Essential fatty acid and lipid profiles in plasma and erythrocytes in patients with multiple sclerosis[1-4]

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ABSTRACT This study was conducted to investigate the possible differences in erythrocyte lipid composition, which might account for the previously reported increase in erythrocyte membrane zinc levels in patients with multiple sclerosis (MS). Compared with healthy control subjects, plasma lipids in patients with MS contained less sphingomyelin but more phosphatidylserine and the cholesterol-phospholipid ratio was 42% higher in the plasma from MS patients ($p < 0.01$). In erythrocytes from MS patients, phosphatidylinositol was lower and erythrocyte cholesterol per milligram protein was significantly lower than concentrations in healthy control subjects ($p < 0.01$). Among the long-chain fatty acids, the ω-3 fatty acids were lower in plasma from MS patients and linoleic acid was lower in erythrocyte ghosts from MS patients ($p < 0.01$). We conclude that altered levels of cholesterol in plasma and erythrocytes from MS patients may contribute to increased erythrocyte-membrane Zn in MS patients. It cannot be stated with certainty whether the altered fatty acid profiles in MS patients were a function of the disease or of altered fatty acid intake. *Am J Clin Nutr* 1989;50:801–6.

KEY WORDS Essential fatty acid, linoleic acid, arachidonic acid, docosahexaenoic acid, lipid, multiple sclerosis, zinc

Introduction

Dore-Duffy et al (1) and Ho et al (2) reported that compared with either healthy control or neurological control subjects, plasma and erythrocytes from patients with multiple sclerosis (MS) have higher zinc levels. Erythrocyte Zn levels were increased specifically in the erythrocyte membrane and, within the membrane, were elevated in the extracts of the lipid fraction. Furthermore, during periods of relapse or exacerbation of MS symptoms, Zn levels in the erythrocytes decreased dramatically (2). Of the total Zn normally present in the erythrocyte, only 20% is present in the membrane (2). However, 67% of the Zn present in the erythrocyte-membrane is bound to the lipid fraction (3). Because long-chain fatty acids such as oleic acid (18:1ω-9) have been shown to markedly increase the uptake of Zn by the erythrocyte membrane (4), we considered it worthwhile to investigate whether oleic acid levels were abnormal in erythrocytes of individuals with MS, which might account for increased Zn uptake by their erythrocytes.

In reviewing the literature it became evident that despite several studies reporting differences in long-chain fatty acid composition in plasma or erythrocytes of patients with MS (5–9), and other studies reporting modest beneficial effects in MS from a reduction of dietary fat intake (10) or supplementation with linoleic acid (11, 12), there appears to be no studies in which the composition of both long-chain fatty acids and lipids in plasma and erythrocyte samples from MS patients have been analyzed in detail and in comparison with data from neurological control subjects. Therefore, in addition to studying the fatty acid composition of erythrocyte membranes from MS patients for a possible abnormality associated with increased Zn levels, we also made a detailed assessment of the fatty acid composition of plasma from MS patients compared with both healthy and neurological control subjects.

Methods

Patients

Patients with MS were chosen from the University of Connecticut MS Center Clinic population. Patients were evaluated

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according to the criteria of McAlpine (13) and the study was approved by the Ethics Committee of the University of Connecticut. Progressive patients had more than one exacerbation per year. An exacerbation was determined upon clinical examination and documentation of the appearance of a new symptom of the disease. Control subjects with neurological disease were chosen from the general neurology clinics at the University of Connecticut; diseases included myasthenia gravis, amyotrophic lateral sclerosis, brain tumors, and pseudotumors.

Blood-sample preparation

Blood samples were collected by venipuncture by using special Zn-free evacuated tubes (Becton-Dickinson, Rutherford, NJ). After centrifugation at 1000 × g for 15 min, plasma was separated and frozen at −20 °C. Erythrocytes were washed three times with 0.15 mol NaCl/L and once with 310 mmol phosphate buffer and either frozen at −20 °C or erythrocyte ghosts were prepared. Erythrocyte ghosts were prepared by the method of Dodge et al (14) with the following modifications. All solutions were carefully prepared to avoid any possible Zn contamination. Membrane ghosts were centrifuged with ice-cold hypotonic phosphate buffer (20 mmol, pH 7.4) at 10 000 × g for 40 min. The washing procedure was repeated 4–5 times until the final pellet was milky white and the supernatant visibly free of hemoglobin. The ghost material was dialyzed overnight against distilled water and lyophilized for storage. Ghosts were stored at −70 °C until analyzed.

Analytical methods

The plasma, erythrocyte, and erythrocyte ghost samples were coded and sent on dry ice to the Efamol Research Institute where the fatty acid, lipid, and trace element levels were analyzed blind. All analytical reagents used were American Chemical Society or high-performance liquid chromatography grade (Fisher Scientific, Dartmouth, Nova Scotia, Canada).

Fatty acids. One-milliliter samples of plasma were extracted in chloroform-methanol (2:1, vol:vol) containing 0.02% butylated hydroxytoluene according to the method of Folch et al (15). Aliquots (0.5 g) of the washed erythrocytes were initially diluted with distilled water and the lipids extracted into methanol containing 0.03% butylated hydroxytoluene, followed by addition of chloroform such that the final ratio of chloroform to methanol was 2:1 according to the method of Dodge and Phillips (16).

Aliquots of the lipid extracts were spotted on silica gel 60 thin-layer chromatography plates (Merck 5734, Mandel Scientific, Rockwood, Ontario, Canada) and developed in either hexane–diethyl ether–acetic acid (80:20:1, vol:vol:vol) for neutral lipid separation or chloroform-methanol–5 mol ammonium hydroxide/L–water (65:25:4:2, vol:vol:vol:vol) for separation of phospholipid classes. Plates were sprayed with 0.02% dichlorofluorescein in methanol (Sigma Chemical Co, St Louis) and viewed under ultraviolet light for identification of lipid bands. The lipid bands were scraped into screw-capped test tubes (Kimax, Toronto, Ontario, Canada) and methylated with boron trifluoride-methanol (Sigma Chemical Co) at 90 °C for 30 min. The percent composition of the fatty acid methyl esters was then determined by gas-liquid chromatography (HP5880 with autosampler, Hewlett-Packard Co, Palo Alto, CA) by using a 0.6 cm × 2 m glass column packed with Gas Chrom Q impregnated on Chromosorb WAW (Applied Science, State College, PA). Operating conditions were as follows: injection temperature, 200 °C; detector temperature, 220 °C; oven temperature gradient, 165–200 °C at 2 °C/min; and carrier gas flow rate, 30 mL/min. Fatty acid methyl ester retention times were determined by comparison with authentic standards (Nuchek Prep, Elysian, MN) with a Level 4 integrator (Hewlett-Packard Co).

Lipids. Total cholesterol and triacylglycerol in plasma were measured by an automated procedure with the Cobas Bio autoanalyzer (Roche Diagnostics, Nutley, NJ) and assay kits (Boeringer-Mannheim, Indianapolis, IN). Total phospholipids in plasma and erythrocytes were quantitated by the method of Bartlett as modified by Christie (17). Total cholesterol in erythrocytes was quantitated by gas-liquid chromatography by using a 1-m glass column packed with OV-1 on Chromasorb WAW under the operating conditions previously described (18).

Trace metals. Zn, copper, and iron levels in plasma and Zn levels in erythrocyte ghost membranes were quantitated by atomic absorption spectrophotometry (Instrumentation Laboratories 457, Wilmington, NJ). Plasma and erythrocyte samples were collected, stored, and assayed under conditions of minimal contamination. Glass surfaces intended for contact with the samples were soaked overnight in 10% nitric acid (Aristar, BDH Chemicals, Dartmouth, Nova Scotia) and rinsed with doubly distilled water. Plasma samples were diluted with doubly distilled water (1:5, vol:vol) and analyzed directly. Lyophilized erythrocyte ghosts were resuspended in phosphate buffer and aspirated directly. No differences in Zn levels were observed whether the ghosts were resuspended in phosphate or barbital buffer (both 20 mmol, pH 7.4).

Protein. Protein levels in the erythrocyte ghost membranes were analyzed according to the method of Lowry et al (19).

Statistics

Comparisons between lipid and fatty acid values in the three groups were made by using one-way analysis of variance and specific differences were identified by using Scheffe's test for multiple comparisons or the Mann-Whitney U test for smaller numbers of samples (erythrocyte ghosts) (20).

Results

Plasma and erythrocyte lipids

The content of lipids in the plasma and the composition of the phospholipid subclasses is shown in **Table 1.** Patients with MS had similar total phospholipid, cholesterol, and triacylglycerol levels in plasma as compared with healthy and neurological control subjects. Nevertheless, the ratio of cholesterol to phospholipid was 42% higher in the patients with MS than in the healthy or neurological control subjects ($p < 0.01$). The distribution of phospholipid classes differed significantly between groups; in the MS patients compared with the healthy control subjects, sphingomyelin was quantitatively lower by 41% and proportionally lower by 34% ($p < 0.01$) whereas phosphatidylserine was proportionally higher by 49% ($p < 0.01$).

Total phospholipids, cholesterol, and cholesterol-phospholipid levels in erythrocytes (based on wet weight) were quantitatively similar in all three groups (**Table 2**). Lysophospholipids represented 16–20% of the erythrocyte phospholipids as separated by thin-layer chromatography. This was probably because some samples were frozen for extended periods (up to 6 mo) while other samples were being collected. Phospholipid subclass dis-

TABLE 1
Plasma lipids in healthy control subjects compared with patients with multiple sclerosis (MS) or unrelated neurological diseases (NC)*

	Control (n = 14)	MS (n = 12)	NC (n = 5)
		mmol/L	
PL	2.17 ± 0.63	1.70 ± 0.26	2.36 ± 0.54
PC	1.20 ± 0.36 (60.1)	1.06 ± 0.11 (63.4)	1.38 ± 0.31 (60.2)
SM	0.34 ± 0.11 (17.0)	0.20 ± 0.11 (11.2)†	0.31 ± 0.18 (12.6)
PS	0.2 ± 0.08 (9.5)	0.24 ± 0.05 (14.2)†	0.28 ± 0.10 (13.3)
PI	0.05 ± 0.03 (2.2)	0.04 ± 0.01 (2.3)	0.05 ± 0.01 (2.2)
PE	0.04 ± 0.03 (1.5)	0.03 ± 0.01 (1.5)	0.04 ± 0.03 (1.8)
CH	4.6 ± 1.2	5.1 ± 0.7	5.3 ± 1.3
TG	1.5 ± 1.4	1.5 ± 0.8	1.5 ± 0.5
CH:PL	2.12 ± 0.37	3.00 ± 0.51†	2.25 ± 0.39

* $\bar{x}$ ± SD; percents in parentheses. PL, total phospholipid; PC, phosphatidylcholine; SM, sphingomyelin; PS, phosphatidylserine; PI, phosphatidylinositol; PE, phosphatidylethanolamine; CH:PL, ratio of cholesterol to phospholipid; and TG, triacylglycerol.

† Significantly different from control subjects, $p < 0.01$ (one-way analysis of variance and Scheffe test for multiple comparisons).

tribution differed significantly between groups; phosphatidylcholine was proportionally higher by 46% ($p < 0.01$) in the neurological control subjects as compared with the healthy control subjects. Phosphatidylinositol was quantitatively lower by 40% and proportionally lower by 57% ($p < 0.01$) in the MS patients as compared with the healthy control subjects.

Long-chain fatty acid composition

The only statistically significant difference between groups in the percent composition of long-chain fatty acids in the total phospholipids in plasma was in palmitic acid (16:0), which was 19% higher in the MS patients than in the healthy control subjects ($p < 0.01$) (**Table 3**). The increased palmitic acid in the MS patients was localized to the phosphatidylserine fraction (data not shown). Although levels of the ω-6 and ω-3 fatty acids in the total phospholipids in plasma were not different between groups, the sum of the ω-3 fatty acids derived from α-linolenic acid (18:3ω-3) was 19% lower in the MS patients than in the healthy control subjects ($p < 0.01$). ω-3 fatty acids were also not detected in the phosphatidylserine fraction of the MS plasma phospholipids but were present at 1.5% in the healthy control subjects and 2.1% in the neurological control subjects. Ratios of linoleic acid to arachidonic acid and α-linolenic acid to docosahexaenoic acid in the total phospholipids (indicative of desaturase activity) did not differ significantly between groups.

In the free fatty acids, triacylglycerols, and cholesteryl esters in plasma, isolated differences in fatty acid values existed between groups but values for linoleic acid or total ω-6 and ω-3 fatty acids did not differ significantly between groups (data not shown).

In the phosphatidylcholine fraction of the erythrocyte phospholipids, the MS patients and neurological control subjects had no detectable levels of docosapentaenoic acid (22:5ω-6), α-linolenic acid, or eicosapentaenoic acid (20:5ω-3); MS patients alone also had 20% lower stearic acid levels compared with the other two groups (**Table 4**). In the phosphatidylinositol fraction, MS patients had no detectable ω-3 fatty acids. In the other erythrocyte phospholipid fractions, very few differences in fatty acid

TABLE 2
Erythrocyte lipids in healthy control subjects compared with patients with multiple sclerosis (MS) and unrelated neurological diseases (NC)*

	Control (n = 14)	MS (n = 12)	NC (n = 5)
		μmol/g	
PL	239 ± 43	258 ± 40	249 ± 33
SM	48 ± 18 (20.9)	54 ± 11 (26.6)	39 ± 11 (21.3)
LPL	46 ± 11 (20.5)	39 ± 13 (19.1)	33 ± 9 (16.4)
PC	39 ± 15 (17.0)	40 ± 9 (19.5)	46 ± 10 (24.9)†
PE	31 ± 15 (13.4)	29 ± 11 (13.4)	18 ± 11 (8.8)
PS	28 ± 8 (11.8)	20 ± 8 (12.3)	21 ± 8 (11.3)
PI	6 ± 1 (4.7)	4 ± 1 (2.0)†	5 ± 1 (3.2)
CH	216 ± 31	224 ± 26	211 ± 18
CH:PL	0.90 ± 0.13	0.87 ± 0.12	0.85 ± 0.09

* $\bar{x}$ ± SD; percents in parentheses. PL, total phospholipid; SM, sphingomyelin; LPL, lysophospholipid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PS, phosphatidylserine; and PI, phosphatidylinositol.

† Significantly different from control subjects, $p < 0.01$ (one-way analysis of variance and Scheffe test for multiple comparisons).

TABLE 3
Long-chain fatty acids (% composition) in plasma total phospholipids of healthy control subjects compared with patients with multiple sclerosis (MS) or unrelated neurological diseases (NC)*

	Control (n = 14)	MS (n = 12)	NC (n = 5)
16:0	21.0 ± 4.2	25.1 ± 3.2†	23.3 ± 4.7
18:0	9.1 ± 2.4	8.6 ± 1.4	8.1 ± 1.5
18:1ω-9	11.4 ± 1.0	12.0 ± 1.4	12.0 ± 3.3
18:2ω-6	31.5 ± 3.4	28.7 ± 4.3	29.4 ± 5.5
20:3ω-6	2.9 ± 0.4	3.1 ± 0.6	3.3 ± 1.0
20:4ω-6	15.4 ± 3.5	15.4 ± 2.1	15.2 ± 2.5
22:4ω-6	0.6 ± 0.2	0.4 ± 0.1	0.5 ± 0.2
22:5ω-6	0.4 ± 0.1	0.4 ± 0.1	0.5 ± 0.2
18:2ω-6/20:4ω-6	2.14 ± 0.61	1.92 ± 0.47	2.03 ± 0.71
18:3ω-3	0.2 ± 0.1	0.3 ± 0.2	0.3 ± 0.2
20:5ω-3	1.0 ± 0.6	0.7 ± 0.2	0.8 ± 0.4
22:5ω-3	0.9 ± 0.2	0.8 ± 0.2	0.8 ± 0.3
22:6ω-3	4.7 ± 1.1	3.8 ± 0.9	4.2 ± 0.5
18:3ω-3/22:6ω-3	0.05 ± 0.02	0.06 ± 0.04	0.08 ± 0.03
Total ω-3 products	6.6 ± 1.5	5.3 ± 1.1†	5.7 ± 1.1

* $\bar{x}$ ± SD.

† Significantly different from control subjects, $p < 0.01$ (one-way analysis of variance and Scheffe test for multiple comparisons).

TABLE 4
Long-chain fatty acids (% composition) in erythrocyte phospholipids of healthy control subjects compared with patients with multiple sclerosis (MS)*

	Phosphatidylcholine		Phosphatidylinositol	
	Control (n = 14)	MS (n = 12)	Control (n = 14)	MS (n = 12)
16:0	38.1 ± 8.1	40.1 ± 3.5	18.5 ± 4.6	16.2 ± 3.1
18:0	13.2 ± 1.6	10.5 ± 0.6†	36.7 ± 3.7	37.6 ± 5.4
18:1ω-9	20.7 ± 2.0	22.4 ± 2.4	15.6 ± 5.1	14.0 ± 2.5
18:2ω-6	20.5 ± 3.9	20.7 ± 2.4	11.3 ± 3.0	11.0 ± 4.0
20:3ω-6	1.3 ± 0.6	1.1 ± 0.3	1.9 ± 0.9	2.1 ± 0.1
20:4ω-6	4.3 ± 2.0	3.4 ± 1.3	19.7 ± 7.4	21.4 ± 3.8
22:4ω-6	0.3 ± 0.1	0.3 ± 0.1	—	1.6 ± 0.5
22:5ω-3	0.4 ± 0.2	0.3 ± 0.1	—	—
22:6ω-3	1.2 ± 0.5	1.5 ± 0.3	1.9 ± 0.4	—

* $\bar{x}$ ± SD.
† Significantly different from control subjects, $p < 0.01$ (one-way analysis of variance and Scheffe test for multiple comparisons).

profiles were found between the three groups (data not shown).

Levels of linoleic acid and arachidonic acid were significantly positively correlated in the erythrocyte phosphatidylcholine fraction of the healthy control subjects but were not correlated in the erythrocytes of the MS patients (**Fig 1**).

Erythrocyte ghost membranes

Erythrocyte ghost membranes were prepared to study the lipid and Zn content. Total protein, lipids, and Zn

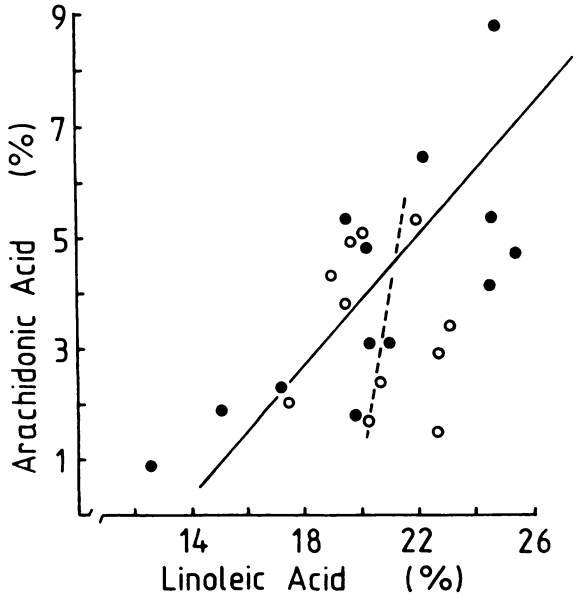

FIG 1. Correlation between the percent composition of arachidonic acid (20:4ω-6) and linoleic acid (18:2ω-6) in erythrocyte phosphatidylcholine in healthy control subjects (●; $r = +0.48$, $p < 0.01$) and patients with multiple sclerosis (○; $r = -0.12$, NS).

TABLE 5
Protein, zinc, and lipids in erythrocyte ghosts of healthy control subjects compared with patients with multiple sclerosis (MS)*

	Control (n = 5)	MS (n = 5)
Protein (mg/g DW)	273 ± 46	365 ± 58
Zn (μmol/g DW)	0.82 ± 0.53	2.60 ± 1.07†
Cholesterol		
(mmol/g DW)	0.22 ± 0.02	0.21 ± 0.02
(mmol/g protein)	0.85 ± 0.18	0.59 ± 0.10†
Phospholipid		
(mmol/g DW)	0.26 ± 0.05	0.26 ± 0.05
(mmol/g protein)	1.00 ± 0.31	0.74 ± 0.23

* $\bar{x}$ ± SD. Values are based on dry weight (DW) of erythrocyte ghosts.
† Significantly different from control subjects, $p < 0.01$ (Mann-Whitney U test).

levels in the erythrocyte ghosts are shown in **Table 5.** Total protein was 34% higher (NS), Zn was three times higher ($p < 0.01$), and total cholesterol (based on protein content but not dry weight) was 30% lower ($p < 0.01$) in the ghost membranes from the MS patients than in that from the healthy control subjects. Total phospholipid levels, although 26% lower in the MS ghost membranes, did not differ significantly between the two groups.

Most significant among the differences in fatty acid composition of the erythrocyte ghost membrane phospholipid fractions was the significantly lower linoleic acid in three of the four fractions studied (**Table 6**); 17% lower in phosphatidylcholine, 31% lower in phosphatidylethanolamine, and 46% lower in sphingomyelin ($p < 0.01$).

Plasma trace metals

Plasma levels of Zn, Cu, and Fe did not differ significantly between groups; in healthy control subjects they were as follows (μmol/L, $\bar{x}$ ± SD): Zn, 14.8 ± 2.4; Cu, 24.3 ± 4.6, and Fe, 37.8 ± 8.4.

Discussion

The single fatty acid most commonly studied in MS in relation to both dietary supplementation and blood lev-

TABLE 6
Linoleic acid composition (% composition) of erythrocyte ghost phospholipids of healthy control subjects compared with patients with multiple sclerosis (MS)*

	Control (n = 5)	MS (n = 5)
Phosphatidylcholine	23.1 ± 1.7	19.2 ± 3.8†
Phosphatidylethanolamine	8.9 ± 2.0	6.1 ± 1.0†
Phosphatidylinositol	6.4 ± 1.0	9.3 ± 2.2
Sphingomyelin	7.1 ± 2.5	3.8 ± 2.0†

* $\bar{x}$ ± SD.
† Significantly different from control subjects, $p < 0.01$ (Mann-Whitney U test).

els is linoleic acid. Most groups studying linoleic acid levels in MS patients have examined its levels in total lipids or phospholipids in plasma. Some have found plasma linoleic acid to be modestly lower in MS patients (21–24), others have found no change (25, 26), and still others have demonstrated a clinically significant therapeutic benefit of linoleic acid supplementation (12, 27, 28) in MS patients.

Navarro and Segura (29) recently completed a detailed study of plasma fatty acid and lipid profiles in MS patients and reported that linoleic acid was significantly lower in plasma cholesteryl esters but only marginally lower in plasma triacylglycerols and phospholipids. They also observed a lower proportion of linoleic acid in patients with other neurological diseases pointing to the comparison of neurological and healthy control subjects as essential in drawing any conclusions about the role of linoleic acid in MS patients. Their data are similar to ours with respect to the modestly lower linoleic acid in plasma phospholipids. Lower linoleic acid concentrations in plasma cholesteryl esters have been reported in MS patients (21) but we did not observe any significant difference in the linoleic acid content of plasma cholesteryl esters in MS patients or in neurological control subjects with respect to healthy control subjects.

In five plasma lipid fractions and in five erythrocyte phospholipid fractions, mean linoleic acid levels in the present study were lower by 8.6% and 5.6%, respectively (NS). In the plasma phosphatidylcholine from 6 of 14 MS patients, linoleic acid levels were at least one SD below the mean of the healthy control subjects. Erythrocyte ghost membrane phospholipids also had significantly lower linoleic acid levels in three of four phospholipids fractions assayed (Table 6). Had the present study included more MS patients we might have observed significantly lower linoleic acid levels in plasma and erythrocyte lipids.

Considerable variability in plasma fatty acid composition can be attributed to differences in analytical technique, patient selection, disability, and, especially, diet. Supplementation studies with linoleic acid have generally been on an outpatient basis and differences in linoleic acid intake alone could create much of the variability in linoleic acid levels in the blood of MS patients. Even when the variability in these factors is excluded, dietary variation in the intake of nutrients affecting linoleic acid metabolism, such as competing fatty acids (eg, saturates) (30) and cofactors in linoleic acid desaturation (31), may markedly alter plasma phospholipid levels of linoleic acid. Therefore, because blood levels of linoleic acid indicate overall linoleic acid status, our data suggest that neither dietary intake nor metabolism of linoleic acid is markedly affected in MS patients. Because of the widely observed but marginal effect of MS on linoleic acid metabolism reported by others (21–29), it may be that blood levels of essential fatty acids (EFA) are an imprecise measure of a functional deficit of EFAs in the body.

Homa et al (7) observed that while linoleic acid and arachidonic acid levels in erythrocyte phospholipids were significantly negatively correlated in healthy control subjects ($r = -0.83$), they were much less well correlated in MS patients ($r = -0.27$) (7). The implication was that in healthy individuals with adequate linoleic acid intake, the degree of conversion of linoleic acid to arachidonic acid is reflected in the relative incorporation of these fatty acids into erythrocyte phospholipids. However, although Homa et al (7) observed an inverse correlation between linoleic acid and arachidonic acid levels in erythrocyte phospholipids from healthy control subjects, we observed a significant positive correlation, $r = 0.48$, $p < 0.01$ (Fig 1). Hence, the correlation data from both our studies suggests that in some MS patients, synthesis or erythrocyte acylation of arachidonic acid may be inappropriately low.

One possible cause of altered synthesis or metabolism of arachidonic acid or docosahexaenoic acid in MS patients is that the cofactors required for desaturase activity, eg, Fe and Zn, could have been insufficiently available. In this study plasma levels of Zn, Cu, and Fe in MS patients were similar to those in the healthy control subjects (1, 2). Zn levels in erythrocytes were previously shown to be increased in MS patients (Table 5) (1, 2). The changes in erythrocyte Zn level in MS patients are restricted to the plasma membrane but it is not known whether membrane-bound Zn is available to the desaturase enzymes controlling long-chain fatty acid synthesis. It is also possible that disease status may be a significant factor in determining the degree to which synthesis of arachidonic acid and docosahexaenoic acid is affected in MS.

Because of the reported effect of oleic acid on increasing erythrocyte uptake of Zn (4), we initially anticipated that oleic acid levels would be increased in erythrocyte phospholipids in the MS patients. We did not observe any significant difference in the oleic acid levels in any erythrocyte phospholipid classes in MS patients. We did however note that in relation to protein content, cholesterol was 30% lower in the erythrocyte ghosts of MS patients (Table 5). Furthermore, plasma cholesterol content was 42% higher in the MS patients, supporting the concept that cholesterol metabolism may be abnormal in MS patients and may contribute to other membrane changes, eg, Zn content in erythrocyte membranes. These differences in cholesterol distribution in plasma and erythrocytes may in turn be related to functional changes in the erythrocyte membranes in MS, eg, their impaired filterability and electrophoretic mobility (32–34).

The etiology of MS has been related to a lower intake or incorporation of ω-3 fatty acids into brain lipids (35). Lower erythrocyte phospholipid content of ω-3 fatty acids has been reported (8) and supplementation with a dietary source of ω-3 fatty acids was recently reported to ameliorate the clinical symptoms of MS (36). This indicates that further research of the metabolism of ω-3 fatty acids in MS patients is required. Our data extend the evidence indicating that intake or metabolism of ω-3 fatty acids may be impaired in MS patients; in total phospholipids and erythrocyte phosphatidylcholine in plasma, ω-

3 fatty acids were significantly lower in MS patients than in control subjects (Tables 3 and 4) and ω-3 fatty acids were not detectable in plasma phosphatidylserine (not shown) or erythrocyte phosphatidylinositol (Table 4).

In conclusion, this study provides additional evidence suggesting that modest abnormalities in linoleic acid and ω-3 fatty acid profiles may exist in individual plasma and erythrocyte lipid fractions in MS patients. Through correlation data we also elaborate on the apparent abnormalities in conversion of linoleic acid to arachidonic acid and synthesis or lipid incorporation of docosahexaenoic acid in MS patients. Our findings are novel in demonstrating that cholesterol levels in plasma and erythrocytes in MS patients are significantly altered in relation to phospholipid and protein, respectively. This may be a basis for altered erythrocyte filterability and increased erythrocyte membrane Zn levels in MS patients. The role of diet compared with disease process in accounting for these differences has not been evaluated.

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