetic	0.018 ± 0.028*	+50.0
Phosphoglucoisomerase	Control	0.770 ± 0.016	–
	Diabetic	0.488 ± 0.025**	−36.6
Phosphofruktokinase	Control	0.681 ± 0.030	–
	Diabetic	0.173 ± 0.016**	−74.6
Aldolase	Control	0.860 ± 0.015	–
	Diabetic	0.312 ± 0.037**	−66.7
Lactate dehydrogenase	Control	1.916 ± 0.026	–
	Diabetic	2.899 ± 0.102**	+84.9

Enzyme activities are expressed as n mole/min/10^6 RBCs

Results are given as mean ± S.E.M of 6 independent observations

* $p < 0.05$ and ** $p < 0.0001$

10]. In spite of the increase in the HK activity in the diabetics, paradoxically the PGI, PFK and ALD activities showed substantial decrease with maximum decrease seen in the activities of the latter two enzymes (Table 1). Despite these decreases the activities of PGI, PFK and ALD are sufficiently high and saturating compared to increased HK activity. It may therefore be assumed that in the diabetic erythrocytes the process of glycolysis could proceed in an unimpaired normal manner or may in fact be augmented due to increased levels of G6P. Not surprisingly then, it has been reported that the levels of ATP and the ATP/ADP ratio are elevated in erythrocytes from diabetic patients [16, 17]. The other factors which could contribute to the observed increases in the level of ATP and ATP/ADP ratio, may be decreased Na^+, K^+-ATPase and Ca^{2+}-ATPase activities which would result in underutilization of ATP [18, 19]. The fallout would be compromised K^+ and Ca^{2+} homeostasis. Ca^{2+} is a known activator of protein kinase C (PKC) therefore it is possible that membrane protein phosphorylation pattern could alter [20].

The maximum decrease in PFK activity in the diabetics may suggest that PFK may be a rate limiting step in the downstream process. It may be pointed out that in the erythrocytes PFK is the only irreversible step since the enzyme fructose 1,6 bis phosphohydrolase (FBPase) is apparently absent in the erythrocytes. Thus PFK can serve as an additional rate limiting step.

It has been reported that the levels of pyruvate and lactate are increased in diabetic erythrocytes leading to lactic acidosis [21]. This is consistent with our presumption that in the diabetic RBCs the process of glycolysis might be proceeding at a higher rate (vide infra). In the erythrocytes LDH functions towards reduction of pyruvate to lactate.

The observed increase in LDH activity in the diabetics (Table 1) is consistent with this observation.

The correlation of enzyme activities with glucose levels (Fig. 1) deserves some comments. In the control group HK, PFK, ALD and LDH showed positive correlation with blood sugar levels. However, there was only marginal increase in the activity of the latter three enzymes. In as much as RBCs are preformed cells released in circulation, it may be suggested that the pattern represents the built-in enzyme levels of individuals. The marginal increase with sugar levels is also consistent with their activities being present in saturating amounts (Table 1). A similar logic would apply for PGI.

In the diabetic group there appears to be built-in increase in HK activity which is not correlated with sugar levels. Possibly diabetic stage itself may be a signal for increase in the HK activity. Diabetic state may also serve as a signal for increase in PFK activity with blood sugar levels although these activities are significantly low compared to the controls.

Animal model and clinical studies have shown that diabetic hyperglycemia leading to non-enzymatic glycosylation of proteins is the major factor contributing to the progressive secondary complications [22, 23]. Increased glycosylation of erythrocytes from diabetics due to high concentrations of glucose and/or G6P has been well documented [6–10]. It was also found that the rate of glycosylation by the intracellular sugars such as fructose, G6P, and glyceraldehyde-3-phosphate is considerably greater than that with glucose [24]. These changes directly or indirectly affect the functional characteristics of diabetic erythrocytes [25]. Glycosylation modifies the structural and functional properties of different proteins including membrane lipoproteins and erythrocyte membrane proteins [26]. Our observations are consistent with this assumption and suggest that observed changes can impose additional burden. These alterations result in the known functional characteristics of erythrocytes such as decreased deformability at individual level and increased aggregation at collective level [25]. Good flow through microvessels and large arteries is attributed to the ability of erythrocyte to undergo shape transformation i.e. deformability [27]. However, decreasing trends of deformability, membrane fluidity and flow of erythrocytes in microvessels are observed in diabetic patients [25]. In diabetic nephropathy occlusion of fragmented RBCs in glomeruli has been reported [28].

Compliance with Ethical Standards

Conflict of interest None.

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