# Higher Levels of Erythrocyte Membrane Microviscosity in Diabetes

YASUTADA BABA, MOTOAKI KAI, TETSURO KAMADA, SHIRO SETOYAMA, AND SHOGO OTSUJI

**SUMMARY**

**Significantly higher levels of erythrocyte membrane microviscosity (MV) [$\bar{\eta}$: 5.22 ± 0.17 (4.70–5.92), mean ± SD (range), poise, N = 67, P < 0.005] measured by fluorescence depolarization using 1,6-diphenyl-1,3,5-hexatriene as a fluorescent probe were found in diabetic patients when compared with normal controls [5.05 ± 0.15 (4.70–5.29), N = 22]. No significant differences in MV existed between males and females, nor was MV significantly correlated with diabetic age, duration of diabetes, plasma cholesterol, cholesterol/phospholipid ratios, and plasma lecithin:cholesterol acyltransferase activities. No significant difference in MV was observed between groups with or without diabetic retinopathy. There was, however, significantly higher MV [5.29 ± 0.19 (5.00–5.92), N = 20, P < 0.05] in the group with fasting blood glucose (FBG) ≥ 140 mg/dl than that [5.19 ± 0.15 (4.70–5.46), N = 47] in the group with FBG < 140 mg/dl. The changes in erythrocyte membrane MV presented in this study appear to be related to the current metabolic control of diabetic patients and are considered to be one of the factors responsible for the reduced erythrocyte deformability in diabetes. DIABETES *28*: 1138–1140, December 1979.**

The dynamic properties of the erythrocyte membrane lipid core in diabetic patients were studied by fluorescence depolarization using 1,6-diphenyl-1,3,5-hexatriene (DPH), which has been established as a fluorescent probe for the hydrocarbon region of the membrane lipid bilayer.[1] Measurement of erythrocyte membrane microviscosity (MV) was applied to patients with diabetes mellitus. Certain hereditary disorders known to have possible dynamic abnormalities[2–5] of the erythrocyte membrane were also studied.

From the Central Clinical Laboratory, The Kagoshima University Hospital, Faculty of Medicine, Kagoshima University, 1208–1 Usuki-Cho, Kagoshima, 890, Japan.

Received for publication 20 June 1979 and in revised form 6 August 1979.

## MATERIALS AND METHODS

A total of 67 patients consisting of 7 juvenile- and 60 maturity-onset diabetes cases were examined, none showing ketoacidosis.

The hemoglobin-free white ghost was prepared by the method of Dodge et al.[6] at 4°C. The isolated membrane [100 μg of membrane protein/ml of phosphate-buffered saline (PBS) (150 mM NaCl buffered with 2 mM sodium phosphate, pH 7.2)] was labeled with an equal volume of $4 \times 10^{-6}$ M DPH/PBS dispersion, and the mixture was incubated for 1 h at 37°C.

The degree of fluorescence polarization (P) was measured using an Elscint Microviscosimeter MV-Ia (Elscint Ltd., Haifa, Israel) as described previously.[1,2,7–10] The equipment simultaneously analyzes $I_{\parallel}$ and $I_{\perp}$, the fluorescence intensities polarized parallel and perpendicular, respectively, to the direction of the polarized excitation beam.

$$P = \frac{I_{\parallel} - I_{\perp}}{I_{\parallel} + I_{\perp}} \quad (1)$$

MV ($\bar{\eta}$) was obtained from the fluorescence polarization properties of DPH as described by the Perrin equation:

$$\frac{r_0}{r} = 1 + C(r)\frac{T\tau}{\bar{\eta}} \quad (2)$$

where r is the fluorescence anisotropy, which is obtained from P by the relationship:

$$r = \frac{I_{\parallel} - I_{\perp}}{I_{\parallel} + 2I_{\perp}} = \frac{(I_{\parallel}/I_{\perp}) - 1}{(I_{\parallel}/I_{\perp}) + 2} = \frac{2P}{3 - P} \quad (3)$$

$r_0$ is the limiting fluorescence anisotropy,[1,7] $\tau$ is the lifetime of the excited state,[7] and T is the absolute temperature. C(r) is a structural parameter of the probe.[1] Based on Eq. (2), a calibration curve[7] of $r_0/r$ versus $T\tau/\bar{\eta}$ was used for evaluation of $\bar{\eta}$. All values of P were determined at 25°C. The results obtained were the average MV values of all the cells present in each assay population and were expressed in poise ($\bar{\eta}$).

The derived MV values were plotted as log $\bar{\eta}$ versus 1/T to determine the fusion activation energy (ΔE).

Plasma lecithin:cholesterol acyltransferase (LCAT) activities were assayed by the method of Nagasaki and Akanuma[11] and expressed in units (one unit is equivalent to 1 nmol of Δ free cholesterol/ml/h/37°C). Protein was measured by the method of Lowry et al.[12]

All data are presented as mean ± SD, with ranges in parentheses. Statistical analysis was determined by Student's *t* test. The criterion of significance is a P value of less than 0.05.

## RESULTS

The normal value of MV was 5.05 ± 0.15 (4.70–5.29) poise (N = 22) at 25°C. At temperatures of 10–40°C, ΔE for MV was 6.59 ± 0.12 (6.46–6.77) kcal/mol (N = 5) in normal controls. These values nearly agreed with the hitherto reported values.[8,13,14]

It was found that erythrocyte membrane MV was distributed in a narrow range in normal controls as well as in each disorder, with relative standard deviations averaging about 3% (Table 1). There were no significant changes in MV and plasma LCAT activities in ages ranging from 22 to 70 yr in normal controls. There were also no significant differences in MV and plasma LCAT activities between males and females in normal controls [MV: males 5.04 ± 0.16 (4.70–5.29) poise (N = 14), females 5.05 ±0.14 (4.75–5.23) poise (N = 8); LCAT: males 79.2 ± 22.1 (50–121) units (N = 14), females 73.1 ± 23.2 (44–104) units (N = 8)].

A significant increase in the erythrocyte membrane MV was found in diabetes, and MV was significantly higher in the group with fasting blood glucose (FBG) ≥140 mg/dl than in the group with FBG <140 mg/dl (Table 1). No significant differences in MV and plasma LCAT activities were revealed between males and females [MV: males 5.25 ± 0.18 (5.00–5.92) poise (N = 33), females 5.20 ± 0.16 (4.70–5.52) poise (N = 34); LCAT: males 86.8 ± 20.6 (56–150) units (N = 33), females 84.9 ± 17.9 (48–126) units (N = 34)]. No significant correlations were demonstrated between MV and diabetic age [54.2 ± 14.2 (18–76) yr], duration of diabetes [4.9 ± 4.6 (0–20) yr], plasma cholesterol [193.4 ± 45.8 (103–315) mg/dl], cholesterol/phospholipid ratios [0.91 ± 0.12 (0.68–1.23)], or LCAT activities [85.9 ± 19.3 (48–150) units] (r = 0.196, 0.151, 0.052, 0.154, and −0.085, respectively). No significant difference in MV was obtained between groups with or without diabetic retinopathy of grade I–III (Scott) (Table 1).

Hereditary spherocytosis and myotonic and Duchenne muscular dystrophies also showed a significant increase in erythrocyte membrane MV (Table 1). It is interesting to note that, unlike in diabetes, there was a significant negative correlation (r = −0.660, N = 11, P < 0.05) between membrane MV and plasma LCAT activities in Duchenne dystrophy.

## DISCUSSION

In this study, an abnormality in the dynamic properties of the hydrophobic core of the erythrocyte membrane was detected in diabetes by measuring the fluorescence polarization properties of DPH. Erythrocyte membrane MV has been known to be affected by environmental lipid constituents such as plasma cholesterol, cholesterol/phospholipid ratios, and plasma LCAT activities. No significant correlations, however, were found between MV and these values. The changes in MV were also not significantly correlated with diabetic age or duration of the disease, nor were there significant differences in MV between males and females. There was a smaller increase of MV in diabetics with retinopathy than in those without retinopathy, but the difference

TABLE 1
Microviscosity of the erythrocyte membrane lipid core in diabetes mellitus and certain hereditary disorders

| | No. of cases | | | | | |
|---|---|---|---|---|---|---|
| | Total | M | F | Age (yr) | Microviscosity $\bar{\eta}$ (poise) | Plasma LCAT activity (units) |
| Normal controls | 22 | 14 | 8 | 44.3 ± 12.8 (22–70) | 5.05 ± 0.15 (4.70–5.29) | 77.0 + 22.7 (44–121) |
| Diabetes mellitus | 67 | 33 | 34 | 54.2 ± 14.2 (18–76) | 5.22 ± 0.17 (P < 0.005)* (4.70–5.92) | 85.9 ± 19.3 (NS)* (48–150) |
| Complication: | | | | | | |
| Retinopathy (−) | 21 | 8 | 13 | 48.3 ± 13.6 (18–70) | 5.20 ± 0.14 (4.97–5.46) (NS) | 81.5 ± 15.4 (48–111) (NS) |
| Retinopathy (+) | 28 | 13 | 15 | 57.9 ± 12.6 (29–76) | 5.25 ± 0.17 (4.91–5.92) | 89.4 ± 21.7 (56–150) |
| FBG: | | | | | | |
| <140 mg/dl [110.3 ± 17.2 (60–136)] | 47 | 22 | 25 | 54.9 ± 13.2 (18–76) | 5.19 ± 0.15 (4.70–5.46) (P < 0.05) | 89.8 ± 15.9 (64–125) (NS) |
| ≥140 mg/dl [191.0 ± 45.9 (147–325) | 20 | 11 | 9 | 52.7 ± 16.1 (19–70) | 5.29 ± 0.19 (5.00–5.92) | 84.2 ± 20.4 (48–150) |
| HS | 2 | 1 | 1 | 10.5 (7, 14) | 5.85, 5.69 | 70.2, 85.0 |
| MMD | 2 | 1 | 1 | 45.0(44, 46) | 5.39, 5.46 | 65.0, 61.1 |
| PMD | 11 | 11 | 0 | 14.7 ± 1.8 (12–17) | 5.39 ± 0.12 (P < 0.005)* (5.23–5.65) | 46.1 ± 8.1(P < 0.005)* (34–57) |

LCAT: lecithin:cholesterol acyltransferase; FBG: fasting blood glucose; HS: hereditary spherocytosis; MMD: myotonic muscular dystrophy; PMD: progressive muscular dystrophy (Duchenne); NS: not significant (P ≥ 0.05).

* Versus normal controls.

All data are expressed as mean ± 1 SD, with ranges in parentheses.

was not significant. There was, however, significantly higher MV in diabetics with FBG ≥140 mg/dl than in those with FBG <140 mg/dl. The highest MV value of 5.92 poise was obtained in a poorly controlled 59-yr-old male diabetic on insulin therapy for the past 10 yr, with 18 yr duration, 182 mg/dl of FBG, and with grade III retinopathy.

Reduced erythrocyte deformability has been demonstrated to occur in diabetes.[15–17] Schmid-Schönbein and Volger[15] reported that the decreased erythrocyte deformability depends critically on the incident metabolic control of the diabetics. Concerning the possible cause of the reduced deformability in diabetes, McMillan et al.,[17] using direct observation of the flow properties of erythrocytes in micropipets, suggested an elevation of either intraerythrocyte or membrane viscosity. Although MV, three-dimensional viscosity of the membrane hydrocarbon interior, is not equivalent to the membrane viscosity (which is a resistance to the motion of the membrane), a certain relationship may exist between these two parameters.[18]

The higher erythrocyte membrane MV presented in this study appears to be related to the current metabolic control of diabetic patients and is considered to be one of the factors responsible for reduced erythrocyte deformability in diabetes.

**ACKNOWLEDGMENTS**

We thank A. Niiuchi for her excellent technical assistance. This study was supported in part by a grant from the Kodama Foundation (T. Kodama, Director), Kagoshima, Japan.

**REFERENCES**

[1] Shinitzky, M., and Inbar, M.: Difference in microviscosity induced by different cholesterol levels in the surface membrane lipid layer of normal lymphocytes and malignant lymphoma cells. J. Mol. Biol. *85*:603–15, 1974.

[2] Aloni, B., Shinitzky, M., Moses, S., and Livne, A.: Elevated microviscosity in membranes of erythrocytes affected by hereditary spherocytosis. Br. J. Haematol. *31*:117–23, 1975.

[3] Kunze, D., Reichmann, G., Egger, E., Leuschner, G., and Eckhardt, H.: Erythrozytenlipide bei Progressiver Muskeldystrophie. Clin. Chim. Acta *43*:333–41, 1973.

[4] Kalofoutis, A., Jullien, G., and Spanos, V.: Erythrocyte phospholipids in Duchenne muscular dystrophy. Clin. Chim. Acta *74*:85–87, 1977.

[5] Butterfield, D. A., Roses, A. D., Cooper, M. L., Appel, S. H., and Chesnut, D. B.: A comparative electron spin resonance study of the erythrocyte membrane in myotonic muscular dystrophy. Biochemistry *13*:5078–82, 1974.

[6] Dodge, J. T., Mitchell, C., and Hanahan, D. J.: The preparation and chemical characteristics of hemoglobin-free ghosts of human erythrocytes. Arch. Biochem. Biophys. *100*:119–30, 1963.

[7] Shinitzky, M., and Barenholz, Y.: Dynamics of the hydrocarbon layer in liposomes of lecithin and sphingomyelin containing dicetylphosphate, J. Biol. Chem. *249*:2652–57, 1974.

[8] Aloni, B., Shinitzky, M., and Livne, A.: Dynamics of erythrocyte lipids in intact cells, in ghost membranes and liposomes. Biochim. Biophys. Acta *348*:438–41, 1974.

[9] Shinitzky, M., Dianoux, A. C., Gitler, C., and Weber, G.: Microviscosity and order in the hydrocarbon region of micelles and membranes determined with fluorescent probes. I. Synthetic micelles. Biochemistry *10*:2106–13, 1971.

[10] Shinitzky, M., and Barenholz, Y.: Fluidity parameters of lipid regions determined by fluorescence polarization. Biochim. Biophys. Acta *515*:367–94, 1978.

[11] Nagasaki, T., and Akanuma, Y.: A new colorimetric method for the determination of plasma lecithin-cholesterol acyltransferase activity. Clin. Chim. Acta *75*:371–75, 1977.

[12] Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J.: Protein measurement with the folin phenol reagent. J. Biol. Chem. *193*:265–75, 1951.

[13] Cooper, R. A., and Leslie, M. H.: Fluidity of red cell membranes possessing more than two cholesterols per phospholipid. Fed. Proc. *35*:604, 1976.

[14] Shinitzky, M., and Inbar, M.: Microviscosity parameters and protein mobility in biological membranes. Biochim. Biophys. Acta *433*:133–49, 1976.

[15] Schmid-Schönbein, H., and Volger, E.: Red-cell aggregation and red-cell deformability in diabetes. Diabetes *25*(Suppl. 2):897–902, 1976.

[16] Barnes, A. J., Locke, P., Scudder, P. R., Dormandy, T. L., Dormandy, J. A., and Slack, J.: Is hyperviscosity a treatable component of diabetic microcirculatory disease? Lancet *2*:789–91, 1977.

[17] McMillan, D. E., Utterback, N. G., and Puma, J. L.: Reduced erythrocyte deformability in diabetes. Diabetes *27*:895–901, 1978.

[18] Evans, E. A., and Hochmuth, R. M.: Mechanochemical properties of membranes. Curr. Top. Membranes Transport *10*:1–64, 1978.