Clinical Significance of Erythrocyte Sorbitol–Blood Glucose Ratios in Type II Diabetes Mellitus

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The polyol pathway has been implicated in the pathogenesis of diabetic complications. To determine the activity of the polyol pathway, the ratio of erythrocyte sorbitol to blood glucose, which reflects aldose reductase activity, was evaluated in 329 patients with type II (non-insulin-dependent) diabetes mellitus and in 100 nondiabetic age-matched control subjects. Although erythrocyte sorbitol levels were markedly elevated, sorbitol-glucose ratios were significantly lower in diabetic patients than in nondiabetic subjects. Sorbitol-glucose ratios in diabetic patients decreased progressively as blood glucose and hemoglobin A_{1c} (HbA_{1c}) levels increased. Sorbitol-glucose ratios were also studied during a 75-g oral glucose tolerance test. Ratios were again lower in diabetic patients than those in nondiabetic subjects and significantly decreased 120 min after glucose loading. The ratio in diabetic patients also fell with increasing age of the patients. In diabetic patients with neuropathy, retinopathy, or nephropathy, however, sorbitol-glucose ratios were significantly higher than in those without these complications; ratios increased further as complications became more severe. Our findings suggest that the affinity of aldose reductase for glucose in patients with diabetic complications may be increased and that the polyol pathway is implicated in the pathogenesis of diabetic complications. *Diabetes Care* 13:461–67, 1990

Among the many proposed hypotheses on the pathogenesis of diabetic complications, the role of the polyol pathway has received much attention from investigators. Recently, numerous lines of evidence have accumulated that support the hypothesis that increased glucose flux through the polyol pathway and the resulting accumulation of sorbitol are involved in the pathogenesis of long-term complications of diabetes (1–4). The eye lens accumulates sorbitol or galactitol in diabetic or galactosemic rats, respectively, and eventually cataract results. However, cataract formation can be prevented or effectively delayed by various aldose reductase inhibitors (5–8), demonstrating that aldose reductase is a crucial factor in the initiation of cataracts (1,2). Aldose reductase inhibitors were also effective in improving both the clinical symptoms and motor nerve conduction velocities in diabetic patients with neuropathy (9–11) and in experimental animals (12), despite the presence of persistent hyperglycemia. Furthermore, proteinuria in diabetic rats has been recently reported to be diminished by an aldose reductase inhibitor (13). On the other hand, conflicting results have been reported regarding aldose reductase activity in diabetes mellitus, suggesting either an increase, a decrease, or no change in activity (14–19).

Because erythrocyte sorbitol levels are linearly related to plasma glucose levels (20,21), the ratio of erythrocyte sorbitol to blood glucose may be a good indicator of polyol pathway activity in erythrocytes (22).

We determined the activity of the polyol pathway in erythrocytes in terms of the sorbitol-glucose ratio in type II (non-insulin-dependent) diabetic patients and evaluated the clinical significance of the sorbitol-glucose ratio.

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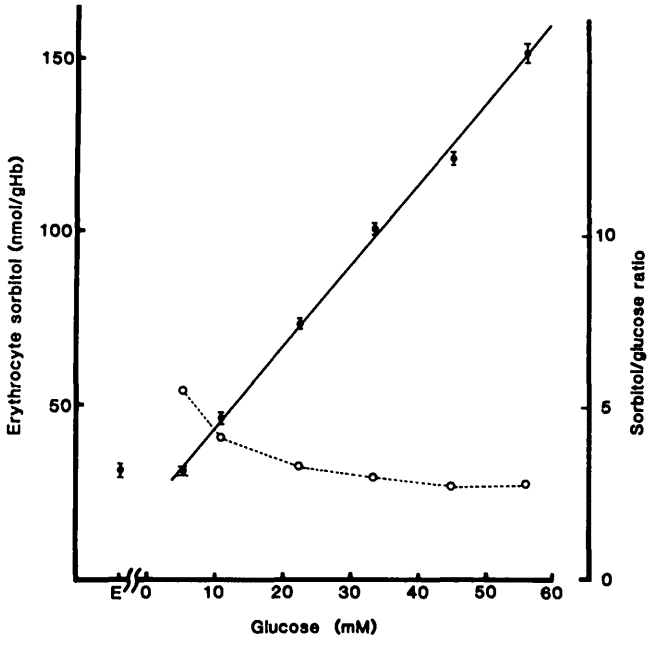

FIG. 1. Sorbitol accumulation in erythrocytes (●) and sorbitol-glucose ratios (○) from in vitro experiments. Erythrocytes from healthy subject were incubated in Krebs-Ringer bicarbonate buffer containing different concentrations of glucose for 2 h (n = 4). E, endogenous sorbitol content of erythrocytes before incubation. Values are means ± SE.

RESEARCH DESIGN AND METHODS

Blood samples were taken ~2 h after breakfast from 329 type II diabetic patients (193 men, 136 women; mean ± SE age 57.0 ± 0.7 yr) and from 100 healthy nondiabetic age-matched control subjects (52 men, 48 women; age 53.3 ± 1.8 yr). Ninety-five diabetic patients had neuropathy (painful paresthesia, dysesthesia, loss of tendon reflexes, or loss of vibratory sense), 26 had nephropathy (reagent-strip–positive persistent proteinuria or serum creatinine >168 μM), and 118 had retinopathy; 95 had simple retinopathy with microaneurisms, dot and blot hemorrhages, and hard or soft exudates, whereas 23 had proliferative retinopathy with neovascularization, vitreous hemorrhage, or retinal detachment. Fifty-five diabetic patients were treated with oral hypoglycemic agents, 106 were treated with insulin, and 168 were maintained on diet alone.

Blood glucose was measured by the glucose oxidase method with a Dri-Chem 1000 analyzer (Fuji Photo Film, Tokyo) (23). Hemoglobin A_{1c} (HbA_{1c}) levels were determined by high-performance liquid chromatography (24). Erythrocyte sorbitol was measured by the fluorometric enzyme assay described previously (20,25,26). The inter- and intra-assay variances of this assay for sorbitol were 4 and 1%, respectively. The sorbitol-glucose ratio was also calculated. Because erythrocyte sorbitol levels have been shown to correlate with coincident plasma glucose concentrations (20,21), Malone et al. (22) expressed these levels as a function of the coincident plasma glucose concentrations. The sorbitol-glucose ratio was applied to standardize the sorbitol content of cells exposed to the wide range of plasma glucose concentrations that characteristically occur in type I (insulin-dependent) diabetes. Malone et al. (22) suggest that the sorbitol-glucose ratio expresses the polyol pathway activity per unit of available glucose.

In vivo experiments. Erythrocytes from heparinized healthy human blood were incubated for 2 h in Krebs-Ringer bicarbonate buffer (KRBB) with different concentrations of glucose as described previously (25,26). Sorbitol content was then measured as described above.

Data are means ± SE. Statistical analysis was carried out with Student's t test.

RESULTS

Figure 1 shows the sorbitol content of erythrocytes incubated in KRBB containing different concentrations of glucose for 2 h. The sorbitol content increased linearly with the increase of glucose concentration, a result similar to that previously reported (21). Therefore, the erythrocyte sorbitol content in vivo was also expected to increase linearly. However, the actual sorbitol-glucose ratios calculated from the data decreased as the glucose level increased. Thus, although absolute sorbitol levels in erythrocytes rose as blood glucose levels increased, less sorbitol was formed relative to the ambient blood glucose level (Fig. 1).

In control subjects, the blood glucose and erythrocyte sorbitol levels were 5.5 ± 0.1 mM and 31.7 ± 1.0 nmol/g Hb, respectively, whereas in diabetic patients, levels were significantly increased to 13.7 ± 0.3 mM ($P < 0.001$) and 47.0 ± 1.0 nmol/g Hb ($P < 0.001$), respectively (Table 1). In diabetic patients, the erythrocyte sorbitol content progressively increased with the increase of blood glucose levels (n = 329; r = 0.36, $P < 0.001$; Fig. 2).

The sorbitol-glucose ratio in diabetic patients was significantly lower than in control subjects (3.93 ± 0.11 vs. 6.11 ± 0.22, respectively, $P < 0.001$; Table 1). The sorbitol-glucose ratio in diabetic patients with blood glucose levels <8.3 mM was 6.28 ± 0.34 (n = 60), which did not differ significantly from the level in control subjects. However, the ratio decreased progressively with the increase of blood glucose (n = 329; $r = -0.55$, $P < 0.001$, $y = -0.19x + 6.56$; Fig. 3), a phenomenon similar to that noted in the in vitro experiments (Fig. 1).

Figure 4 shows the inverse correlation present between the sorbitol-glucose ratio and HbA_{1c} levels. Again, as the percentage of HbA_{1c} increased, the ratio became lower. It was also found that the ratio in patients with an HbA_{1c} level <6% was not different from that in control subjects.

We next analyzed the erythrocyte sorbitol content and

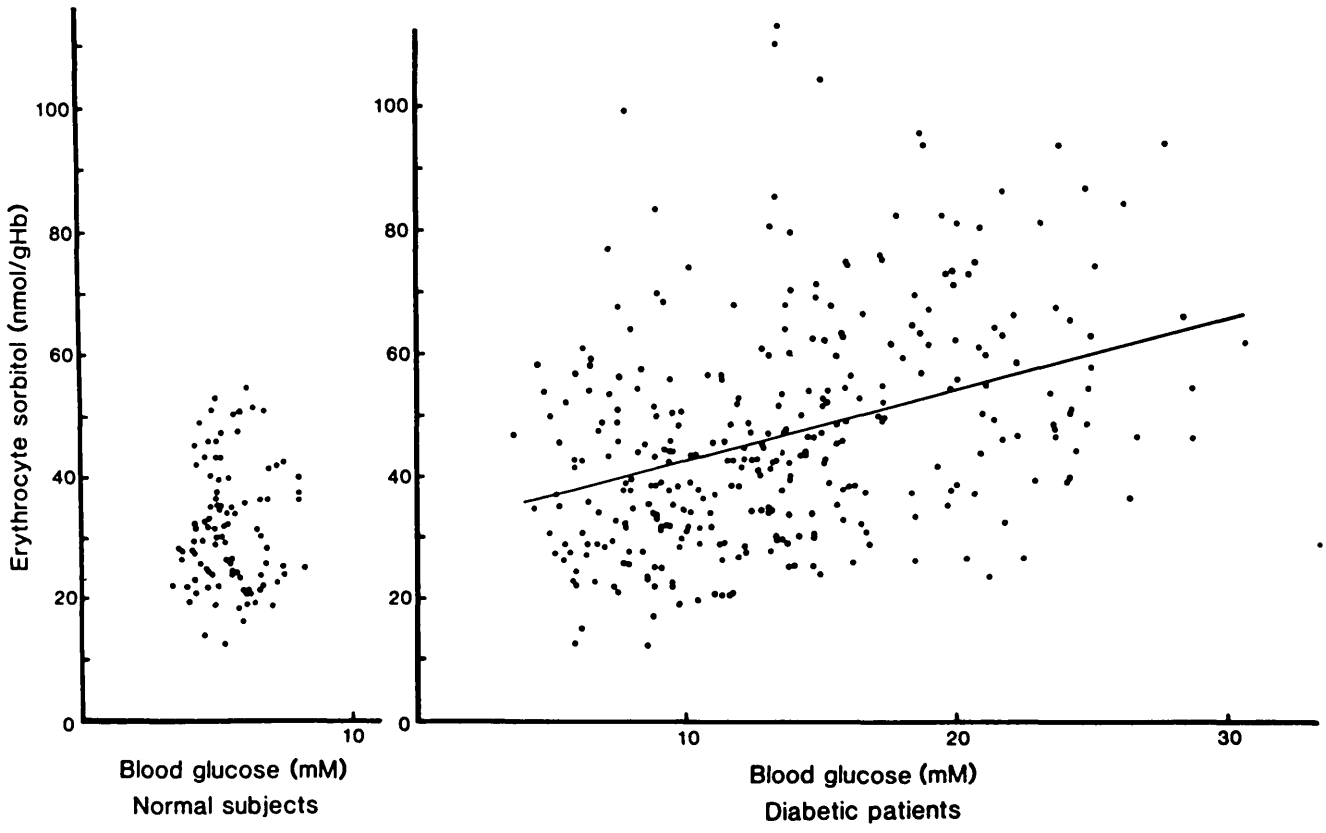

FIG. 2. Correlation between blood glucose levels and erythrocyte sorbitol content in nondiabetic (normal) subjects (n = 100) and diabetic patients (n = 329; r = 0.36, $P < 0.001$, $y = 1.13x + 31.3$).

the sorbitol-glucose ratio with respect to sex. No significant difference was found in blood glucose levels, sorbitol content, or sorbitol-glucose ratio between diabetic men and women or nondiabetic men and women (data not shown).

We compared the sorbitol-glucose ratio during an oral glucose tolerance test (OGTT) in small groups, because Malone et al. (22) reported that after an 8-h fast, the sorbitol-glucose ratio increased in type I diabetic patients, even in those with normal glucose and/or HbA_{1c} levels. Sixteen control subjects (6 men, 10 women; age 55.4 ± 3.4 yr) and 25 newly diagnosed age-matched

TABLE 1
Sorbitol-glucose ratios in nondiabetic subjects and in diabetic subjects with and without diabetic complications

	n	Blood glucose (mM)	Erythrocyte sorbitol (nmol/g Hb)	Sorbitol-glucose ratio
Nondiabetic subjects	102	5.5 ± 0.1	31.7 ± 1.0	6.11 ± 0.22
Diabetic patients	329	13.7 ± 0.3	47.0 ± 1.0	3.93 ± 0.11
Neuropathy				
Without	234	13.6 ± 0.3	45.5 ± 1.2*	3.72 ± 0.12†
With	95	13.8 ± 0.7	50.7 ± 1.8*	4.44 ± 0.24†
Nephropathy				
Without	303	13.8 ± 0.3	46.9 ± 1.1	3.84 ± 0.11†
With	26	11.9 ± 1.3	47.8 ± 3.7	4.99 ± 0.62†
Without retinopathy	211	13.3 ± 0.4*	46.2 ± 1.4	3.85 ± 0.13‡
Retinopathy				
Simple	95	15.0 ± 0.7*	48.3 ± 1.7	3.89 ± 0.23‡
Proliferative	23	11.8 ± 1.2*	48.4 ± 3.3	4.79 ± 0.52‡

Values are means ± SE. $P < 0.001$ vs. nondiabetic subjects.
*$P < 0.05$, †$P < 0.01$, ‡$P < 0.025$, for comparisons within complications (i.e., neuropathy, nephropathy, and retinopathy).

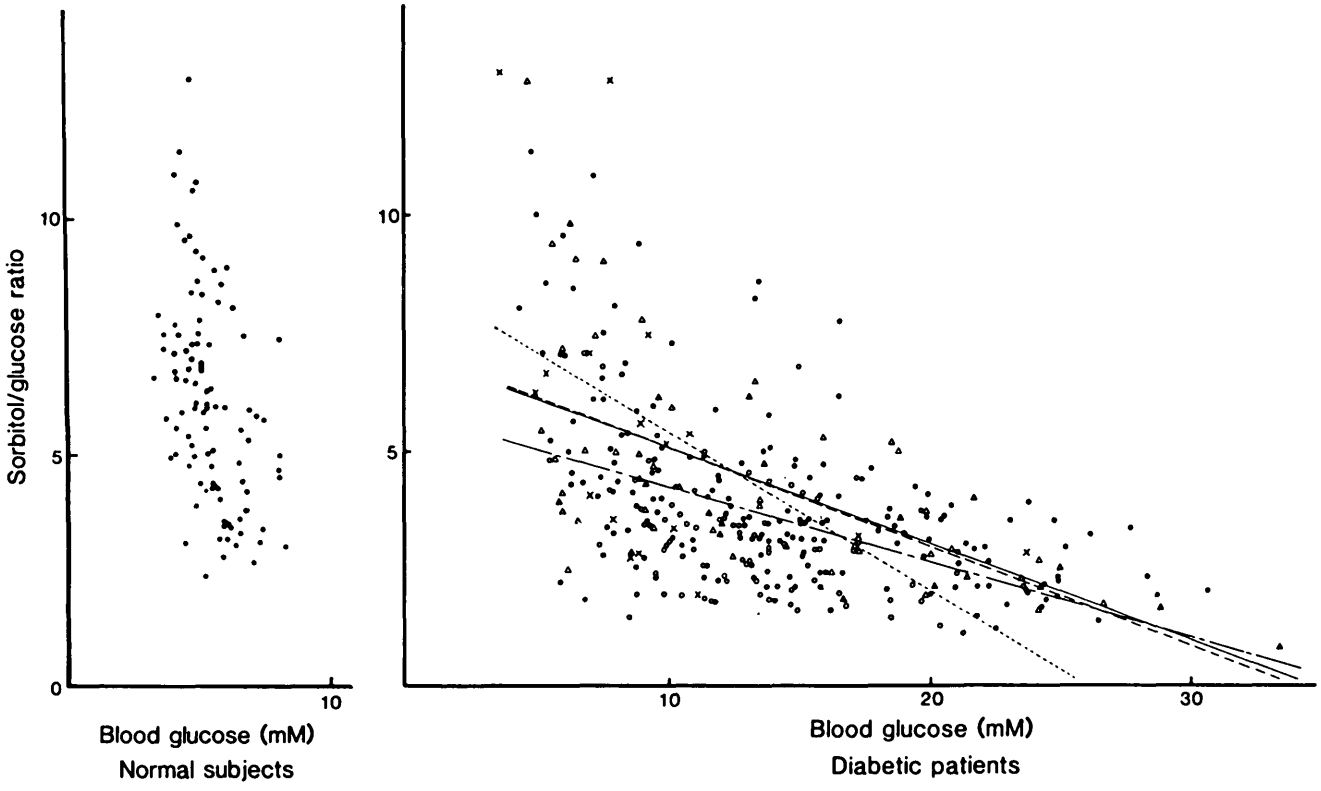

FIG. 3. Correlation between blood glucose levels and sorbitol-glucose ratios in normal (nondiabetic) subjects (n = 100) and diabetic patients (n = 329). — · —, Patients without diabetic complications (○, r = −0.45, $P < 0.001$, y = −0.16x + 5.38). ———, Patients with 1 complication (△; r = −0.62, $P < 0.001$, y = −0.20x + 7.12). – – – –, Patients with 2 complications (●; r = −0.72, $P < 0.001$, y = −0.21x + 7.17). · · · · , Patients with 3 complications (×; r = −0.50, $P < 0.05$, y = −0.34x + 8.76). Regression line for all diabetic patients was y = −0.19x + 6.56 (n = 329; r = −0.55, $P < 0.001$).

diabetic patients (20 men, 13 women; age 56.7 ± 2.2 yr) underwent the 75-g OGTT. The erythrocyte sorbitol content was determined in blood taken both before and 120 min after ingestion of the glucose load, and then the sorbitol-glucose ratio was calculated (Table 2). In control subjects, the erythrocyte sorbitol content and sorbitol-glucose ratio did not alter. However, in diabetic patients, the fasting sorbitol-glucose ratio (4.33 ± 0.39) was again significantly lower than in control subjects (5.69 ± 0.36, $P < 0.05$), and the ratio further decreased to 2.97 ± 0.25 at 120 min after glucose loading ($P <$ 0.001).

Sorbitol-glucose ratios in patients with diabetic complications are shown in Table 1. In diabetic patients with neuropathy, the erythrocyte sorbitol level and the sorbitol-glucose ratio were significantly higher than in those without neuropathy. The same pattern was also seen for nephropathy. The sorbitol-glucose ratio in diabetic patients with proliferative retinopathy was also significantly higher than in those without retinopathy. There were no significant differences in blood glucose and erythrocyte sorbitol levels between diabetic patients with or without nephropathy or retinopathy.

We then compared the sorbitol-glucose ratios in accordance with the number of diabetic complications present (Fig. 5). The ratio in patients completely free of diabetic complications was 3.68 ± 0.13 (n = 187). In those with only one complication (n = 62; neuropathy, 20; retinopathy, 42), the ratio was 4.34 ± 0.29, which

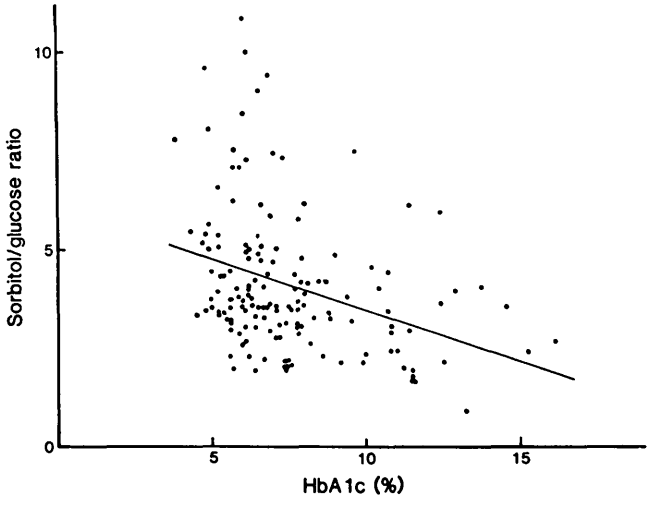

FIG. 4. Correlation between HbA_{1c} and sorbitol-glucose ratios in diabetic patients (n = 151; r = −0.34, $P < 0.001$, y = −0.26x + 6.01).

TABLE 2
Sorbitol-glucose ratios before and after 120-min 75-g oral glucose tolerance test (OGTT)

	Before OGTT	After OGTT	*P* (paired *t* test)
Nondiabetic subjects (*n* = 16)			
Blood glucose (mM)	5.3 ± 0.1	5.8 ± 0.2	NS
Erythrocyte sorbitol (nmol/g Hb)	29.9 ± 1.8	31.7 ± 4.3	NS
Sorbitol-glucose ratio	5.69 ± 0.36	5.51 ± 0.69	NS
Diabetic patients (*n* = 25)			
Blood glucose (mM)	8.4 ± 0.6*	16.5 ± 1.1*	<0.001
Erythrocyte sorbitol (nmol/g Hb)	34.4 ± 3.2	45.8 ± 3.7†	<0.001
Sorbitol-glucose ratio	4.33 ± 0.39‡	2.97 ± 0.27§	<0.001

Values are means ± SE.
*$P < 0.001$, †$P < 0.002$, ‡$P < 0.05$, §$P < 0.01$, vs. nondiabetic subjects.

was significantly higher than in patients without complications ($P < 0.05$), whereas there were no significant differences in the blood glucose or erythrocyte sorbitol levels between groups. The ratio in patients with three complications ($n = 17$) was 5.52 ± 0.79, which was also significantly higher ($P < 0.05$) than in patients with two complications (3.82 ± 0.25, $n = 63$; neuropathy + nephropathy, 4; neuropathy + retinopathy, 55; nephropathy + retinopathy, 4). In diabetic patients with three complications, the ratio was the highest, although blood glucose levels were low (9.6 ± 1.2 mM). We next compared ratios between patients with three complications and those without any complications who had similar blood glucose levels. The ratio in the patients without any complications (average blood glucose 9.6 ± 0.2 mM, range 4.8–13.2 mM; $n = 93$) was 4.16 ± 0.19, which was again significantly lower than in patients with three complications ($P < 0.05$).

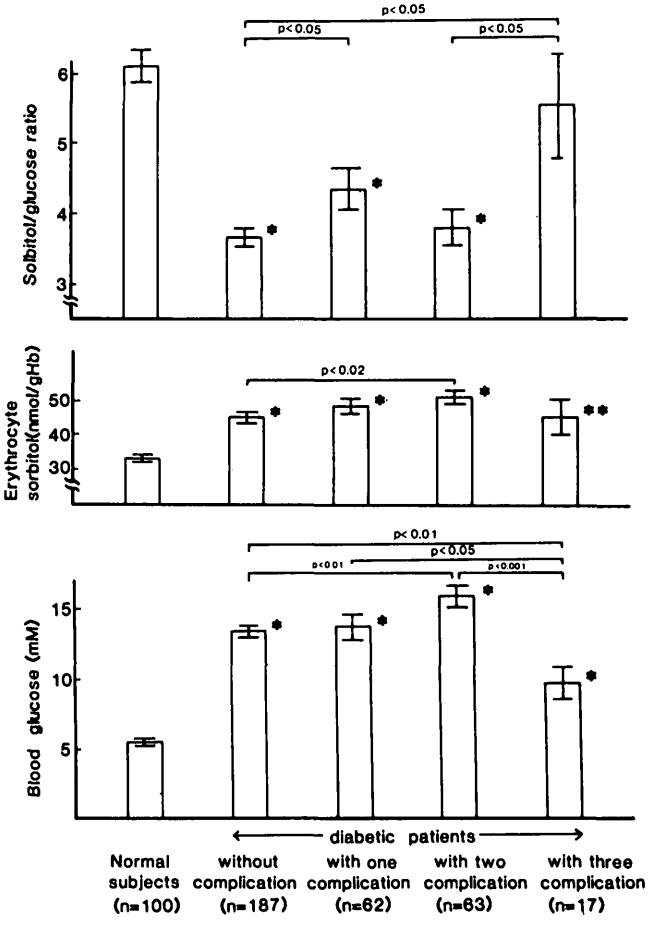

FIG. 5. Blood glucose, erythrocyte sorbitol, and sorbitol-glucose ratios in nondiabetic subjects and diabetic patients ranged in order of severity of diabetic complications. Values are means ± SE. *$P < 0.001$ vs. nondiabetic subjects. **$P < 0.02$ vs. nondiabetic subjects.

Figure 3 also shows the regression analysis of the sorbitol-glucose ratios in patients with or without diabetic complications. As the number of complications increased, the slopes of the regression lines and the intercepts on the ordinate became greater.

Table 3 shows the sorbitol-glucose ratios according to the type of therapy. No significant differences were found in the sorbitol-glucose ratios between groups.

We also studied the correlation between age and the sorbitol-glucose ratio in diabetic patients. Because the ratio was related to the blood glucose level, we analyzed the correlations in three groups formed according to their blood glucose levels. Figure 6 shows the significant correlation between age and the ratio in the group with an average blood glucose ≥14 and <19.6 mM. A significant correlation was also found in patients with blood glucose levels >19.6 mM ($n = 60$; $r = -0.30$, $P < 0.02$). However, no correlation was found in patients who had average blood glucose levels <14 mM. On balance, we found that the ratio was lower in older patients.

DISCUSSION

Intracellular sorbitol accumulation has been reported to be an etiological factor in diabetic complications (1–13). Malone et al. (27) demonstrated that the erythrocyte sorbitol concentration paralleled the sorbitol content of both lenses and peripheral nerves over a wide range in hyperglycemia, indicating

TABLE 3
Sorbitol-glucose ratios in diabetic patients according to type of treatment

Treatment	*n*	Blood glucose (mM)	Erythrocyte sorbitol (nmol/g Hb)	Sorbitol-glucose ratio
Diet alone	168	12.6 ± 0.34*	43.7 ± 1.5*	3.86 ± 0.15
Oral hypoglycemic agent	55	13.1 ± 0.72†	47.2 ± 2.6†	3.88 ± 0.21
Insulin	106	15.7 ± 0.70	52.0 ± 1.6	4.06 ± 0.22

Values are means ± SE. $P < 0.001$ vs. nondiabetic subjects.
*$P < 0.001$; †$P < 0.001$, vs. insulin groups.

that erythrocytes provide a convenient model for assessing the activity of the polyol pathway.

Erythrocyte sorbitol levels increased along with the degree of hyperglycemia (Fig. 2), and similar findings have been reported by others (3,20,27,28). Lapolla et al. (28) also reported that the erythrocyte sorbitol level correlated not only with the coincident blood glucose level but also with the HbA_{1c} level.

Malone et al. (22) have applied the sorbitol-glucose ratio to the standardization of the erythrocyte sorbitol content for erythrocytes exposed to varying blood glucose concentrations. They found that the fasting sorbitol-glucose ratio was elevated in type I diabetic patients even when the plasma glucose and/or HbA_{1c} levels were normal, suggesting that the aldose reductase activity is greater in erythrocytes from type I diabetic patients than in normal erythrocytes. However, we found that the sorbitol-glucose ratio in type II diabetic patients was quite close to normal when blood glucose levels were <8.3 mM and that the ratio in diabetic patients with HbA_{1c} levels <6% was not higher than in control subjects (Fig. 4).

Furthermore, we compared sorbitol-glucose ratios in control subjects and diabetic patients who underwent a 75-g OGTT. The fasting sorbitol-glucose ratio was again lower in diabetic patients than in control subjects, and the ratio significantly decreased 120 min after glucose loading in diabetic patients. Thus, in type II diabetic patients, sorbitol-glucose ratios were lower than in control subjects and fell when the blood glucose concentration increased, even over the short term. The reasons for the discrepancies between our findings and the data of Malone et al. are not clear. The ages of the patients studied may be a factor, because the younger patients had a higher ratio than the older ones in our study (Fig. 6). Juvenile diabetic patients have also been reported to have a significantly higher erythrocyte glyceraldehyde reductase level than older-onset diabetic patients (29).

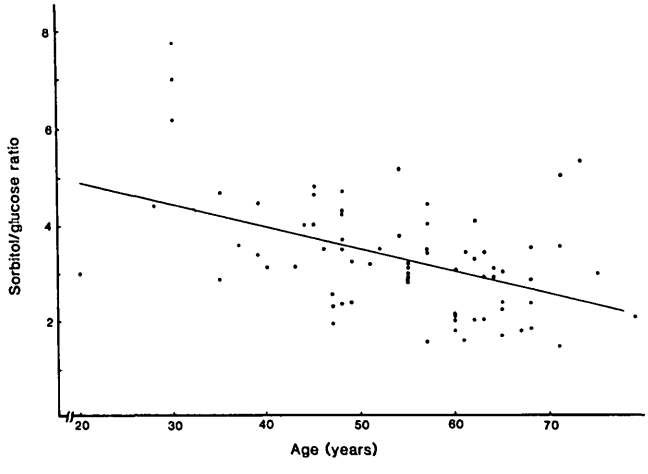

FIG. 6. Correlation between ages and sorbitol-glucose ratios in diabetic patients (blood glucose ≥14 and <19.6 mM). $n = 74$; $r = 0.48$, $P < 0.001$, $y = -0.047x + 5.86$.

We also found that the sorbitol-glucose ratio decreased when erythrocytes were exposed to high blood glucose concentrations, whereas the absolute erythrocyte sorbitol content increased progressively both in vitro and in vivo (Figs. 1–3). The same was found when the ratio was correlated with the HbA_{1c} level. This may have occurred because the ratio is an arbitrary criterion. However, it is still a good indicator of polyol pathway activity in erythrocytes at any given glucose level.

Interestingly, however, in diabetic patients with complications, sorbitol-glucose ratios were significantly higher than in patients without complications, despite no significant difference in blood glucose concentrations between groups (Table 1).

From the regression analysis, it became obvious that diabetic patients with one or two complications had greater slopes of the regression lines and higher intercepts on the ordinate compared with patients without complications (Figs. 3 and 5). These results may indicate that sorbitol is more easily formed in patients with complications than in patients without complications. Crabbe et al. (29) reported that diabetic patients with retinopathy or both retinopathy and cataract had significantly higher erythrocyte aldehyde reductase activity levels than control subjects.

A strain of congenitally hyperglycemic mice does not develop cataracts because the lenses of these mice have a very low aldose reductase activity level (only 10% of that present in the rat lens; 30,31). On the other hand, *Octodon degus* develops cataract quickly, and the *Octodon degus* lens has about four times the level of aldose reductase as a rat lens (31,32). These reports further support the hypothesis that aldose reductase is involved in the initiation of diabetic complications.

Our findings more specifically suggest that the affinity

of aldose reductase for glucose may be greater in patients with diabetic complications, indicating again that the polyol pathway is implicated in the pathogenesis of diabetic complications. Because the functional significance of aldose reductase under physiological conditions is not yet precisely understood, further studies are still required to elucidate the fundamental pathophysiology of diabetic complications.

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