References

1. Andersen L, Dinesen B, Jørgensen PN et al. (1993) Enzyme immunoassay for intact human insulin in serum or plasma. Clin Chem 39: 578–582
2. Kjems LL, Røder ME, Dinesen B et al. (1993) Highly sensitive enzyme immunoassay of proinsulin immunoreactivity with use of two monoclonal antibodies. Clin Chem 39: 2146–2150
3. Matthews DR, Hosker JP, Rudenski AS et al. (1985) Homeostasis model assessment: insulin resistance and β-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 28: 412–419

Stability of the glucose transporter in plasma membranes of human erythrocytes

Dear Sir,

The facilitative glucose transporter (GLUT-1) glycoprotein attains its highest density in human erythrocyte membranes, while erythrocytes of most other mammals have little if any glucose transporters [1]. The functional importance of the high density of the glucose transporter in erythrocytes is unknown, particularly in view of their relatively low glucose metabolism. However, this plethora of glucose transporters allows near instantaneous glucose equilibration across the erythrocyte membrane, thus considerably increasing the glucose-carrying capacity of human blood [2]. The stability of the glucose transporter during the lifespan of human erythrocytes is unknown although such knowledge may have important implications. For example, we recently reported that erythrocytes from diabetic subjects have a higher density of the glucose transporter [3], but could not ascertain whether this finding was related to the possible decreased erythrocyte lifespan in diabetic subjects [4].

Venous blood drawn from four healthy men was defibrinated with glass beads and the serum and buffy coat discarded. Aliquots (11 ml) of semipacked erythrocytes from each subject were fractionated as described by Murphy [5]. The top (light) and the bottom (heavy) 5 % of the erythrocytes from each subject were harvested. Samples of the harvested erythrocytes were analysed for their mean corpuscular volume, mean corpuscular haemoglobin content, and mean corpuscular haemoglobin concentration by automatic analyser. The remainder of the harvested erythrocytes were washed, lysed, and their membranes were used for cytochalasin B binding [3]. D-glucose-displaceable [^{3}H]cytochalasin B binding was performed at ligand concentrations of 0.1 to 1.8 µmol/l. Saturation binding isotherms were analysed according to Scatchard [6] to obtain the dissociation constant (K_d), in µmol/l, and the maximum density of binding (B_{max}), in pmol/mg of erythrocyte membrane protein. Comparisons between the results obtained from the "light" and "heavy" erythrocyte fractions from each subject were performed by the paired Student's t-test (2-tailed). Significance was considered at $p < 0.05$.

Fractionation of erythrocytes according to their density yields "light" fractions enriched with reticulocytes and new erythrocytes and "heavy" fractions enriched with old erythrocytes. This is explained by the loss of water and ions from aging erythrocytes. Our findings of a higher mean corpuscular volume and a lower mean corpuscular haemoglobin concentration in the "light" fraction than in the "heavy" fraction of erythrocytes obtained from the same subjects was therefore expected (Table 1). However, we found no significant differences between "light" and "heavy" erythrocytes in their B_{max} or K_d values for cytochalasin B binding (Table 1).

Table 1. Mean corpuscular volume and mean corpuscular haemoglobin concentrations in heavy and light erythrocytes

	"Light" erythrocytes	"Heavy" erythrocytes	P. value
Mean corpuscular volume (µm^3)	91.3 ± 4.8	81.6 ± 4.9	$p < 0.001$
Mean corpuscular haemoglobin (pg)	30.2 ± 1.6	31.1 ± 2.2	NS
Mean corpuscular haemoglobin concentration (g/dl)	33.2 ± 0.9	38.2 ± 1.0	$p < 0.001$
Cytochalasin B binding			
B_{max} (pmol/mg protein)	326 ± 44	381 ± 89	NS
K_d (µmol/l)	0.14 ± 0.03	0.17 ± 0.04	NS

The values denote means ± SD for light and heavy erythrocytes obtained from four men. Statistical differences were calculated by the Student's paired t-test (2-tailed)

These results, given that no appreciable protein synthesis occurs in mature erythrocytes, suggest a stable glucose transporter glycoprotein in erythrocyte membranes. The stability of the glucose transporter in erythrocytes may not be a universal property of glucose transporter glycoproteins in other cell membranes but may reflect decreased protein degradation in erythrocytes. These results also suggest that the glucose-carrying capacity of erythrocytes does not change with age.

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References

1. Jones MN, Nickson JK (1981) Monosaccharide transport proteins of the human erythrocyte membranes. Biochim Biophys Acta 650: 1–20
2. Jacquez JA (1984) Red blood cell as glucose carrier: significance for placental and cerebral glucose transfer. Am J Physiol 246: R289–R298
3. Harik SI, Behmand RA, Arafah BM (1991) Chronic hyperglycemia increases the density of glucose transporters in human erythrocyte membranes. J Clin Endocrinol Metab 72: 814–818
4. Peterson CM, Jones RL, Koenig RM, Melvin ET, Lehrman ML (1977) Reversible hematologic sequelae of diabetes mellitus. Ann Intern Med 86: 425–429
5. Murphy JR (1973) Influence of temperature and method of centrifugation on the separation of erythrocytes. J Lab Clin Med 82: 334–341
6. Scatchard G (1949) The attractions of proteins for small molecules and ions. Ann NY Acad Sci 51: 660–672

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