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Cell membrane fatty acid composition in Type 1 (insulin-dependent) diabetic patients: relationship with sodium transport abnormalities and metabolic control

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Summary. We have studied the fatty acid composition of erythrocyte membrane phospholipids in nine Type 1 (insulin-dependent) diabetic patients and nine healthy control subjects. Cell membranes from the diabetic patients showed a marked decrease in the total amount of polyunsaturated fatty acids (19.0 % ± 2.2 vs 24.6 % ± 1.4, $p < 0.0001$) mainly at the expense of docosahexaenoic acid C22:6(n3) (2.9 % ± 1.1 vs 5.3 % ± 1.3, $p < 0.001$), and arachidonic acid C20:4 n6 (12.0 % ± 1.6 vs 15.1 % ± 0.6, $p < 0.0005$). Conversely, the total amount of saturated fatty acids was significantly increased ($p < 0.05$) and the polyunsaturated/saturated ratio was decreased in the Type 1 diabetic patients ($p < 0.00005$). Neither the time from diagnosis, nor C-peptide levels, correlated with parameters indicating a poor metabolic control of Type 1 diabetes. However, C22:6(n-3) and total n-3 content significantly correlated with HbA_{1c} ($r = -0.79$ and $r = -0.88$, respectively, $p < 0.01$), fructosamine ($r = -0.71$ and $r = -0.74$, respectively, $p < 0.05$), and Na^+-K^+ ATPase activity (maximal rate/K_m quotient) ($r = 0.78$ and $r = 0.71$, respectively, $p < 0.05$). In conclusion we have found marked alterations of cell membrane lipid composition in Type 1 diabetic patients. These cell membrane abnormalities in lipid content were related to sodium transport systems and to poor metabolic control. Either diet, or the diabetic state, might be responsible for the observed cell membrane abnormalities. A dietary intervention study might differentiate the role of diet and diabetes in the reported cell membrane alterations.

Key words: Phospholipids, fatty acid composition, sodium transport system, cell membrane, diabetes mellitus.

Several ion transport abnormalities have been reported in patients with essential hypertension [1–3] and are related to a greater cardiovascular risk [4], but the underlying molecular mechanisms leading to these phenomena remain unknown. Patients with Type 1 (insulin-dependent) diabetes mellitus also have anomalies in their sodium transport systems, related to the degree of target organ damage [5, 6] and a more atherogenic lipoprotein profile [7]. The mechanisms underlying these observations are equally unknown.

In 1986 Bing and colleagues [8] hypothesised that altered composition of the lipid fraction of cell membrane was the common underlying factor in hypertension. Many other authors have demonstrated a significant correlation between cell membrane fatty acid composition and sodium transport abnormalities in different diseases [9–11]. In vitro experiments [12] have shown that small changes in fatty acid composition of cell membrane phosphatydylcholine cause considerable changes in the sodium transport systems.

We have previously reported that diet composition and other exogenous factors may influence fatty acid composition of phospholipids in the cell membrane [13–17]. Diet supplementation with increasing doses of linoleic acid may change the activity of membrane transport systems in normotensive humans [18]. Field et al. [19, 20] have recently demonstrated that diet composition alters membrane phospholipid composition, and the cellular response to insulin in streptozotocin-induced diabetic rats, and Storlien et al. [21] found that dietary fat composition influences development of insulin resistance in rats. The modification of cellular lipid content may also induce an insulin-resistant state [22].

Type 1 diabetic patients follow a restricted diet, which could alter cell membrane lipid content, thus causing sodium transport abnormalities. These events might be related to a worsened metabolic control, the presence of increased target organ damage, and a greater cardiovascular risk. Exogenous interventions may be able to improve these alterations.

We have studied the erythrocyte membrane fatty acid composition in Type 1 diabetic patients and control subjects, to assess its contribution to abnormalities in the sodium transport systems and to the degree of metabolic control in these patients.

Table 1. General data of both populations studied

Parameter	Controls ($n = 9$)	Patients ($n = 9$)
Age (years)	30.5 ± 3.8	24.7 ± 7.0
Sex (M/F)	(5/4)	(4/5)
BMI (kg/m^2)	22.9 ± 1.3	22.8 ± 2.1

Values are mean ± SD. p = NS for all comparisons

Table 2. Plasma lipid levels

Variable	Control subjects ($n = 9$)	Diabetic patients ($n = 9$)
Cholesterol (mmol/l)	5.2 ± 1.3	4.6 ± 0.9
HDL (mmol/l)	1.1 ± 0.4	1.0 ± 0.3
LDL (mmol/l)	3.2 ± 1.1	2.5 ± 0.6
HDL_2 (mmol/l)	0.25 ± 0.03	0.25 ± 0.03
HDL_3 (mmol/l)	0.88 ± 0.31	0.75 ± 0.21
Triglycerides (mmol/l)	0.8 ± 0.6	1.0 ± 0.6
Phospholipids (mmol/l)	1.9 ± 0.4	1.9 ± 0.3

Values are mean ± SD. p = NS for all comparisons

Table 3. Erythrocyte sodium transport kinetic parameters

Parameters	Control subjects ($n = 9$)	Diabetic patients ($n = 9$)
Na^+-K^+ pump[a]		
K_m	5.7 ± 2.5	6.8 ± 3.7
V_{max}	10635 ± 8474	11125 ± 5516
V_{max}/K_m ratio	1711 ± 508	1694 ± 411
Na^+-K^+ Cotransport[a]		
K50	15.4 ± 4.8	9.6 ± 6.9
V_{max}	392.8 ± 225	381.4 ± 40.4
Na^+-Li^+ Countertransport[a]		
K_m	4.4 ± 4.5	2.0 ± 1.1
V_{max}	147.2 ± 83	180.4 ± 111
Passive Na^+ permeability[a]		
KpNa	18.2 ± 4.9	16.0 ± 3.9
Intracellular Na^+[b]	7.2 ± 2.3	6.2 ± 1.4

Values are mean ± SD. [a] $\mu mol \cdot l^{-1} \cdot cell^{-1} \cdot h^{-1}$. [b] $mmol/l \cdot cell$

Subjects and methods

Patients

The first two patients who attended our Type 1 diabetes out-patient clinic each morning for 1 week ($n = 10$), were informed of the purpose of the study, and nine of them agreed to participate.

Mean duration of diabetes was 10 years and all the patients were free of chronic complications as determined by routine clinical and eye examinations. Mean overnight albumin excretion rate was 27.6 µg/min (range 0–91 µg/min) and mean serum creatinine levels were 74.3 µmol/l (range 57.5–97.2 µmol/l). The control group comprised nine healthy subjects, chosen from members of the staff, with no family history of diabetes, hypertension or hypercholesterolaemia.

The patients had no disease other than diabetes and were not taking any medication. They were advised to eat a diet of 2500 kcal, containing 30% fat, 15% protein and 55% carbohydrate. Control subjects ate an unrestricted diet composed of approximately 35% fat, 15% protein and 50% carbohydrate. The approximate composition of dietary fats, obtained by inquiry, was in % of polyunsaturated (PUFA), monosaturated and saturated, respectively 10,10,10 in diabetic patients and 10,15,10 in control subjects. Extra virgin olive oil was the only oil consumed by both groups.

Table 4. Relative percentages of individual neutral lipids and phospholipids extracted from erythrocyte plasma membrane

	(weight/weight)	
	Control subjects	Diabetic patients
Cholesterol	35.8 ± 2.3	33.3 ± 4.2
Phosphatidylethanolamine	29.2 ± 1.2	28.4 ± 2.4
Phosphatidylcholine	14.3 ± 0.2	19.1 ± 3.9
Lysophosphatidylcholine	3.6 ± 0.6	3.4 ± 1.2
Sphingomyelin	17.0 ± 1.7	15.5 ± 1.3

Results are given as mean ± SD. p = NS for all comparisons

Medical history and physical examination

Height and weight were measured with the subjects not wearing shoes. Mean systolic and diastolic (fifth Korotkoff sound) blood pressures were obtained by averaging two blood pressure measurements taken with the patient in the supine position. A medical history was taken and a complete physical examination performed for each patient. Eye examination by an ophthalmologist was performed, and dosage of insulin and number of injections was also recorded.

Biochemical measurements

Levels of blood glucose, fructosamine, serum urea, creatinine, sodium, potassium, alanine aminotransferase (ALT), aspartate aminotransferase (AST), total cholesterol, plasma phospholipids and triglycerides were measured by conventional enzymatic methods; HDL, HDL_2 and HDL_3 by precipitation with addition of polyethylene glycol (Quantolip inmuno-GMBH, Heidelberg, FRG) and LDL-cholesterol by precipitation with reagent precipitating polycyclic anionic (Biomérieux, Marcy L'etoile, France); HbA_{1c} by HPLC chromatography, apolipoproteins A-I and B by laser nefelometry, and C-peptide levels by RIA using polyclonal antibodies obtained from rabbit and human standards (CIS-B10, Gif-sur Yvette, France), using fasting venous blood obtained on the day of the examination.

Preparation of erythrocytes

Fasting blood samples were collected in heparinized tubes and centrifuged at 1750 g at 4 °C for 10 min. The plasma and buffy coat were aspirated, and the erythrocyte pellet was washed twice with 110 mmol/l $MgCl_2$and used immediately. Aliquots of these cells were separated for simultaneous assay of sodium transport kinetics and fatty acid composition of cell membrane.

Sodium transport kinetics

Sodium loading procedure: in each experiment four aliquots of cells containing different concentrations of intracellular sodium [Na_i] were prepared using a method based on the stimulation of physiological $NaHPO_4^-$ influx through the anion carrier. Briefly, four different aliquots of washed erythrocytes were resuspended to a haematocrit of about 5% in four different Na^+-loading media containing (in mmol/l): 1) 75 $MgCl_2$, 85 sucrose, 10 glucose and 10 4-morpholinopropanesulphonic acid-Tris buffer (pH 7.4 at 37 °C), 2) 150 NaCl, 2.5 Na^+ phosphate (pH 7.4 at 37 °C), 1 $MgCl_2$, 10 glucose, 3 inosine, and 2 adenine, 3) 75 NaCl, 50 Na_2HPO_4, 35 sucrose, 10 glucose, 3 inosine and 2 adenine, and 4) 100 Na_2HPO_4 and 75 sucrose. Erythrocytes in medium 1 were immediately processed and those in media 2, 3 and 4 were incubated at 37 °C for 120 min and then resuspended to a haematocrit of 10% in medium 2 and incubated at 37 °C for 120 min. At the end of this loading period the cells were centrifuged at 1750 g at 4 °C for 5 min and then stored at 4 °C until the next day.

In a control experiment we verified that cell pH, inorganic phosphate content, and haemoglobin absorbance per litre of cells (an indirect indicator of cell volume) were normal.

Measurements of sodium efflux: erythrocytes were washed five times with cold 110 mmol/l $MgCl_2$ and resuspended at a haematocrit of 25 % in medium 1. A portion of each cell suspension was set aside to measure haematocrit, intracellular sodium and potassium by atomic absorption spectrophotometry, and haemoglobin absorbance at 540.5 nm. The $[Na_i]$ content of unloaded cells was taken as the basal erythrocyte Na^+ content.

Next 0.5 ml of the erythrocyte suspension was added (final haematocrit 4–5 %) to tubes containing 2 ml of cold medium 1 plus the following additions: a) 2 mmol/l KCl , b) 1 mmol/l ouabain, c) 1 mmol/l ouabain + 0.1 mmol/l bumetanide, and d) 1 mmol/l ouabain + 0.1 mmol/l bumetanide + 10 mmol/l LiCl. The osmolalities were maintained at 295 ± 5 mosmol.

At time zero, the tubes were transferred to a 37 °C water bath for further incubation. At 0 (media c and d), 30 (medium a) and 60 (media b,c,d) min, the tubes were chilled at 4 °C for 5 min and centrifuged at 1750 *g* at 4 °C for 4 min. External Na^+ concentrations were measured in the supernatants by atomic absorption spectrophotometry (Perking-Elmer 460, Norwalk, Conn., USA). In control experiments no evidence of cell lysis during the incubation in the efflux media was detected.

Na^+ efflux (V) was computed using the following equation:

$$V = D_{Na}\,(100\text{–}Hct)/Hct \times t. \quad (1)$$

where D_{Na} is the difference in external Na^+ concentration before (time zero) and after incubation at 37 °C, Hct is the final haematocrit and t is the incubation time.

Na^+-K^+ pump activity was taken as the ouabain-sensitive Na^+ efflux (Vp); calculated by subtracting Na^+ efflux in medium b from that of medium a. Na^+-K^+ cotransport was equated to the bumetanide-sensitive Na^+ efflux (Vc); calculated by subtracting Na^+ efflux in medium c from that in medium b. Na^+-Li^+ countertransport was taken as the Li^+ stimulated Na^+ efflux (Vcn); calculated by subtracting Na^+ efflux in medium c from that in medium d. Na^+ efflux in medium c was equated to passive Na^+ permeability.

Calculation of apparent affinity for internal sodium and maximal rate of sodium efflux: Na^+ efflux catalysed by each transport system studied was plotted as a function of $[Na_i]$. Regarding the Na^+-K^+ pump in human erythrocytes, this function can be fitted by a modified Hanes equation:

$$Vp = Vp_{max}/(1 + K_{pNa}/[Na_i])^3 \quad (2)$$

where Vp represents the ouabain-sensitive sodium efflux, Vp_{max} is the maximal rate of Vp (V_{max}), Kp_{Na} represents the apparent dissociation constant for $[Na_i]$ (K_m), and 3 is the number of inner pump sites for $[Na_i]$.

Equation 2 can be rearranged as follows:

$$[Na_i]/(Vp)^{1/3} = Kp_{Na}/(Vp_{max})^{1/3} + [Na_i]/(Vp_{max})^{1/3} \quad (3)$$

The left side of equation 3 was plotted against $[Na_i]$ (Hanes plot). Kp_{Na} and Vp_{max} were obtained from the intercept with the abscissa and slope, respectively [23].

Outward Na^+-K^+ cotransport (Vc) as a function of $[Na_i]$ also follows an equation similar to equation 2 in human erythrocytes [24].

$$Vc = Vc_{max}/(1 + Kc_{Na}/[Na_i])^3 \quad (4)$$

where Vc_{max} is the maximal rate of bumetanide-sensitive Na^+ efflux (V_{max}) and Kc_{Na} represents the apparent dissociation constant for $[Na_i]$ and both were obtained by a similar method to the one used for the Na^+-K^+ pump (Hanes plot in equation 3).The exponent 3 does not necessarily mean that the Na^+-K^+ cotransport has three inner sites for Na^+. Equation 4 only describes a phenomenological function that fits the sigmoidal dependence of outward cotransport fluxes as a function of $[Na_i]$.

$Kc_{50\%}$ the $[Na_i]$ required for half-stimulation of outward Na^+-K^+ cotransport, was obtained by using the following equation (3):

$$Kc_{50\%} = 3.85\,Kc_{Na} \quad (5)$$

Na^+-Li^+ countertransport is a Michaelis-like function of $[Na_i]$ in human erythrocytes [25]. It follows the equation:

$$Vcn = Vcn_{max}/(1 + Kcn_{Na}/[Na_i]) \quad (6)$$

where Vcn_{max} is the maximal rate of Li^+-stimulated Na^+ efflux (V_{max}) and Kcn_{Na} represents the apparent dissociation constant for internal Na^+ (K_m) and both were obtained from a Hanes plot of the data [24].

Passive Na^+ permeability was obtained by dividing passive Na^+ efflux from unloaded cells by $[Na_i]$ (similar values were obtained by calculating the slope of passive Na^+ efflux as a function of $[Na_i]$ for the four erythrocyte Na^+ concentrations).

Phospholipid fatty acid composition of the erythrocyte cell membrane

Lipids were extracted from erythrocyte membranes using the method of Rose and Oklander [26].

Lipids were separated on commercial silica gel 60 plates using thin layer chromatography (Kiesegel 60 F-254: Merck, Darmstadt, FRG). Plates were developed using a solvent system composed of hexane-ether-acetic acid (80: 20: 1), respectively. Lipid bands were visualized by exposing plates to iodine vapor. Phospholipids were estimated by measurement of total phosphate using the procedure of Barlett with slight modification [27].

Individual phospholipids were separated on thin layer chromatography silica gel 60 plates according to Parsons and Patton [28]. Lipid extract (200–400 µg) was loaded and the plates were developed using chloroform-methanol-water-0.88 sp.gr. ammonia 112: 50: 4: 5 (v/v/v/v) in the first dimension and chloroform-acetone-methanol-glacial acetic acid-water 3: 4: 1: 1: 0.5 (v/v/v/v/v) in the second dimension. Individual phospholipids were identified by reference to known standards (Sigma Chemical Co, St. Louis, Mo., USA). Additional confirmation of the identity of the lipids was obtained using a ninhydrin spray reagent [29]. Individual phospholipids were eluted from the silica with two 10-ml portions of chloroform-methanol-water 5: 5: 1 (v/v/v). The solvent was evaporated in a stream of nitrogen and the sample was dessicated prior to further analysis. Phospholipids were estimated by measurement of total phosphate according to the procedure of Barlett with slight modifications [27].

Analysis of fatty acid methyl esters (FAME): the phospholipids were transmethylated according to a modified method of Morrison and Smith [30]. The lipid bands on silica gel 60 plates were sprayed lightly with a solution of 0.1 % (w/v) 2,6-tert-butyl-p-cresol (butylate hydroxy toluene, BHT) obtained from Sigma (Poole, Dorset, UK) in methanol prior to visualization. The phospholipids were eluted from the silica gel with two 15-ml aliquots of chloroform-methanol-water. The solvent was evaporated in a stream of nitrogen and, 10 µg tricosanoic acid (23: 0 internal standard) was added prior to the addition of 40 µl chloroform-methanol (1: 1 v/v) after which, 200 µl complex were immediately added. The sample was flushed with nitrogen, sealed in a vial fitted with a teflon-lined cap, and heated at 120 °C for 1 h. After the sample was cooled, the FAME were extracted with 500 µl hexane.

For fatty acid analysis a gas chromatograph (Hewlett Packard series 5890; Avondale, Pa., USA) with a flame ionisation detector and heated injection ports, was used. A Supelcowax 10 fused silica capillary column (60 mat × 0.25 inner diameter film thickness 0.25 µm) was obtained from Supelco (Bellafonte, Pa., USA). The sample (1 µl injection of test material was made) was injected into the gas chromatograph. Following injection, the oven temperature was maintained at 200 °C for 10 min, then programmed to increase at a rate of 2 °C per min to a final temperature of 260 °C for 30 min. The flow-rate of helium was 2 ml/min, column head pressure 250 kPa, split ratio 1: 25, the detector and injector temperature 275 °C, and detector auxiliary flow rate 25 ml/min. Each FAME present in the extract was identified by comparison of its retention time with those of known standard FAME, obtained from Larodan Fine Chemicals (Malmö, Sweden).

Table 5. Fatty acid composition (relative molar percentages) of erythrocyte plasma membrane

Fatty acid	Controls ($n = 9$)	Patients ($n = 9$)
C16:0	22.5 ±0.9	28.0 ±4.3[b]
C16:1(n7)	0.12 ± 0.09	0.20 ± 0.06[a]
C16:1(n9)	0.07 ± 0.03	0.18 ± 0.08[c]
C18:0	18.9 ±2.1	16.5 ±1.8
C18:1(n9)	16.5 ±0.8	17.1 ±2.3
C18:1(n7)	1.5 ±0.3	1.5 ±0.2
C18.1(Trans)	0.20 ± 0.07	0.22 ± 0.1
C18.2(n6)	12.7 ±1.6	13.6 ±1.4
C20:0	0.51 ± 0.3	0.30 ± 0.1
C20:1(n9)	0.30 ± 0.08	0.51 ± 0.2[b]
C20:2(n6)	1.68 ± 0.3	1.26 ± 0.2[b]
C20:3(n3)	0.08 ± 0.05	0.16 ± 0.1
C20:3(n6)	0.32 ± 0.05	0.35 ± 0.14
C20:4(n6)	15.1 ±0.6	12.0 ±1.6[e]
C22:0	0.27 ± 0.16	0.31 ± 0.2
C22:4(n6)	2.12 ± 0.6	2.37 ± 0.5
C22:5(n3)	0.92 ± 0.5	0.92 ± 0.5
C22:5(n6)	0.81 ± 0.7	0.20 ± 0.1
C22:6(n3)	5.31 ± 1.3	2.97 ± 1.1[d]
C24:0	0.13 ± 0.04	1.54 ± 0.5[e]
Σ Saturated	42.4 ±1.7	47.2 ±4.7[a]
Σ Monounsaturated	18.7 ±0.9	19.7 ±2.4
Σ Diunsaturated	14.4 ±1.8	14.9 ±1.6
Σ Polyunsaturated	24.6 ±1.4	19.1 ±2.2[f]
P/S ratio	0.58 ± 0.04	0.41 ± 0.07[g]
Σ n-3	6.31 ± 1.3	4.05 ± 1.4[b]
Σ n-6	32.3 ±1.7	29.5 ±2.4[a]
C20:4/C18:2 ratio	1.2 ±0.6	0.89 ± 0.2[d]

Values are mean ± SD. P/S ratio, Polyunsaturated/saturated fatty acid ratio. [a] $p < 0.05$, [b] $p < 0.01$, [c] $p < 0.005$, [d] $p < 0.001$, [e] $p < 0.0005$, [f] $p < 0.0001$, [g] $p < 0.00005$

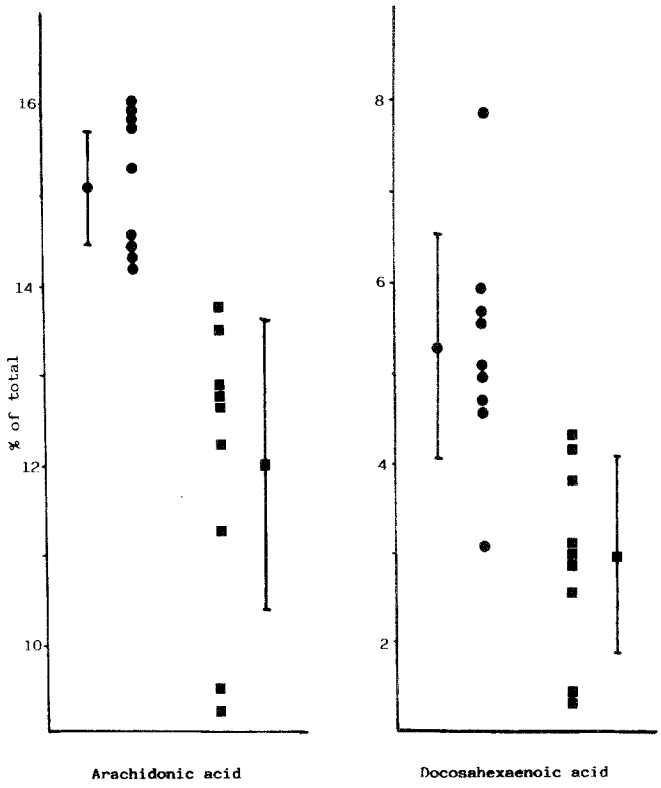

Fig. 1. Phospholipids arachidonic acid (left panel) and docosahexaenoic acid (right panel) content in cell membrane of Type 1 diabetic (■, $n = 9$) and control subjects (●, $n = 9$); $p < 0.0005$ for arachidonic acid content; $p < 0.001$ for docosahexaenoic acid content in Type 1 diabetic patients vs control subjects

Statistical analysis

Data were evaluated comparing means for independent samples (Student's *t*-test) and single linear regression analysis. Kolmogorow test was used to estimate the normality of populations.

Results

Age, sex and BMI were similar in both populations (Table 1). Plasma levels of lipids were also comparable despite a slight, non-significant, elevation of triglycerides in the diabetic group (Table 2).

All the patients were normotensive and there were no obese patients (BMI range: 20.2–27.2). All the diabetic patients were receiving exogenous human insulin at a mean dose of 46.3 ± 6.7 IU/day (Regular: 8.3 ± 6.9 IU/day, NPH: 38 ± 7.9 IU/day) in 2.1 ± 0.3 (range 2–3) subcutaneous injections per patient per day. Mean C-peptide was 0.23 µg/l (range 0–0.58). The level of blood fasting glucose was 10.9 ± 5.9 µmol/l , HbA_{1c} was 8.4 ± 2.2%, and fructosamine 402.1 ± 94.7 µmol/l. Serum AST, ALT, urea, creatinine, sodium and potassium were within the normal range. There were four patients with albumin excretion rates between 20 µg/min and 200 µg/min. There were no patients with albumin excretion rates over 200 µg/min.

Basal sodium content of erythrocyte was lower in diabetic patients although this difference was not statistically significant (Table 3). Sodium transport kinetic parameters were also similar in both groups.

Relative percentages of individual neutral lipids and phospholipids were identical for both groups (Table 4). However the fatty acid composition of erythrocyte cell membranes was clearly different between diabetic patients and control subjects (Table 5).

The amount of docosahexaenoic acid C22: 6(n-3) and arachidonic acid C20: 4(n-6) was markedly decreased in diabetic patients (Fig. 1). The arachidonic/linoleic ratio was also lower in these patients, as was the total amount of PUFA and the summation of n-3 and n-6 PUFA. The content of C20: 2(n-6), another PUFA, was also lower (Table 5).

Conversely, the total amount of saturated fatty acid was increased, and the polyunsaturated/saturated (P/S) ratio was decreased in diabetic patients. The replacement of PUFA was mainly at the expense of the saturated fatty acid C16: 0 and C24: 0, and the mono-unsaturated fatty acid C16: 1(n-7), C16: 1(n-9) and C20: 1(n-9).

Neither the time after diagnosis nor C-peptide levels significantly correlated with parameters indicating poor metabolic control of diabetes. However, the amount of C22: 6(n-3) (a fatty acid deficient in cell membrane of Type 1 diabetic patients, was negatively and significantly correlated with HbA_{1c} ($r = 0.79$, $p < 0.01$) and fructosamine ($r = -0.71$, $p < 0.05$) values (Fig. 2). A similar relationship was observed between C22: 5(n-3) and HbA_{1c} ($r = -0.80$, $p < 0.01$) or fructosamine ($r = -0.68$, $p < 0.05$). Likewise, the total content of n-3 PUFA, correlated with the levels of HbA_{1c} ($r = -0.88$, $p < 0.01$) and fructosamine ($r = -0.74$, $p < 0.05$) (Fig. 3).

Na^+-K^+ ATPase activity (V_{max}/K_m ratio) was significantly related to C22: 6(n-3) ($r = 0.79, p < 0.05$), the total content of n-3 fatty acids ($r = 0.71, p < 0.05$) (Fig. 4), the total content of n-6 PUFA (maximal rate $r = 0.70$, $p < 0.05$), the total amount of PUFA ($r = 0.69, p < 0.05$) and, C18: 1 trans (K_m $r = 0.67$, $p < 0.05$ V_{max} $r = 0.73$, $p < 0.05$). Whereas, C22: 5(n-6) related to the maximal rate of Na^+-Li^+ countertransport ($r = 0.92, p < 0.01$) and passive permeability ($r = -0.80, p < 0.05$). A negative relationship was found between Na^+-K^+ ATPase and intracellular sodium ($r = -0.69, p < 0.05$). There was no significant relationship between microalbuminuria and sodium transport systems, or between Na^+-K^+ ATPase activity and HbA_{1c} or fructosamine.

Plasma triglycerides were related to cell membrane content of C16: 0 ($r = 0.76, p < 0.05$), C20: 1 ($r = 0.68, p < 0.05$) and the total content of saturated fatty acids ($r = 0.67$, $p < 0.05$). Plasma phospholipids were related to the C18: 1 trans cell membrane content ($r = 0.88, p < 0.01$).

Discussion

Using a highly sensitive method to quantify the fatty acid composition of phospholipids, we observed a marked decrease in the total amount of PUFA in the erythrocyte cell membrane of Type 1 diabetic patients. This decrease was mainly due to docosaexaenoic C22: 6(n-3) and arachidonic C20: 4(n-6) acids, and was compensated by a parallel increase of total saturated fatty acid content, in particular by the long-chain saturated fatty acid C24: 0. These observations confirm those of Kamada et al. [31] with regard to total PUFA, P/S ratio and C22: 6(n-3) content of cell membrane (C20: 4(n-6) amounts were decreased but this was not statistically significant), and those of Baldini et al. [32] with regard to a decrease in C20: 4(n-6) content in erythrocyte membrane phosphatidylcholine in Type 1 diabetic patients. These abnormalities might be related to the inhibitory effect of a glucose-rich medium or insulin deficiency on desaturase activity [33].

Similar findings regarding PUFA, saturated fatty acids, P/S ratio and C20: 4(n-6) content of other cell membrane phospholipids, have been reported in studies performed with diabetic rats [19, 34, 35]. However, only one report [34] quantified C22: 6(n-3) and a decrease in this fatty acid was not seen.

Rats with chemically-induced diabetes and fed on a standard diet have changes in the cell membrane lipid content which are similar to those found in Type 1 diabetic humans with the exception of C22: 6(n-3). Thus, we believe that diet, not the diabetic state, might be responsible for the decreased cell membrane content of C22: 6(n-3).

We also found a significant correlation between C22: 6(n-3) cell membrane content and parameters indicating poor metabolic control, whereas no correlation was observed between these parameters and C-peptide levels or duration of diabetes. This observation is supported by previous data showing that diet supplementation with n-3 PUFA significantly increased C22: 6(n-3) content in cell membrane phosphatidylcholine and phosphatidyethanolamine, as well as the in vivo insulin-stimulated glucose uptake [36], and therefore Hb_{A1c} and fructosamine levels

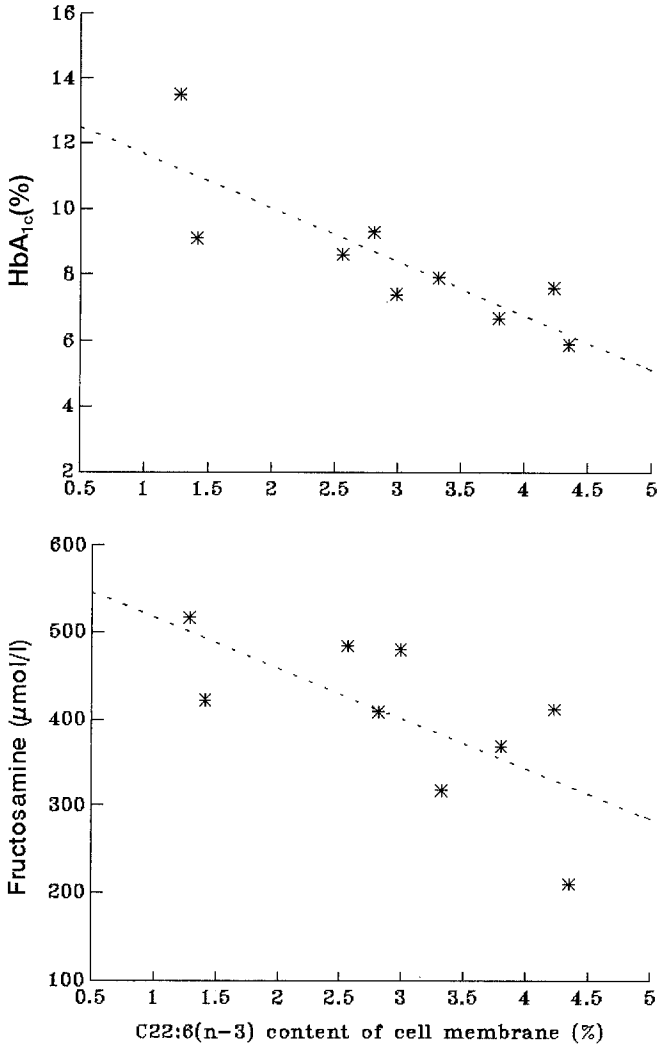

Fig. 2. Comparison of cell membrane C22: 6(n-3) content and parameters of metabolic control of diabetes. Upper panel $r = 0.83$, $p < 0.01$; lowel panel $r = 0.68, p < 0.05$

may be improved. Data indicating that fatty acid composition of adipocyte cell membrane increased insulin binding and glucose metabolism after consuming a high PUFA diet have been also reported [19, 20]. Since we did not directly control the diet of our patients, the possibility of restricted n-3 PUFA consumption (essentially fish oil consumption) by erroneous choices in regard to diet in diabetes cannot be excluded. In this sense, we have retrospectively questioned our patients and control subjects concerning their fish oil consumption (sardine, herring, mackerel, whitebait, anchovy and salmon are the most commonly available). While control subjects did not recall any restriction, two diabetic patients reported very infrequent consumption, and four, an infrequent consumption of this kind of fish. Interestingly the two patients with very infrequent consumption have the lowest levels of C22: 6(n-3) and total n-3 PUFA and one of them has high levels of HbA_{1c} and fructosamine. Only a dietary intervention study will be able to clarify this point.

We have also found a remarkable decrease in arachidonic acid C20: 4(n-6) content of cell membrane in diabetic patients, with no significant changes in its precursor, linoleic acid C18: 2(n-6), and therefore with a de-

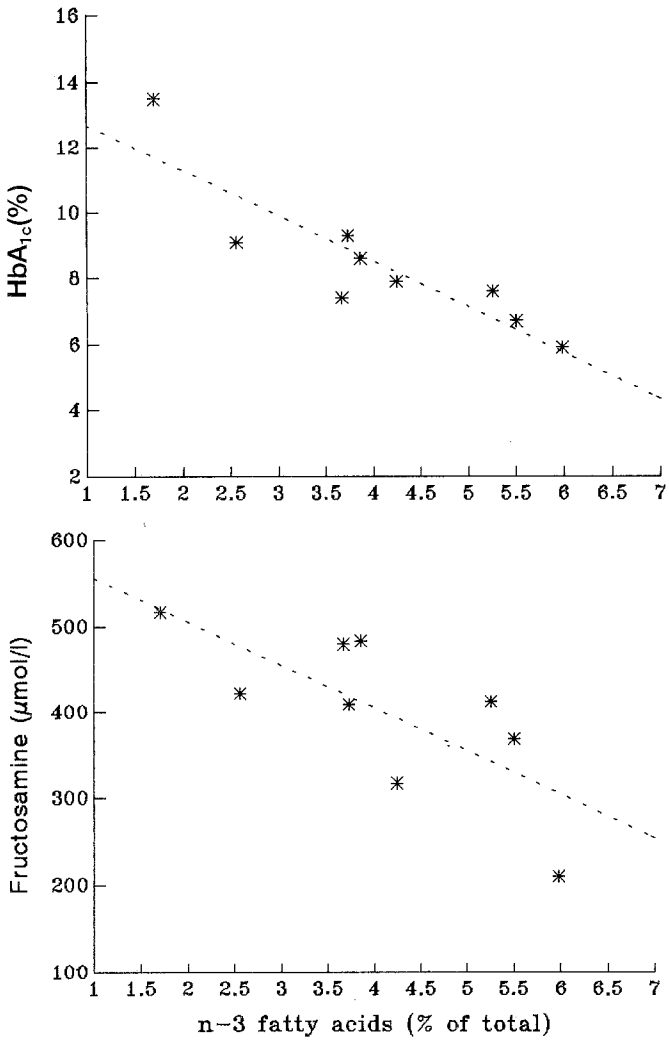

Fig. 3. Total cell membrane n-3 fatty acid content and parameters of metabolic control of diabetes. Upper panel $r = 0.88, p < 0.01$; lower panel $r = 0.74, p < 0.05$

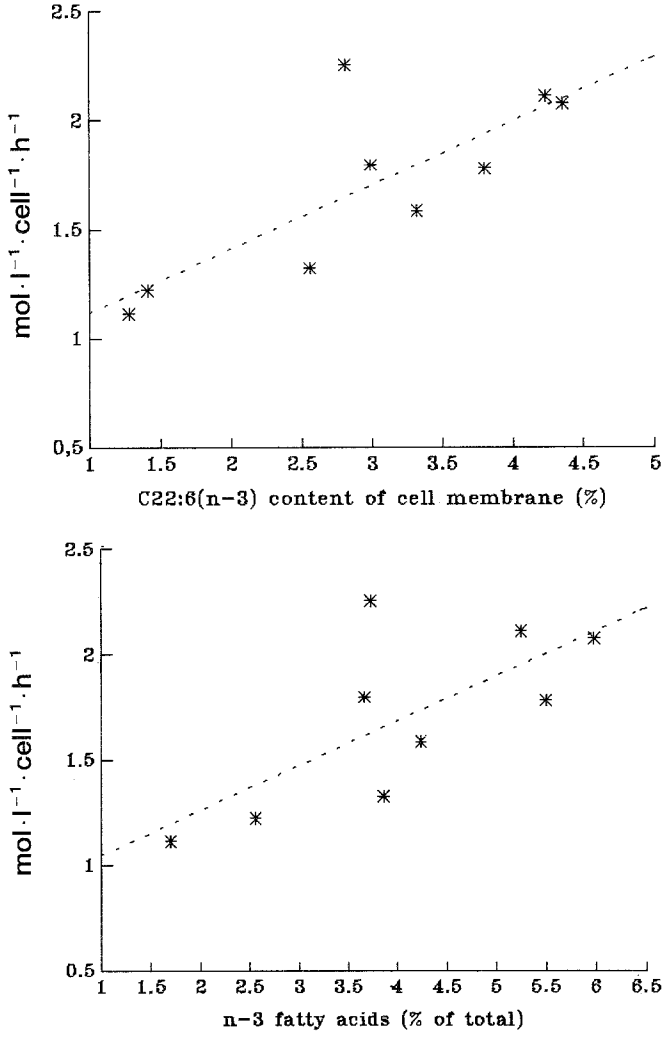

Fig. 4. Relationship between Na^+-K^+ ATPase activity (maximal rate/K_m quotient) and C22: 6(n-3) or total n-3 fatty acid cell membrane content. Upper panel $r = 0.79, p < 0.05$; lower panel $r = 0.71$, $p < 0.05$

crease in the C20: 4/C: 18: 2 ratio. This finding may be explained by the diabetes [20] and dietary associated reduction [37, 38] of delta-6 or delta-5 desaturase activity.

When the fluid mosaic model for cell membrane was formulated, evidence was becoming available that membrane lipids can influence the function of certain membrane proteins, such as those involved in the ion transport systems [39]. We have analysed four sodium transport systems together with the lipid composition of cell membrane and have found a significant relationship between docosahexaenoic acid C22: 6(n-3), total n-3 PUFA, and total PUFA with Na^+-K^+ ATPase activity measured by the V_{max}/K_m quotient. Baldini et al. [32] have found a significant decrease in ouabain-binding capacity of the Na^+-K^+ ATPase in Type 1 diabetic patients and a relationship between this parameter and the cholesterol to phospholipid ratio in cell membrane.These authors have not reported a similar relationship between C22: 6(n-3) or total n-3 PUFA. A discrepancy with our results is possible, since Baldini et al. [32] did not find a significant decrease in the amount of C22: 6(n-3) in cell membrane and their patients had a mean value of HbA_{1c} of $7.1 \pm 1.3\%$, indicating excellent metabolic control. Corrocher et al. [11] have found a similar finding between this carrier and the average degree of fatty acid unsaturation, measured as a P/S ratio in psoriatic patients. These authors have hypothesized that the Na^+-K^+ ATPase activity may be facilitated by the altered membrane fluidity, due to unbalanced P/S ratio. Nevertheless, the possibility that the surrounding lipids affect the conformation of certain carriers, thereby enhancing or reducing the accessibility of their binding sites [38] should be also taken into account. Finally Na^+-Li^+ countertransport, a transport system which is related to the amount of PUFA and P/S ratio in cell membrane in other diseases [9–11] when measured as Na^+-induced Li^+ efflux, it was significantly correlated with the amount of C22: 5(n-3) according to our methodology.

In conclusion, we have found marked differences in fatty acid composition of cell membrane in Type 1 diabetic patients and a relationship between these alterations and parameters which indicate poor metabolic control, and thus greater cardiovascular risk. The contribution of sodium ion transport systems in these alterations and the possibility of dietary intervention should be considered in future studies.

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