Lower Levels of Erythrocyte Membrane Fluidity in Diabetic Patients

A Spin Label Study

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SUMMARY

The dynamic properties of intact erythrocyte membrane in diabetic patients were investigated by means of electron spin resonance using three stearic acid spin labels (SAL): 5-, 12-, and 16-SAL. Significantly lower levels of erythrocyte membrane fluidity were revealed with 16-SAL as a probe in diabetic patients when compared with normal controls. However, there were no significant differences in fluidity values using 5- or 12-SAL between the two groups. Therefore, it became obvious that the decrease in fluidity was located in deeper sites (hydrophobic region) of the erythrocyte double membrane in diabetic patients. It was strongly suggested that changes in the membrane cholesterol to phospholipid molar ratios are not a principal factor contributing to the fluidity change. A significant increase of sphingomyelin and decrease of phosphatidylethanolamine were found in the erythrocyte membrane of diabetic patients and an alteration in membrane phospholipid classes and their acyl-chains could conceivably be related to the fluidity change.

There were no significant correlations between change in membrane fluidity and most plasma lipids, plasma lecithin-cholesterol acyl-transferase activities, erythrocyte glycosylated hemoglobin, erythrocyte adenosine triphosphate, fasting blood glucose or duration of the disease. Plasma cholesterol of high-density lipoprotein showed a significant negative correlation with the membrane fluidity values.

Some of the possible factors contributing to and the significance of the lower levels of erythrocyte membrane fluidity were discussed in conjunction with both metabolic and clinical aspects in diabetic patients. DIABETES *32*:585–591, July 1983.

The occurrence of changes in the erythrocytes of diabetic patients has become evident from several aspects. Excessive aggregation,[1] reduced deformability,[1,2] decreased membrane surface electric charge,[3] elevated glycosylated hemoglobin (HbA_1)[4] and protein,[5,6] and changes in 2,3-diphosphoglycerate levels[7,8] are all seen in the erythrocytes of diabetic patients. These changes have been suggested to contribute to the pathogenesis of diabetic microangiopathy and abnormalities of microcirculation in diabetic patients.[9] Recently, we reported that an increased erythrocyte membrane microviscosity* in diabetic patients was observed by means of fluorescence depolarization spectroscopy.[10,11]

In the present communication, the dynamic physical properties of the intact erythrocyte membrane were investigated by means of a spin label method and lower levels of membrane fluidity* were further verified in diabetic patients. Since erythrocyte membrane fluidity is associated with lipids or other constituents of the erythrocytes or plasma, the possible relationships of the membrane fluidity changes to membrane and plasma lipids, plasma lecithin-cholesterol acyl-transferase (LCAT) activities, erythrocyte HbA_1, and adenosine triphosphate (ATP) were also investigated in diabetic patients.

MATERIALS AND METHODS

The diabetic subjects investigated in this study were those whose history and laboratory findings had been carefully investigated at our diabetic clinic. None showed any clinical history or signs of anemia and other conditions which might cause reticulocytosis or leukocytosis. They also showed no ketoacidosis or severe nephropathy with renal failure.

Venous blood was obtained in the fasting state from sex- and age-matched healthy subjects and type II, maturity-onset diabetics with different durations of the disease, varying metabolic status, and with or without diabetic retinopathy (background retinopathy without any proliferative changes).

This study was presented in part at the international workshop "On Pathogenetic Concepts of Diabetic Microangiopathy," held at Garmisch-Grainau, Germany, October 16–18, 1980.

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*An increase in microviscosity is equivalent to a decrease in fluidity.

A total of 16 diabetic patients, ranging in age from 22 to 70 yr, was studied in experiment 1. Six were maintained on insulin therapy, seven on sulfonylureas, and three on diet alone. Eight patients were females and eight were males. In experiment 2, a total of 45 patients ranging in age from 18 to 76 yr were studied. Nineteen were managed with insulin, 16 with sulfonylureas, and ten by diet alone. Twenty-three were females and 22 were males. Experiment 1 is shown in the *Membrane Fluidity* section of RESULTS. The remainder of the study makes up experiment 2.

Blood was anticoagulated with EDTA (−2K, −2Na) at a concentration of 1.5 mg/ml and centrifuged at 3000 rpm for 10 min at 4°C. The plasma and buffy coat were removed. Erythrocytes were washed three times with 5 vol of isotonic phosphate buffered saline (pH 7.4)(PBS) at 4°C, and the buffy coat was completely eliminated each time. Washed erythrocytes were then added to the same volume of PBS and mixed thoroughly. The suspensions of erythrocytes were immediately subjected to spin labeling and to measurement of membrane lipids, erythrocyte HbA_1, and ATP. Contamination by reticulocytes and leukocytes in the erythrocyte suspensions was less than 0.2% and 0.001%, respectively.

Measurements of plasma lipids and LCAT activities were performed on the same day as the blood was drawn.

Spin labeling and electron spin resonance (ESR) measurement. Three stearic acid spin labels (SAL): 5-, 12- and 16-SAL, were purchased from Syva Co. (Palo Alto, California). These are stearic acid analogues and each has a nitroxide radical ring at the 5th, 12th, and 16th carbon position counted from the carboxyl group of the acyl-chain, respectively (Figure 1). The spin label technique has been established as a valuable tool to obtain conformational and dynamic data concerning the physical state of the biologic membrane.[12–18] These spin labels embedded in the biologic membrane exhibit their freedom of anisotropic motion in conformity with the position of the nitroxide ring on the alkyl fatty acid chain.[12,18] This anisotropic motion reflects the molecular motion of the lipid bilayer, the so-called membrane "fluidity."[12–14,16–18] We measured parameters from the ESR spectra of spin labels embedded in intact erythrocyte membrane to estimate the dynamic states of the lipid bilayer of the membrane.

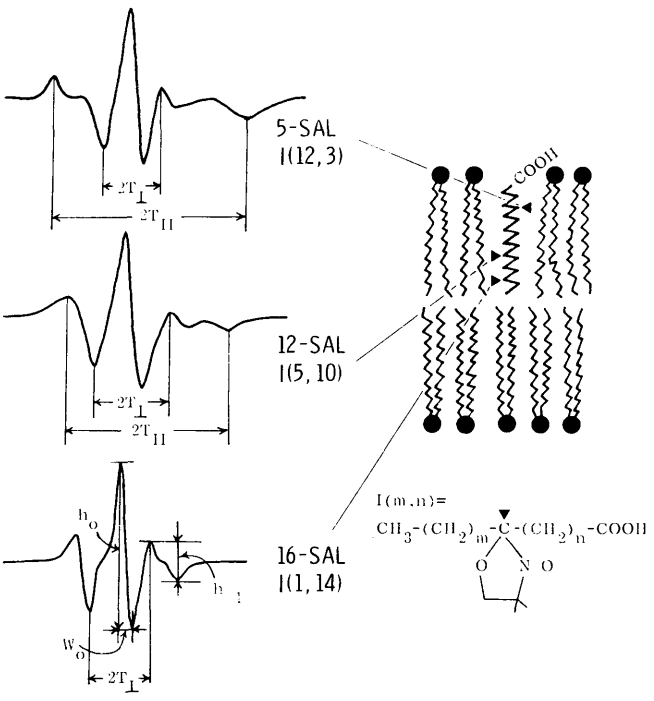

FIGURE 1. Chemical formulae for 5-, 12-, and 16-SAL and the representative electron spin resonance spectra of SAL embedded in the erythrocyte membrane. SAL: stearic acid spin label. I(m,n): general molecular formula for fatty acid spin labels; ⟿: phospholipid; $2T_{||}$: outer hyperfine splitting; $2T_{\perp}$: inner hyperfine splitting; h_0: mid-field height; h_{-1}: high-field height; W_0: line width.

The incorporation of these spin labels into the erythrocyte membrane bilayer was readily accomplished by the following procedure.[13] Four microliters of 5-μg/μl spin label solutions in 100% ethanol were diluted with 500 μl of PBS and the resultant label solution was added to 500 μl of the erythrocyte suspension in PBS. After incubation for 20 min at 37°C by gentle shaking, erythrocytes were washed three times with 20 vol of PBS to eliminate free spin labels. The labeled erythrocytes were packed by centrifugation at 3200 rpm for 20 min and used immediately for ESR measurements. About 50 μl was transferred to a capillary quartz cell and ESR spectra were obtained at 37°C controlled by a variable temperature controller (JES-VT-3A2) on a JEOL X-band spectrometer, Model JES FEIX (JEOL Ltd., Tokyo, Japan). There were no morphologic changes in the labeled erythrocytes when the analysis was performed.

The representative spectrum of SAL spin labels embedded in the erythrocyte membrane is shown in Figure 1. The observed values of the outer ($2T_{||}$) and inner ($2T_{\perp}$) hyperfine splitting (in Gauss) were used to calculate the order parameter (S) in 5- and 12-SAL according to formula (1) of Gaffney.[14] However, in the case of 16-SAL, the outer splitting value could not be measured since the low-field peak was not resolved.[15] The order parameter was determined from the observed inner splitting value and the calculated outer splitting value using the relation: $T_{||} = 3a - 2T_{\perp}$ where a is the isotropic hyperfine splitting.[15] 3a was taken to be 44.5G.

$$S = \frac{T_{||} - T_{\perp} + -c}{T_{||} + 2T_{\perp} + +2c} \times 1.723 \qquad (1)$$

where $c = 1.4G - 0.053(T_{||} - T_{\perp})$.

The motion parameter (τ_0) was calculated from formula (2) of Henry and Keith,[16] where W_0 is the line width, h_0 is the mid-field height and h_{-1} is the high-field height (Figure 1).

$$\tau_0 = K \cdot W_0[(h_0/h_{-1})^{\frac{1}{2}} - 1]. \qquad (2)$$

The constant $K = 6.5 \cdot 10^{-10}$ (seconds) is dependent on the anisotropic hyperfine coupling values and the g-tensor terms when the correlation time $\tau_c > 10^{-9}$.[16] Although equation 2 was derived for the analysis of isotropic motion, the peak height ratio (h_0/h_{-1}) and motion parameter could be used as parameters of 16-SAL anisotropic mobility for the purpose of comparison.[16,17] Both the peak height ratio and motion parameter were used for comparison of membrane fluidities throughout the present study except for experiment 1 since they have higher sensitivities than the outer hyperfine splitting and order parameter in the measurements with 16-SAL.

TABLE 1
Outer hyperfine splitting ($2T_{II}$) and order parameter (S) of three different stearate spin labels in erythrocyte membranes of normal controls and diabetes mellitus

	Normal controls		Diabetes mellitus	
SAL*	$2T_{II}$(G†)	S	$2T_{II}$(G)	S
5-SAL	57.01 ± 0.18	0.667 ± 0.006	57.03 ± 0.16	0.666 ± 0.009
12-SAL	49.31 ± 0.26	0.496 ± 0.004	49.24 ± 0.14	0.493 ± 0.006
16-SAL	40.04 ± 0.44	0.286 ± 0.014	40.72 ± 0.42‡	0.306 ± 0.010§

Normal controls: N = 14 (7 males, 7 females), age 49.50 ± 11.09 (22–70) yr. Diabetes mellitus: N = 16 (8 males, 8 females), age 48.50 ± 12.02 (22–70) yr, 10 with retinopathy, 6 without retinopathy. The ESR spectra were obtained at 37°C.
*SAL: stearate spin label.
†G:gauss.
‡$P < 0.005$.
§$P < 0.01$ versus normal controls.

Greater values of membrane fluidity, i.e., greater freedom of motion of spin labels in the double membrane, are associated with smaller values of outer hyperfine splitting, order parameter, peak height ratio, and motion parameter.

Erythrocyte membrane lipids. Lipids of the erythrocyte membrane were extracted with isopropanol-chloroform (11:7 by vol) followed by chloroform-methanol (2:1 by vol) according to a modification of the method of Rose and Oklander.[19] The anti-oxidant, butyrated hydroxytoluene (BHT), was added to the solvent used for extraction at a concentration of 50 mg/L. The total amount of phospholipids in the extract was measured by the method of Bartlett.[20] Individual phospholipid classes were quantitatively separated by thin layer chromatography on a HPTLC plate (silica gel 60 precoated plate, Merck) activated at 200°C for 2 h with a developing solvent of chloroform-methanol-acetic acid-water (25:14:4:1 by vol), containing BHT at a concentration of 50 mg/L. The spots were detected by spraying with Dittmer reagent according to the method of Dittmer and Lester.[21] Quantitation of individual phospholipids was performed at 730 nm with a dual-wavelength TLC scanner, Model CS-910 (Shimadzu Ltd., Kyoto, Japan) in comparison with known amounts of each phospholipid class developed on the same plate. Corrections for background were performed at 360 nm. Cholesterol was measured by the method of Zak.[22] The free cholesterol to phospholipid (C/PL) molar ratios were calculated from an authentic mixture of both lipids containing known amounts.

Plasma lipids and LCAT activities. Plasma cholesterol (total and free) and phospholipid were measured by means of enzymatic methods.[23,24] Cholesterol of high-density lipoprotein (HDL) was measured by the dextran sulfate-$MgCl_2$ precipitation method.[25] Plasma LCAT activities were assayed by the method of Nagasaki and Akanuma.[26]

Erythrocyte HbA_1 and ATP. Erythrocyte HbA_1 was measured according to the micro-scale column procedure of Welch and Boucher.[27] Erythrocyte ATP measurements were carried out by a modification of the method of Bücher.[28] Levels of erythrocyte ATP were expressed in terms of nmol/mg of erythrocyte protein. Erythrocyte proteins were measured according to the method of Lowry et al.[29]

Statistical analyses. All data are presented as mean value ± standard deviation, with ranges in parentheses. Linear regression analysis was used to assess the degree of correlation. Significant differences were tested by Student's *t* test. The criterion of significance was a P value of less than 0.05.

RESULTS

MEMBRANE FLUIDITY

Experiment 1. The membrane fluidity of diabetic erythrocytes was significantly decreased in the measurement using 16-SAL as a probe with the outer hyperfine splitting and order parameter (Table 1). However, there was no significant difference between the fluidity values measured with 5- and 12-SAL, which represents the fluidity of rather shallow sites of the erythrocyte membrane.

Experiment 2. To study further the fluidity changes observed in experiment 1, the membrane fluidity values measured with 16-SAL were compared in normal and diabetic erythrocytes using the peak height ratio and motion parameter. The significant decrease of membrane fluidity of diabetic erythrocytes was further verified (Table 2).

TABLE 2
Peak height ratio (h_o/h_{-1}) and motion parameter (τ_o) of 16-stearate spin label in erythrocyte membranes of diabetes mellitus with or without retinopathy

	N	Sex	h_o/h_{-1}	$\tau_o \times 10^{10}$(s)
Normal controls	16	(8 M, 8 F)	4.96 ± 0.12	20.88 ± 0.43
Diabetes mellitus				
Total	45	(22 M, 23 F)	5.10 ± 0.17*	21.26 ± 0.65†
Rp(−)	27	(14 M, 13 F)	5.07 ± 0.19†	21.18 ± 0.71
Rp(+)	18	(8 M, 10 F)	5.13 ± 0.14‡	21.39 ± 0.54*

M: males. F: females. Rp: retinopathy. Normal controls: age 48.50 ± 12.02 (22–70) yr. Diabetes mellitus: age 53.31 ± 12.10 (18–76) yr [Rp(−) 53.24 ± 12.23 (18–74) yr, Rp(+) 54.10 ± 11.12 (23–76) yr.] The ESR spectra were obtained at 37°C.
*$P < 0.01$, †$P < 0.05$, ‡$P < 0.005$ versus normal controls.

TABLE 3
Cholesterol to phospholipid molar ratios and phospholipid classes of erythrocyte membranes in normal controls and diabetes mellitus

	N	Sex	C/PL	PE (mol %)	PS (mol %)	PC (mol %)	SM (mol %)
Normal controls	16	(8 M, 8 F)	0.89 ± 0.05	35.89 ± 2.56	13.41 ± 1.34	27.24 ± 2.06	23.86 ± 2.75
Diabetes mellitus							
Total	45	(22 M, 23 F)	0.92 ± 0.06	32.58 ± 3.67*	13.80 ± 1.38	27.02 ± 3.08	26.40 ± 4.10‡
Rp(−)	27	(14 M, 13 F)	0.92 ± 0.05	32.29 ± 4.32†	13.88 ± 1.53	27.00 ± 3.42	26.59 ± 4.71‡
Rp(+)	18	(8 M, 10 F)	0.93 ± 0.07	33.02 ± 2.41*	13.67 ± 1.16	27.04 ± 2.59	26.12 ± 3.08‡

M: males. F: females. Rp: retinopathy. C/PL: free cholesterol to phospholipid molar ratios. PE: phosphatidylethanolamine. PS: phosphatidylserine. PC: phosphatidylcholine. SM: sphingomyelin. The same subjects shown in Table 2 were investigated.
*$P < 0.005$, †$P < 0.01$, ‡$P < 0.05$ versus normal controls.

The change was more apparent in diabetics with retinopathy than in those without retinopathy, but the difference was not significant. There were no significant correlations between the changes of membrane fluidity and metabolic control states such as fasting blood glucose and erythrocyte HbA_1 levels or duration of the disease. There were no significant differences in membrane fluidity values among groups with different managements consisting of diet alone, sulfonylureas, or insulin.

ERYTHROCYTE MEMBRANE LIPIDS

There was a small but not significant increase in the erythrocyte membrane C/PL molar ratios in diabetic patients when compared with normal controls. Among the individual phospholipids, a significant increase of sphingomyelin (SM) and decrease of phosphatidylethanolamine (PE) were found in diabetic patients when compared with normal controls (Table 3). There were no significant correlations between erythrocyte membrane fluidity values and the level of individual phospholipid classes. Retinopathy had no detectable influence.

PLASMA LIPIDS

Plasma total cholesterol was slightly increased in diabetic patients when compared with normal controls, but the difference was not significant. There were also no significant differences in plasma free cholesterol, phospholipid and C/PL molar ratios between diabetic patients and normal controls (Table 4). There was a significant positive correlation between plasma and erythrocyte membrane C/PL molar ratios among both diabetic patients and normal controls (Figure 2). There was no significant correlation between individual plasma lipid constituents and erythrocyte membrane fluidity values.

Plasma HDL-cholesterol was slightly decreased in diabetic patients when compared with normal controls, but the difference was not significant (Table 4). However, there was a significant negative correlation between plasma HDL-cholesterol and membrane fluidity values (Figure 3), which indicates that patients with higher HDL-cholesterol concentrations in plasma have higher membrane fluidity of erythrocytes.

PLASMA LCAT ACTIVITIES

Plasma LCAT activities were slightly increased in diabetic patients when compared with normal controls, but the difference was not significant (Table 4). Plasma LCAT activities demonstrated no significant correlation with erythrocyte membrane fluidity values.

ERYTHROCYTE HbA_1

Erythrocyte HbA_1 of diabetic patients was significantly increased when compared with normal controls (Table 4). There was no significant correlation between HbA_1 and erythrocyte membrane fluidity values in diabetic patients.

ERYTHROCYTE ATP

There were no significant differences in erythrocyte ATP concentrations between diabetic patients and normal controls (Table 4). Erythrocyte ATP showed no significant correlation with erythrocyte membrane fluidity values in diabetic patients.

DISCUSSION

Membrane fluidity has been shown to be involved in various membrane functions such as permeability;[30] transport of ions,[31,32] glucose,[33] or oxygen;[34] membrane-associated enzymes;[35,36] hormone receptor functions;[37-39] and osmotic fra-

TABLE 4
Plasma lipids, LCAT activities, erythrocyte HbA_1 and ATP in normal controls and diabetes mellitus

	N	Sex	Total C (mg/dl)	Free C (mg/dl)	Total PL (mg/dl)	Free-C/PL molar ratio	HDL-C (mg/dl)	LCAT* activities	HbA_1 (mg/dl)	ATP†
Normal controls	16	(8 M, 8 F)	185.5 ± 33.2	61.5 ± 8.6	228.1 ± 33.3	0.53 ± 0.07	55.3 ± 11.6	86.9 ± 29.6	8.02 ± 0.75	3.17 ± 0.43
Diabetes mellitus										
Total	45	(22 M, 23 F)	195.3 ± 39.5	61.4 ± 14.3	214.8 ± 36.5	0.54 ± 0.10	50.1 ± 13.3	99.2 ± 36.1	11.43 ± 2.50‡	3.35 ± 0.41
Rp(−)	27	(14 M, 13 F)	186.5 ± 29.8	57.8 ± 10.8	210.5 ± 33.7	0.53 ± 0.11	49.3 ± 14.6	92.4 ± 36.2	11.09 ± 2.27‡	3.35 ± 0.40
Rp(+)	18	(8 M, 10 F)	208.5 ± 48.6	66.8 ± 17.4	232.4 ± 34.9	0.56 ± 0.08	50.9 ± 11.7	109.5 ± 34.3	11.95 ± 2.82‡	3.35 ± 0.44

M: males. F: females. Rp: retinopathy. C: cholesterol. PL: phospholipid. HDL: high-density lipoprotein. LCAT: lecithin-cholesterol acyl-transferase. The same subjects shown in Table 2 were investigated.
*Units (one unit is equivalent to 1 nmol of free cholesterol/ml/37°C).
†nmol/mg of erythrocyte protein.
‡$P < 0.005$.

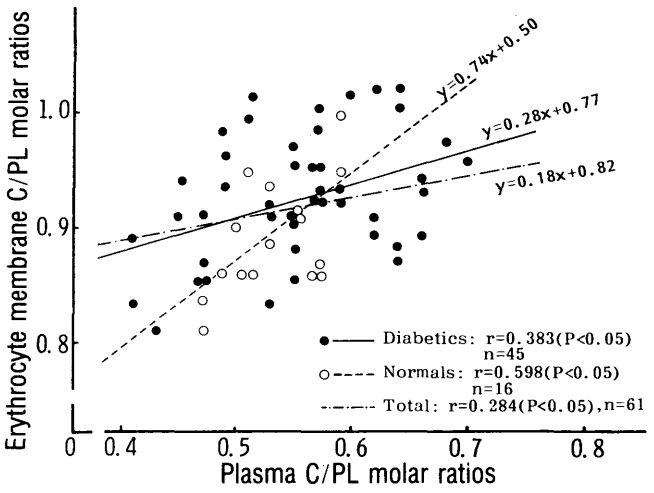

FIGURE 2. Significant correlations of C/PL molar ratio between in erythrocyte membrane and in plasma. The same subjects shown in Table 2 were analyzed. C/PL: free cholesterol to phospholipid.

gility.[40] Some of these functions have been shown to be changed in diabetes mellitus. Therefore, it is conceivable that decreased membrane fluidity in diabetic erythrocytes has important effects on the metabolic and clinical status of diabetic patients.

Lipid composition and lipid-protein interactions are the major contributing factors to changes in the erythrocyte membrane fluidity. The major membrane lipid components related to erythrocyte membrane fluidity have been reported as follows:[41,42] C/PL molar ratio, classes of phospholipid, the length of phospholipid acyl-chains, and the degree of unsaturated double bonds of phospholipids.

Membrane C/PL molar ratios of diabetic erythrocytes were observed to be higher than those of normal controls, but the difference was not significant (Table 3). There was a significant positive correlation between plasma and erythrocyte membrane C/PL molar ratios among both diabetic patients and normal controls (Figure 2). Cholesterol is a major constituent of many biologic membranes and previous studies have established that cholesterol affects the motion of the paraffin chains of membrane lipids and that it plays an important role in maintaining membrane fluidity.[41,43] Our preliminary observations[44] demonstrated that the outer hyperfine splittings of 12-SAL and of 16-SAL were decreased by 4.87% (from 49.25 Gauss to 46.85) and by −0.70% (from 39.84 to 40.12), respectively, when the membrane C/PL molar ratio of 0.90 was decreased to 0.74 by the incubation of intact erythrocytes with egg phosphatidylcholine (PC) vesicles. Therefore, the restricting effects of cholesterol on erythrocyte membrane fluidity were found to be most apparent in 12-SAL but not very apparent in 16-SAL. This positional effect of cholesterol on phospholipid acyl-chains has also been demonstrated by a nuclear magnetic resonance study.[45] In this communication, decreases in membrane fluidity values of diabetic erythrocytes were demonstrated only with 16-SAL but not with 5- or 12-SAL (Table 1) and were not significantly correlated with membrane C/PL molar ratios. Therefore, it is strongly suggested that the decreased fluidity values of diabetic erythrocyte membrane measured with 16-SAL were not derived principally from changes in membrane C/PL molar ratios.

Variation in the degree of saturation of the acyl-chain is another important factor regulating membrane fluidity.[41,42] Phospholipids with saturated acyl-chains form highly ordered membranes in which fluidity is low. Conversely, phospholipids with unsaturated acyl-chains form disordered membranes in which fluidity is high.[41] It has been demonstrated[46–48] that decreased unsaturation of free fatty acids and phospholipid acyl-chains occurs in diabetes. It is very likely that the decreased fluidity in the deeper sites (hydrophobic region) of diabetic erythrocyte membrane demonstrated with 16-SAL in the present study was derived from decreased unsaturation of the phospholipid acyl-chains, since the perturbing influence of the conformation of the unsaturated bond on the ordered packing of alkyl chains is more effective in the region between unsaturated sites and terminal methyl groups.[18]

Generally speaking, the choline-containing phospholipids (PC and SM), particularly SM, have markedly larger fractions of saturated and longer acyl-chains than those of other phospholipids. Conversely, other phospholipids (PE and phos-

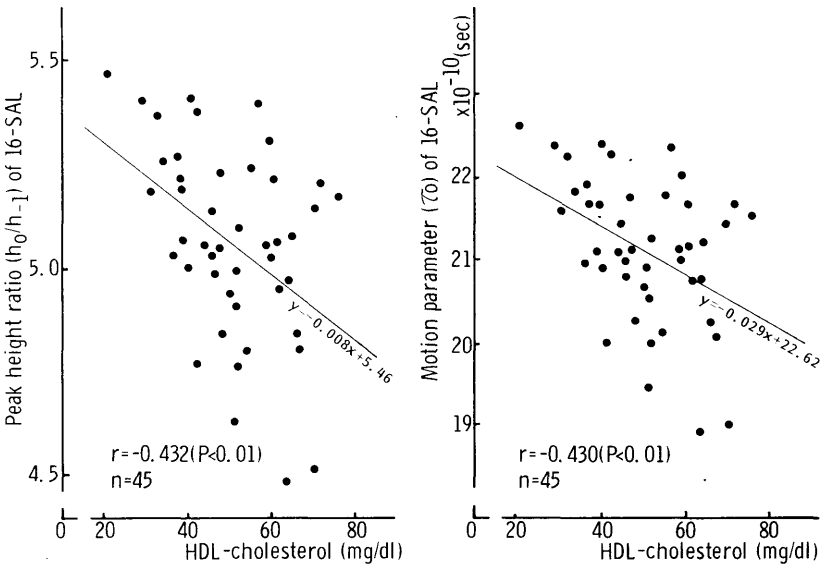

FIGURE 3. Significant correlation between erythrocyte membrane fluidities and plasma HDL-cholesterol in diabetic patients. The same subjects shown in Table 2 were analyzed. h_0: mid-field height (see Figure 1); h_{-1}: high-field height (see Figure 1); τ_0: see the text. HDL: high-density lipoprotein.

phatidylserine) have unsaturated and shorter acyl-chains.[49] Therefore, it is conceivable that the decrease of PE and increase of SM observed in the present study are contributing factors to the decreased membrane fluidity in diabetic erythrocytes. A decreased membrane fluidity measured by the fluorescence polarization of 1,6-diphenyl-1,3,5-hexatriene and an increased SM content have also been reported in the erythrocytes of abetalipoproteinemia.[50]

Lipid-protein interaction[42] is also a determinant factor regulating membrane fluidity. In this study, however, there was no significant correlation between the erythrocyte membrane fluidity and erythrocyte HbA_1 or ATP in diabetic paitents.

Plasma HDL-cholesterol has been discussed widely in relation to ischemic heart disease in which one of the risk factors is diabetes mellitus.[51] Interestingly, there was a significant negative correlation between plasma HDL-cholesterol and the erythrocyte membrane fluidity values in diabetic patients (Figure 3).

Recently, it has been shown that insulin[37,52] and sulfonylureas[53] play an important role in the change in plasma membrane fluidity. However, in our study, there were no significant differences in the erythrocyte membrane fluidity with diet alone, sulfonylureas, or insulin.

It has been reported, using the spin label method with 12- or 16-SAL, that younger red cells have a higher membrane fluidity than older cells,[54] and also that the erythrocyte survival time was reversibly decreased in correlation with glucose regulation in diabetic patients.[55] In this study, erythrocyte membrane fluidity was found not increased but decreased in diabetic patients with 16-SAL. It should be emphasized that because of the presence of hyperglycemia, the role of HbA_1 or glycosylated protein was to be considered a possible contributing factor to the membrane changes. This must await further investigation.

In conclusion, diabetes mellitus causes changes in erythrocyte membrane fluidity, which appear to be primarily located in the deeper (hydrophobic) region. The cause of the change has not yet been identified.

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