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The abnormality of glucose transporter in the erythrocyte membrane of Chinese type 2 diabetic patients

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Abstract

Type 2 diabetes mellitus is characterized by impaired glucose uptake. With a photometric method of recording the erythrocyte suspension absorption during the course of glucose transport across the membranes, we observed that the initial rate of glucose zero-*trans* entry was decreased significantly in 30 Chinese type 2 diabetic patients as compared to 25 healthy controls. The rate of glucose infinite-*cis* efflux exhibited no difference between the patients and controls. The measurement of temperature dependence of glucose transport showed that the activation energy for glucose entry was increased in diabetic patients. The inhibitory constant of glucose entry by cytochalasin B (CB) in patients was similar to that of the controls. However, we found that the inhibitory constant was increased significantly in the patient erythrocytes after phloretin treatment. After the erythrocytes were made into stripped white ghosts, the fluorescence quenching experiment was performed. Glucose, CB and phloretin can quench the fluorescence of tryptophan residues in the glucose transporter 1, GLUT1. The abnormality of fluorescence quenching in the erythrocyte membranes of patients was observed. The transfer tendency of tryptophan residues from the hydrophilic environment to the hydrophobic environment was decreased in patient ghosts as binding with glucose, and the opposite tendency appeared as CB and phloretin instead of glucose. We conclude that the decreased in glucose entry in the erythrocyte membranes of diabetic patients was due to the GLUT1 change in structure – mostly the outer domain of the glucose transporter.

Keywords: GLUT1; Type 2 diabetes; Erythrocyte; Glucose transport

1. Introduction

In type 2 diabetes, normal glucose homeostasis cannot be maintained because of insulin resistance and an inadequate compensatory insulin secretion. Chronic hyperglycemia is a characteristic of diabetes disordered glucose metabolism. Glucose transporter 1 (GLUT1) presents in low levels in nearly all tissues. With five other identified homologous glucose transporter isoforms, GLUT1 plays pivotal roles in glucose and its analogue metabolism and it is responsible for basal glucose uptake [1,2].

Glucose utilization occurs as a result of both insulin-mediated glucose uptake and non-insulin-mediated glucose uptake. It was indicated that reduction in non-insulin-mediated glucose uptake plays an im-

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portant pathogenic role in disorders of carbohydrate metabolism in diabetic patients [3]. Many studies have addressed how glucose transporters might be involved in the pathogenesis of diabetes. Though mutations in the genes that constitute GLUT family could explain some observed physiological defects in type 2 diabetes, nearly all studies did not support this attractive presumption [4–7].

The blood vessel should be regarded as the primary target organ in diabetes mellitus. In diabetic patients, both the macrocirculation and microcirculation are affected [8,9]. GLUT1 is most abundant in the erythrocytes. Besides, Concha et al. [10] reported recently that the human erythrocytes also express the GLUT5 hexose transporter, which operates primarily as a fructose transporter. Because of lacking mitochondria and ribosomes, the content of GLU1 in erythrocyte membrane is steady and there is no new protein biosynthesis. So the erythrocyte is an idle model to study the role of GLUT1 in glucose metabolism in diabetes. Some studies [11–13] indicated that glucose uptake was changed significantly in the erythrocytes of diabetic patients, but the reason of this change has not yet been defined. In this study, we attempted to determine an unambiguous abnormality of glucose transport across the erythrocyte membranes in Chinese type 2 diabetic patients. The most striking result that emerges from this study is that the change in glucose transport was due to the GLUT1 structure change – mostly the outer domain of the glucose transporter.

2. Materials and methods

2.1. Materials

Freshly drawn Chinese type 2 diabetes patients' (treated with diet and oral hypoglycemic) blood samples were obtained from the Xinhua Hospital in Shanghai. The fresh healthy bloods were obtained from the Shanghai Red Cross Blood Center or from healthy volunteers. The samples from both patients and controls were chosen randomly. Cytochalasin B (CB), cytochalasin E (CE), phloretin and *N*-acetyltryptophanamide (NATA) were purchased from Sigma. All other reagents were of analytical grade.

2.2. Erythrocyte preparation

The erythrocytes were washed three times with PBS (155 mM NaCl, 5 mM sodium phosphate, pH 7.4). In transport study, the erythrocytes were incubated at 37°C for 30 min with PBS in zero-*trans* influx measurement or were incubated with PBS containing 80 mM -glucose in infinite-cis efflux measurement. In inhibitor binding experiment, before transport study the erythrocytes were incubated with PBS containing inhibitors at 37°C for 30 min (for CB binding study, 5 μM CE incubation for 30 min was performed before CB treatment for competitive inhibiting the CB binding site at erythrocyte skeleton [14]). The treated erythrocytes were kept in ice and used within 6 h. All measurements were performed at 35°C besides the temperature dependence study.

2.3. Glucose transport

A modified Sen and Widdas method [15,16] was used in D-glucose transport study. For measurement of zero-*trans* influx, 50 μl of the treated erythrocytes ($\sim 3\times10^8$ cell/ml) was rapidly mixed with 2 ml PBS containing 200 mM D-glucose in 1 cm cuvette which contained a stirring bar. The time course of the change in absorbances of the cell suspension at 660 nm on a spectrometer (type 722, Shanghai No. 3 Analytical Instrument Factory) was measured. The spectrometer was modified to set up a magnetic stirrer at the bottom of the photometric cell holder and the circular water around the cell was used. The output electrical signal was through 12 bit A/D converter fed to a microcomputer. The sampling point for each measurement was 1000. The initial transport rate V_0 was determined from the recorded curve (Fig. 1A) and fitting over 5 times of measurement, according to Eq. 1 [17]:

$$V_0 \equiv \left.\frac{dC_S}{dt}\right|_{t=0} = C\cdot\frac{OD_\infty}{OD_0}\cdot\frac{1}{(OD_\infty - OD_0)}\cdot\left.\frac{dOD}{dt}\right|_{t=0} \quad (1)$$

where C_s is glucose concentration in the cell, OD_0 is the absorbance of the cell suspension at the beginning of the cell shrinkage when the erythrocytes were put into the medium containing C concentration of glucose, and OD_∞ is the absorbance after a long time while the glucose concentration on both sides of the membrane reaches an equilibrium state.

For measurement of infinite-*cis* efflux, the treated erythrocytes were injected into 1 cm cuvette containing only 2 ml PBS. The initial rate of glucose efflux, V_m, can be determined from the curve of absorbance vs. time (Fig. 1B) as previously described [16].

2.4. Temperature dependence

The activation energy for glucose transport in the temperature region of 20–40°C can be obtained from an equation deduced from Arrhenius equation [18]:

$$\ln V = -\frac{E}{RT} + \ln V_{273\,K} + \frac{E}{273\,R} \quad (2)$$

where V is the transport rate at temperature of T, $V_{273\,K}$ is transport rate at 273 K, E is transport activation energy and R is the gas constant.

2.5. CB and phloretin inhibition

When there is an inhibitor I, according to the glucose transport kinetic equation of Michaelis–Menten, the relationship between the transport rate with $[I]$ is [19]:

$$\frac{V_P}{V} = 1 + \frac{[I]}{K_i} \quad (3)$$

where V_p and V are transport rate without and with inhibitor, respectively. $[I]$ is inhibitor concentration. The K_i is apparent inhibitory constant and it can be obtained from the slope of a plot of $1/V$ vs. $[I]$.

2.6. Intrinsic fluorescence quench of white-stripped ghosts

2.6.1. Preparation of white-stripped ghosts

In order to reduce the fluorescence contribution from other than membrane proteins, the erythrocytes were prepared into white-stripped ghosts, which were prepared as a modification of Carruthers [20]. After washing three times with PBS, the erythrocytes at 1:20 (v/v) were hemolyzed in 5P7.4 (5 mM sodium phosphate, pH 7.4) at 4°C for 30 min. When membrane fraction became a pearly white appearance after several times washing and centrifuging at $25\,000 \times g$, they were exposed to ice-cold 5P12.0 (5 mM sodium phosphate, pH adjusted to 12.0 by adding dilute NaOH) for 20 s to deplete peripheral proteins. After washing off alkaline by 5P7.4 the ghosts were frozen rapidly and thawed three times, and then stored at −25°C in 5P7.4. In our experimental condition, there were still a minor peripheral proteins on the ghosts as checked by SDS-PAGE (data not shown). But it did not interfere the fluorescence quench study because we needed only the value of fluorescence change after binding the inhibitors with GLUT1. The membrane protein concentration was calibrated according to Lowry et al. [21].

2.6.2. Fluorescence measurements

Fluorescence measurements were performed at 35°C with a Hitachi M850 spectrofluorometer. The wavelength of excitation was 295 nm with a bandwidth of 3 nm and the emission was at 340 nm with a bandwidth of 8 nm to minimize the contribution of tyrosine residues. A 310-nm filter was used. Fifty microliters of D-glucose or transport inhibitors were injected into 2 ml ghosts with 120 μg total membrane proteins in a 1-cm cuvette with a stirrer.

2.6.3. Data analysis

The unspecific effects of dilution, inner filter and ligands fluorescence reabsorption were corrected by a standard, NATA, which was measured as a function of added ligand [20,22].

When there are two kinds of luminescence in fluorescence quench, just as in glucose transporter, some tryptophan residues in hydrophilic and others in hydrophobic circumstance, a part quench factor f was introduced in the Stern–Volmer equation [23]:

Table 1
Initial glucose transport rate across the erythrocyte membranes at 35°C from 25 healthy human and 30 type 2 diabetic patients

Sample	Influx rate V_0 (mM/min)	Efflux rate V_m (mM/min)
Healthy control ($n=25$)	40.56 ± 9.04	251.92 ± 50.38
Type 2 diabetic patients ($n=30$)	31.36 ± 13.67[a]	250.03 ± 51.47

Values are the mean ± S.D. Student's t-test was employed to compare the difference between means.
[a] $P < 0.01$ as compared with controls.

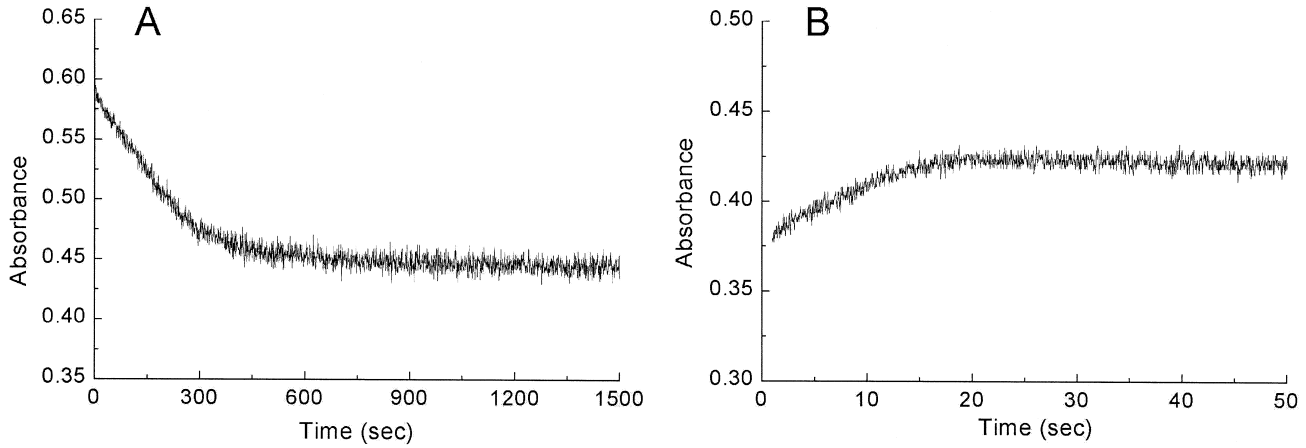

Fig. 1. Time course of D-glucose zero-*trans* influx (A) and infinite-*cis* efflux (B) across the erythrocyte membranes at 35°C. The ordinate gives the absorbance of cell suspension at 660 nm during glucose transport. The records are the accumulation of at least five identical experiments. The D-glucose concentration in the medium was 200 mM in record A, and glucose concentration inside the erythrocytes was 80 mM in record B. The glucose transport rate can be calculated from these record curves as described in Section 2.

$$\frac{F_0}{F} = 1 + fK_q[Q] \quad (4)$$

and hence,

$$\frac{1}{\Delta F} = \frac{1}{fF_0} + \frac{1}{fF_0K_q[Q]} \quad (5)$$

where $\Delta F(=F_0-F)$ is fluorescence change as quencher $[Q]$ binding, F_0 and F are the fluorescence intensity with and without quencher, respectively. K_q is apparent quenching constant and can be obtained from a double reciprocal plot [24].

3. Results

We have measured 25 healthy blood samples and 30 Chinese type 2 diabetic patient erythrocytes. The results of glucose transport across the erythrocyte membranes are shown in Table 1. As can be seen, the glucose entry rate in patient erythrocytes was reduced to 77% of the controls, but no significant difference was observed for the glucose efflux. The result is in accordance with Comi and Hamilton [11], but conflicts with Bistritzer et al. [12], whose result was obtained from nine black female patients.

The activation energy for glucose transport can be obtained from the Arrhenius plot (Eq. 2). We examined the temperature dependence of glucose transport of six patients and five healthy controls. The result is summarized in Table 2. It shows that the activation energy of glucose entry for the patient glucose transporter was increased by 32% over the controls, which is in reasonable agreement with the change in the rate of glucose entry for the patient erythrocytes as shown in Table 1. But the activation energy for glucose efflux has no significant difference, though the mean value for patients increased slightly as compared with healthy erythrocytes (Table 2).

CB causes an inhibition effect for glucose trans-

Table 2
The activation energy for glucose transport in the erythrocytes of five healthy controls and six type 2 diabetic patients

Sample	Activation energy (kJ/mol)	
	Glucose influx	Glucose efflux
Healthy controls ($n=5$)	58.89 ± 4.89	76.54 ± 18.51
Type 2 diabetic patients ($n=6$)	77.53 ± 8.87[a]	91.94 ± 19.82

The temperature region is from 20 to 40°C. Values are the mean ± S.D. Student's *t*-test was employed to compare the difference between means.

[a] $P < 0.01$ as compared with controls.

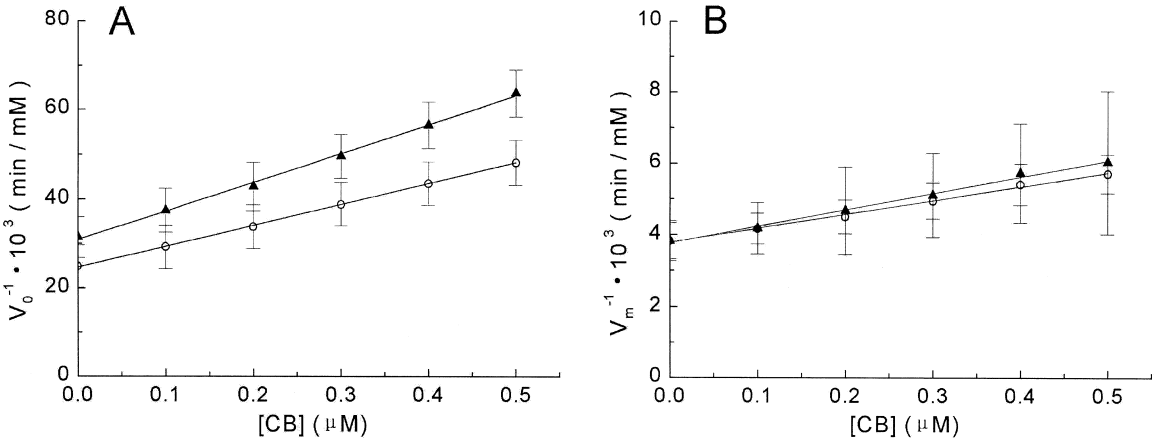

Fig. 2. Inhibitory effect of CB on glucose zero-*trans* influx (A) and infinite-*cis* efflux (B) for healthy controls (circle) and type 2 diabetic patients (triangle). Mean values and standard deviation are indicated from six control and ten patient determinations.

port after binding to the intracellular domain of GLUT1. Fig. 2 shows the relationship between the reciprocal of transport rate and CB concentration. The CB inhibitory constant of glucose entry in 10 patients was 0.46 ± 0.27 μM, which had no significant difference as compared with the value of 0.53 ± 0.40 μM for six healthy controls. For glucose efflux, the inhibitory constants also had no significant difference: 0.81 ± 0.34 μM for patients and 1.00 ± 0.45 μM for controls.

Phloretin is another kind of inhibitor of glucose transport across the erythrocyte membranes. The apparent inhibitory constant for glucose entry in six patients was 8.05 ± 4.27 μM, but even 10 μM phloretin had no significant inhibition effect on the glucose entry in another six patient erythrocytes. The phloretin inhibitory constant for glucose entry in 11 healthy controls was 2.14 ± 1.56 μM. The inhibitory constant for glucose efflux in 11 healthy controls was 10.02 ± 4.33 μM and 9.75 ± 1.87 μM for 12 diabetic patients.

Fig. 3 shows the excitation and emission spectra of white stripped ghosts. There was no spectrum difference between the controls and patients, and the spectra are mainly contributed by the tryptophan residues of the ghosts. Fig. 4 shows the relationship of reciprocal of fluorescence change with reciprocal of quencher concentration. The apparent quench constants calculated from Fig. 4 are shown in Table 3. The concentrations of glucose, CB and phloretin in study are 10–350 mM, 2–15 μM and 0.5–4 μM, respectively. As compared with healthy ghosts, it was shown that the apparent quench constant by glucose

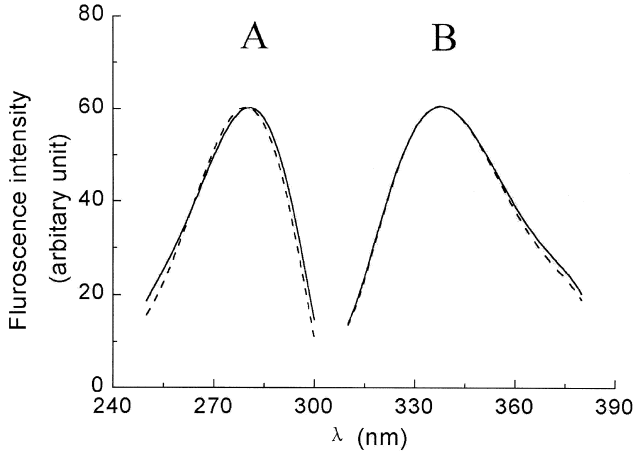

Fig. 3. The excitation (A) and emission (B) spectra of stripped white ghosts from a healthy control (solid) and a type 2 diabetic patients (dash) ghosts.

Table 3
The apparent quench constants, K_q, for the ghosts of 6 healthy controls and 10 type 2 diabetic patients at 35°C

Quencher	Quench constant K_q (M^{-1})	
	Control ghosts	Diabetic ghosts
Glucose	15.68 ± 1.01	10.59 ± 1.54[a]
CB	(6.96 ± 0.84) × 10^3	(48.02 ± 9.12) × 10^{3b}
Phloretin	(1.04 ± 0.24) × 10^5	(1.77 ± 0.19) × 10^{5a}

Values are the mean ± S.D. 120μg of total membrane proteins were used in each measurement. Student's *t*-test was employed to compare the difference between means.
[a] $P < 0.01$ as compared with controls.
[b] $P < 0.001$ as compared with controls.

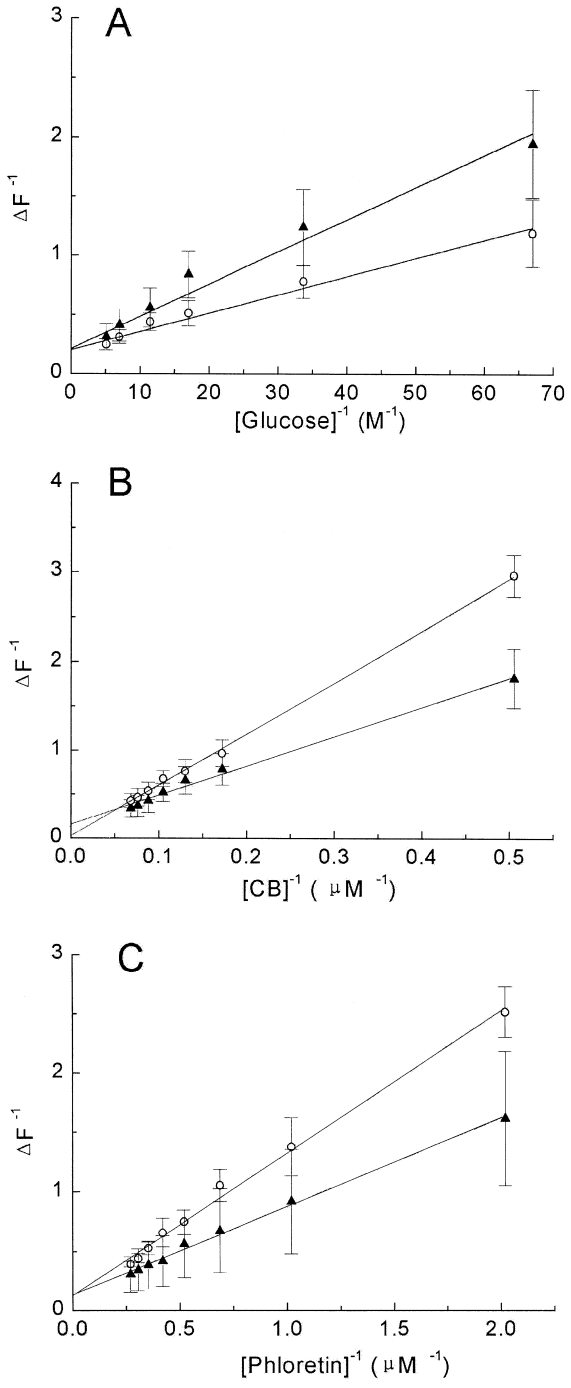

Fig. 4. Double reciprocal plots of GLUT1 fluorescence quench in the presence of glucose (A), CB (B) and phloretin (C). Mean values and standard deviation are indicated from six control (circle) and 10 patient (triangle) determinations. The apparent fluorescence quench constants can be obtained from the plots as described in Section 2.

for patients was decreased to about 40%, and the apparent quench constants by CB and phloretin were increased by 6-fold and 70%, respectively.

4. Discussion

The present results reveal that the rate of glucose entry was decreased in the erythrocyte membranes of Chinese type 2 diabetic patients as compared to the controls. The possible reasons would be as follows: (1) the alteration of the phospholipid bilayers in diabetes erythrocyte membranes; (2) change in GLUT1 content; (3) abnormality of other proteins in the erythrocyte membranes; and (4) the alteration of structure or conformation of GLUT1. We prefer the fourth reason as a most important factor.

The lipid–protein interaction can change the activity of membrane proteins [25,26] and it was reported that GLUT1 has some preferential affinity for specific lipid [27]. It was also reported that there were some changes in lipid component, stiffness and fluidity of diabetic erythrocyte membranes [28–30]. But our studies show that in patient erythrocytes there was no significant change in glucose efflux (Table 1), there was no significant change in the activation energy for glucose efflux (Table 2), the increase in activation energy for glucose entry was in accordance with the decrease in entry rate. These results suggest that the membrane surroundings play minor roles in the alteration of glucose transport in patient erythrocytes.

It was reported that GLUT1 content had been increased by about 50% [31] in human fibroblasts and skeletal muscle from the plasma membrane of non-insulin-dependent diabetes and obese subjects, and in skeletal muscle of streptozocin-induced diabetic rats [32]. As in human erythrocytes, though Harik et al. [33] reported that chronic hyperglycemia increased the density of GLUT1, there was no further study to support the result. Other reports showed that the content of GLUT1 had no changed in diabetic erythrocytes and our unpublished SDS-PAGE data also supported this. Moreover, an increase in the density of GLUT1 could not explain why glucose entry was affected and efflux was not. The content alteration of GLUT1 does not explain

the result of the temperature dependence study (Table 2) because these results did not depend on the protein contents.

Some other membrane proteins can change the activity of GLUT1 through 'protein–protein' interaction. Of particular interest is band 3. Bosman and Kay [34] reported that cells with an increased (sensitivity to) breakdown of band 3 protein display an increased asymmetry of glucose transport. We have also described that an increase in the concentration of Cl^- (the substrate of band 3) in the medium caused an increase in infinite-*cis* efflux of glucose transport [35]. Our study showed that the NO_2^- transport mediated by band 3 in the erythrocyte membranes has no significant difference between Chinese type 2 diabetic patients and the controls (data not shown). Insulin receptors are another important protein for glucose transporter activity. Longo et al. [36] reported that a mutation in arginine-86 of the insulin receptor can increase glucose transport, but there was not enough information to demonstrate the defect of this protein in diabetes. Tegos and Beutler [37] reported that the glycolytic intermediates in diabetes erythrocytes were altered as compared with healthy control, but no evidence for hormonal or metabolic regulation of sugar transport had been obtained.

Regarding the factor of the individual age, Forbes et al. [3] found that glucose uptake was reduced by about 20% in type 2 diabetic patients when using healthy elderly controls. Magnani et al. [38] reported that human erythrocytes of different age possess similar abilities to transport glucose [38]. Our measurement also indicated that the zero-*trans* entry rate has no significant relevance to sex and age for adults (data not shown). So that the age-dependent factor in glucose transport can be exclude.

Phloretin is an inhibitor of glucose transporter, which is bound to the extracellular domain of GLUT1. Our experiment showed that the inhibitory constant was increased after phloretin treatment, but no such change was observed for CB treatment. The result strongly supports that the decrease in glucose entry in the erythrocyte membranes of Chinese type 2 diabetic patients was due to the GLUT1 structure change – mostly the outer domain of the glucose transporter.

GLUT1 consists of 492 amino acid residues which spans the membrane bilayer at least 12 times. There are six tryptophan residues in the GLUT1, among them four tryptophan residues are located in the hydrophobic environment and another two tryptophan residues are in the hydrophilic environment [39]. GLUT1 alters its conformation after binding with glucose, CB or phloretin so that the intensity and the emission peak of the tryptophan fluorescence will be changed. The fluorescence intensity will decrease after the tryptophan residue moves from the hydrophilic environment to the hydrophobic environment [40]. The abnormality of fluorescence quenches in the erythrocyte membranes of patients was observed in our experiment (Fig. 4). Because the fluorescence quench came from binding of GLUT1 with their specific inhibitors or substrates, so we can exclude the contribution of the tryptophan residues of other membrane proteins from the value of fluorescence change (ΔF). On account of the lack of a suitable method to eliminate the scattering effect of the ghosts from the intrinsic fluorescence, the total amount of membrane proteins for each sample of both patients and controls was the same in the experiment. Under this condition, It was still possible to estimate the apparent quench constant according to Eq. 5 from Fig. 4 even if we did not know the exact value of F_0. The transfer tendency of tryptophan residues from the hydrophilic environment to the hydrophobic environment was decreased in patient erythrocyte membranes as binding with glucose, and the opposite tendency was observed as CB and phloretin instead of glucose (Fig. 4 and Table 3). There was no effect of freeze–thawing on the fluorescence analysis results, as this became a routine method of sample preparation for fluorescence experiments [20,41].

The regulation of glucose transport is through an unknown signal [42] in response to increased metabolic demand, such as inhibition of oxidative phosphorylation and glucose starvation in diabetes. The defect of glucose transport in diabetes can take place at this signal pathway. Condorelli et al. [43] reported that PED/PEA-15 gene can increase the content of GLUT1 in diabetes. Our study suggested that the GLUT1 itself has been changed in diabetes erythrocyte membranes, and the altered site is mostly at the outer domain rather than the inner domain of the GLUT1 protein.

Acknowledgements

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References

[1] B.B. Kahn, Lilly lecture 1995 – Glucose transport: pivotal step in insulin action, Diabetes 45 (1996) 1644–1654.
[2] M. Mueckler, Family of glucose transporter genes: implications for glucose homeostasis and diabetes, Diabetes 39 (1990) 6–11.
[3] A. Forbes, T. Elliott, H. Tildesley, D. Finegood, G.S. Meneilly, Alterations in non-insulin-mediated glucose uptake in the elderly patient with diabetes, Diabetes 47 (1998) 1915–1919.
[4] K. Kaku, A. Matsutani, M. Mueckler, M.A. Permutt, Polymorphisms of HepG2/erythrocyte glucose-transporter gene, Diabetes 39 (1990) 49–56.
[5] S.C. Elbein, M.D. Hoffman, A. Matsutani, M.A. Permutt, Linkage analysis of GLUT1 (HepG2) and GLUT2 (liver/islet) genes in familial NIDDM, Diabetes 41 (1992) 1660–1667.
[6] S. Ghosh, N.J. Schork, Genetic analysis of NIDDM, Diabetes 45 (1996) 1–14.
[7] S. Lesage, H. Zouali, N. Vionnet, A. Philippi, G. Velho, P. Serradas, P. Passa, F. Demenais, P. Froguel, Genetic analyses of glucose transporter genes in French non-insulin-dependent diabetic families, Diabetes Metab. 23 (1997) 137–142.
[8] G.E. Plante, J. Alfred, M. Chakir, The blood vessel, linchpin of diabetic lesions, Metabolism 48 (1999) 406–409.
[9] A.S. Krolewski, L.M.B. Laffel, M. Krolewski, M. Quinn, J.H. Warram, Glycosylated hemoglobin and the risk of microalbuminuria in patients with insulin-dependent diabetes mellitus, New Engl. J. Med. 332 (1995) 1251–1255.
[10] I.I. Concha, F.V. Velasquez, J.M. Martinez, C. Angulo, A. Droppelmann, A.M. Reyes, J.C. Slebe, J.C. Vera, D.W. Golde, Human erythrocytes express GLUT5 and transport fructose, Blood 89 (1997) 4190–4195.
[11] R.J. Comi, H. Hamilton, Reduction of red cell glucose transporter intrinsic activity in diabetes running, Horm. Metab. Res. 26 (1994) 26–32.
[12] T. Bistritzer, L.M. Roeder, L. Hanukoglu, P.A. Levin, Non-insulin dependent diabetic patients have increased glucose uptake in red blood cells, Horm. Metab. Res. 23 (1991) 70–73.
[13] X.J. Hu, Z.H. Zhang, F. Peng, H.Q. Zhou, Studies on the abnormality of glucose transport across the erythrocyte membranes in non-insulin-dependent diabetic patients, Acta Biophys. Sinica 14 (1998) 221–226.
[14] S.W. Cushman, L.J. Wardzala, Potential mechanism of insulin action on glucose transport in the isolated rat adipose cell, J. Biol. Chem. 255 (1980) 4758–4762.
[15] A.K. Sen, W.F. Widdas, Determination of the temperature and pH dependence of glucose transfer across the human erythrocyte membrane measured by glucose exit, J. Physiol. 160 (1962) 392–403.
[16] Z.H. Zhang, F. Peng, H. Xu, A method for rapid measurement of glucose transport across the erythrocyte membranes, Acta Biophys. Sinica 9 (1993) 402–406.
[17] F. Peng, Z.H. Zhang, Effects of DIDS on function of glucose transport protein in red cell membranes, Chinese Sci. Bull. 41 (1996) 68–72.
[18] A.R. Walmsley, The dynamics of the glucose transporter, Trends Biochem. Sci. 13 (1988) 226–231.
[19] R.M. Krupka, D. Rosa, An experimental test for cyclic versus linear transport models: the mechanism of glucose and choline transport in erythrocytes, J. Biol. Chem. 256 (1981) 5410–5416.
[20] A. Carruthers, Anomalou asymmetric kinetics of human red cell hexose transfer: role of cytosolic adenosine 5′-triphosphate, Biochemistry 25 (1986) 3592–3602.
[21] O.H. Lowry, N.J. Rosebrough, A.L. Farr, R.J. Randall, Protein measurement with the folin phenol reagent, J. Biol. Chem. 193 (1951) 265–275.
[22] A.B. Pawagi, C.M. Deber, Ligand-dependent quenching of tryptophan fluorescence in human erythrocyte hxose transport protein, Biochemistry 29 (1990) 950–955.
[23] S.S. Lehrer, Solute perturbation of protein fluorescence – the quenching of the tryptophyl fluorescence of model compounds and of lysozyme by iodide ion, Biochemistry 10 (1971) 3254–3263.
[24] R.M. McCarthy, P. Farmer, D. Sheehan, Binding of 2-hydroxy-5-nitrobenzyl alcohol to rat alpha class glutathione S-transferases; evidence for binding at tryptophan 21, Biochim. Biophys. Acta 1293 (1996) 185–190.
[25] A.G. Lee, How lipids interact with an intrinsic membrane protein: the case of the calcium pump, Biochim. Biophys. Acta 1376 (1998) 381–390.
[26] W. Dowhan, Genetic analysis of lipid–protein interactions in *Escherichia coli* membranes, Biochim. Biophys. Acta 1376 (1998) 455–466.
[27] S. Naderi, K. Doyle, D.L. Melchior, Preferential association of membrane phospholipids with the human erythrocyte hexose transporter, Biochim. Biophys. Acta 1236 (1995) 10–14.
[28] M. Hofmann, P. Mainka, H. Tritschler, J. Fuchs, G. Zimmer, Decrease of red cell membrane fluidity and –SH groups due to hyperglycemic conditions is counteracted by alpha-lipoic acid, Arch. Biochem. Biophys. 324 (1995) 85–92.
[29] S.I. Muzulu, R.F. Bing, R.I. Norman, A.C. Burden, Human red cell membrane fluidity and calcium pump activity in normolipidaemic type II diabetic subjects, Diabet. Med. 11 (1994) 763–767.
[30] D.E. McMillian, N.G. Uterback, J. LaPuma, Red cell deformability in diabetes, Diabetes 27 (1978) 895–901.
[31] C. Miele, P. Formisano, G. Condorelli, M. Caruso, F. Oriente, F. Andreozzi, C.G. Tocchetti, G. Riccardi, F. Beguinot, Abnormal glucose transport and GLUT1 cell-surface

content in fibroblasts and skeletal muscle from NIDDM and obese subjects, Diabetologia 40 (1997) 421–429.
[32] A. Klip, A. Marette, D. Dimitrakoudis, T. Ramlal, A. Giacca, Z.Q. Shi, M. Vranic, Effect of diabetes on glucoregulation. From glucose transporters to glucose metabolism in vivo, Diabetes Care 15 (1992) 1747–1766.
[33] S.I. Harik, R.A. Behmand, B.M. Arafah, Chronic hyperglycemia increased the density of glucose transporters in human erythrocyte membranes, J. Clin. Endocrinol. Metab. 72 (1991) 814–818.
[34] G.J.C.G.M. Bosman, M.M.B. Kay, Alterations of band 3 transport protein by cellular ageing and disease: erythrocyte band 3 and glucose transport share a functional relationship, Biochem. Cell Biol. 68 (1990) 1419–1427.
[35] Z.H. Zhang, F. Peng, X.Q. Li, H.Q. Zhou, X.J. Hu, Interaction between the anion exchange protein and the glucose transport protein in human red cell membranes, Acta Biochim. Biophys. Sinica 28 (1996) 665–670.
[36] N. Longo, S.D. Langley, M.J. Still, Role of arginine 86 of the insulin receptor in insulin binding and activation of glucose transport, Biochim. Biophys. Acta 1402 (1998) 86–94.
[37] C. Tegos, E. Beutler, Red cell glycolytic intermediates in diabetic patients, J. Lab. Clin. Med. 96 (1980) 85–89.
[38] M. Magnani, E. Piatti, N. Serafini, F. Palma, M. Dacha, G. Fornaini, The age-dependent metabolic decline of the red blood cell, Mech. Ageing Dev. 22 (1983) 295–308.
[39] J.J. Chin, B.H. Jhun, C.Y. Jung, Structural basis of human erythrocyte glucose transporter function pH effect on intrinsic fluorescence, Biochemistry 31 (1992) 1945–1951.
[40] R.M. Krupka, Asymmetrical binding of phloretin to the glucose transport system of human erythrocytes, J. Membr. Biol. 83 (1985) 71–80.
[41] A. Janoshazi, G. Kifor, A.K. Solomon, Conformational changes in human red cell membrane proteins induced by sugar binding, J. Membr. Biol. 123 (1991) 191–207.
[42] F. Ismail-Beigi, Metabolic regulation of glucose transport, J. Membr. Biol. 135 (1993) 1–10.
[43] G. Condorelli, G. Vigliotta, C. Lavarone, M. Caruso, C.G. Tocchetti, F. Andreozzi, A. Cafieri, M.F. Tecce, P. Formisano, L. Beguinot, F. Beguinot, PED/PEA-15 gene controls glucose transport and is overexpressed in type 2 diabetes mellitus, EMBO J. 17 (1998) 3858–3866.