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Changes in Erythrocyte Sorbitol Concentrations Measured Using an Improved Assay System in Patients With Diabetic Complications and Treated With Aldose Reductase Inhibitor

An accelerated polyol pathway may induce the development of diabetic complications (1-10). In previous reports, the erythrocyte sorbitol concentration was measured both enzymatically and by gas-liquid chromatography. These assay systems were complicated, however, and provided contradictory results on the role of sorbitol metabolism, probably because of the poor reproducibility of the assay methods (11-16). We devised an improved assay system for the measurement of sorbitol concentration (17). Our method enabled us to measure the erythrocyte sorbitol concentration with good

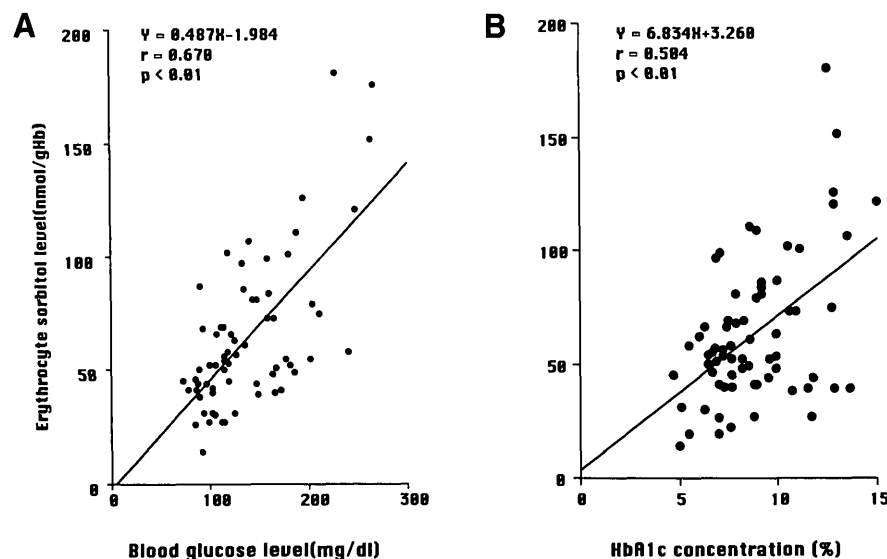


Figure 1—A significant correlation was observed between erythrocyte sorbitol concentration and both blood glucose level (A) and HbA_{1c} concentration (B).

reproducibility and to analyze the correlation between the sorbitol concentration and diabetic complications and the influence of an aldose reductase inhibitor on its concentration.

Of 68 diabetic patients (12 from the outpatient clinic and 56 from admitted patients), 5 had IDDM and the others had NIDDM. The mean age of IDDM patients (2 men and 3 women) was 29.2 ± 11.8 (12-44) years, while that of NIDDM patients (32 men and 31 women) was 56.0 ± 12.4 (22-77) years. There were 39 healthy volunteers (29 men and 10 women, mean age 35 ± 8 [22-56] years) studied.

The erythrocyte sorbitol concentrations were compared between the patients with and without the diabetic complications of ophthalmopathy (more severe than proliferative retinopathy), nephropathy (positive microalbuminuria or proteinuria and/or lowered creatinine clearance), and peripheral neuropathy (numbness and low tibial motor nerve conductivity [MCV]).

Before and after the treatment with aldose reductase inhibitor (ARI) (epalrestat, 5-[(1Z, 2E)-2-methyl-3-phenylpropenyli-dene]-4-oxo-2-thioxo-3-thiazolidineacetic acid (Ono Pharmaceutical, Chuō-ku, Osaka, Japan), the sorbitol concentrations in the erythrocytes of the patients were determined.

Enzymatic measurement of sorbitol concentration

To remove the protein components of the erythrocyte suspension, 1.0 ml of 0.475

mol/l NaOH and 1.0 ml of 0.5 mol/l ZnSO₄ were added and mixed to a uniform consistency. The mixture was then centrifuged at 1,000g for 10 min at 4°C. Then 2 ml of this supernatant was mixed with 1.0 ml of 0.165 mol/l Tris-HCl buffer (pH 8.6) containing 0.02 mol/l EDTA, 1.0 ml (250 mg/dl) NAD, and 0.05 ml (40 U/ml) sorbitol dehydrogenase. After a 30-min incubation of the reaction mixture at 37°C, the NADH concentration produced was determined by spectrophotometry (excitation at 366 nm and emission at 452 nm), and then the concentration of sorbitol was calculated. The reproducibility and linearity in this assay system were more reliable than those of the previously reported method (17). The intra- and interassay coefficients of variance were 3.9 and 3.6%.

We investigated the relationship between erythrocyte sorbitol levels and complications in diabetic patients. The erythrocyte sorbitol concentrations in diabetic patients (68.43 ± 34.29 nmol/gHb in NIDDM; 50.20 ± 18.21 nmol/gHb in IDDM) were significantly increased compared with those in healthy control subjects (38.50 ± 8.10 nmol/gHb). A significant correlation was observed between the erythrocyte sorbitol concentrations and blood glucose levels or HbA_{1c} concentrations (Fig. 1). Treatment with ARI effectively reduced the increased erythrocyte sorbitol concentrations in the NIDDM patients (14 patients, decreased from 98 ± 12 to 58 ± 8 nmol/gHb, $P <$

0.05). The sorbitol concentrations were significantly high in the patients with diabetic retinopathy (26 patients, 90.7 ± 46.1 , $P < 0.01$) and neuropathy (34 patients, 80.9 ± 44.3 , $P < 0.05$) compared with those in patients without these complications (21 patients, 47.9 ± 24.7 and 16 patients, 48.6 ± 26.6).

Thus, this modified assay system may be the best way to measure sorbitol at the present time. Precise measurement of erythrocyte sorbitol content may assist in the understanding of the pathophysiological condition of diabetes and may be useful for certification of distorted sorbitol metabolism and prevention of the development of diabetic complications in clinical practice.

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Glucose Tolerance, Insulin Sensitivity, and the Homeostasis Model Assessment Method

Matsumoto et al. (1) have recently published an impressive study of oral glucose tolerance test (OGTT) data from obese and nonobese Japanese subjects. They studied insulin secretion and insulin sensitivity findings derived from OGTT data and homeostasis model assessment (HOMA).

As they described briefly in the METHODS section, insulin resistance (R) was assessed as the R determined with HOMA, with $R = \text{insulin}/(22.5 e^{-\ln \text{glucose}})$. However, simple mathematics dictate that this calculation is identical to $(\text{insulin} \times \text{glucose})/22.5$.

It would be simpler to refer to this HOMA method as a mere multiplication of baseline values of insulin and glucose.

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Response to van Haeften

The formula for the insulin resistance index in homeostasis model assessment

In our article (1), the formula for insulin resistance (R) assessed by homeostasis model assessment (HOMA) is presented as $\text{HOMA } (R) = \text{insulin}/(22.5 e^{-\ln \text{glucose}})$. As van Haeften mentions (2), the formula $\text{insulin}/(22.5 e^{-\ln \text{glucose}})$ is equal to $(\text{insulin} \times \text{glucose})/22.5$. In the original report