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Deficiencies of polyunsaturated fatty acids and replacement by nonessential fatty acids in plasma lipids in multiple sclerosis

(acyl exchange/mean chain length/mean melting point/membrane fluidity/viral disease)

RALPH T. HOLMAN*†, SUSAN B. JOHNSON*, AND EMRE KOKMEN‡

*The Hormel Institute, University of Minnesota, Austin, MN 55912; and ‡The Mayo Clinic, Rochester, MN 55905

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ABSTRACT **Fatty acid compositions of plasma phospholipids, cholesteryl esters, triacylglycerols, and nonesterified fatty acids of 14 clinically proven and graded cases of multiple sclerosis were determined by capillary gas chromatography and compared with the values obtained for 100 normal, healthy subjects. In phospholipids, linoleic acid (18:2ω6; 18 carbon atoms, 2 double bonds, 6 carbon atoms beyond last double bond) was normal and 18:3ω6 was increased, but all subsequent ω6 acids were subnormal ($P < 0.001$), indicating impairment of chain elongation. All ω3 acids were subnormal. The paucity of polyunsaturated fatty acids was compensated mass-wise by an increase in saturated acids. Disproportionate increases in short-chain, saturated, and monounsaturated acids, decreases in long-chain homologs, and increases of branched and odd-chain acids were observed. Loss of polyunsaturated fatty acids and replacement by nonessential acids lowered mean chain length and raised mean melting point significantly, suggesting that lowered membrane fluidity was only partially compensated by endogenous synthesis of lower-melting, nonessential acids. This phenomenon was not observed in cholesteryl esters or triacylglycerols. Nonesterified fatty acids showed significant changes in pattern of possible autacoid precursors. The abnormal profile of fatty acids in multiple sclerosis has features in common with profiles of other syndromes involving viral infections.**

In 1963, polyunsaturated fatty acid (PUFA) profiles of fatty acids (FA) in lipids of cerebrospinal fluid were measured in patients with multiple sclerosis (MS) (1). Low levels of linoleic acid (18:2ω6) in lipids were reported (2–4). Other studies found little difference between MS patients and normal individuals for 18:2ω6 or other FA in serum lipids (5–8). One study concluded that FA patterns in MS and other illnesses resembled that of essential fatty acid (EFA) deficiency and that this may be a general phenomenon of illness (9). The early studies analyzed total serum lipids by isothermal gas chromatography (GC) with packed columns and so did not detect and measure minor FA or FA that emerged from the GC column after arachidonate (20:4ω6). This laboratory has pursued study of the PUFA patterns of plasma lipids in disease by using state-of-the-art capillary GC, which detects and measures minor components as low as 0.1% of FA ranging from 12:0 through 24:1. This program, summarized recently (10), has revealed abnormal patterns of FA in Reye syndrome (11), a genetic syndrome with severe EFA deficiency (12), linolenic acid (18:3ω3) deficiency (13), and cirrhosis with alcoholism (14). Our studies have confirmed the prediction of Love *et al.* (9) that abnormal patterns observed in diseases are a general phenomenon, and many diseases display unique features of EFA deficiency when the whole range of FA is considered.

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Preliminary studies on a few undescribed cases of MS indicated PUFA abnormalities, so a study of clinically proven and graded MS patients was undertaken. The early focus upon 18:2ω6 was unfortunate, because deficiencies of minor PUFA of both ω6 and ω3 families proved to be of greater significance. The former use of total lipids was also unfortunate because, in our present study, few significant abnormalities were found in cholesteryl esters (CE) or triacylglycerols (TG), which dilute and obscure changes in PUFA patterns in phospholipids (PL).

SUBJECTS AND METHODS

MS Patients. Patients in this study were diagnosed at the Mayo Clinic, and plasma used was excess remaining from routine examinations. This study was approved by the University of Minnesota Committee on the Use of Human Subjects in Research. Clinical records of laboratory studies and medical neurological examinations were reviewed in detail. Diagnostic and classification criteria described by Poser *et al.* (15) were used. For all 14 cases, the patients' functional abilities were estimated using the Kurtzke disability status scale (16), in which higher numbers indicate greater disability. The MS patients are described in Table 1. Mean age was 39.5 ± 13.8 yr (SD). Mean duration of illness was 9.9 ± 6.3 yr (SD), and mean Kurtzke score was 4.4 ± 2.2 (SD).

Control Subjects. In another study (17), 100 omnivors were recruited as normal controls from the staff or student body of the University of Minnesota. Each completed a health and diet history. This control group had a mean age of 29.3 yr, male/female ratio of 37/63, mean serum cholesterol of 192 mg/dl, and mean TG level of 80 mg/dl. Plasmas from the control group and MS patients were subjected to the same lipid analysis described below, except that 17:0 was added as internal standard for quantification of the lipid classes in the control group. An earlier study of normal FA profiles (18) indicated no significant change due to sex or age.

Methods of Analysis. Plasmas were kept frozen until use. Lipids from 2-ml samples were extracted with 6 ml of chloroform/methanol, 2:1 (vol/vol), and centrifuged. The aqueous layer was drawn off and the extract was filtered to remove protein, dried under N_2, and redissolved in 100 μl of chloroform. Extracts were applied to silica gel thin-layer plates and developed in 30–60°C petroleum ether/diethyl ether/acetic acid, 80:20:1 (vol/vol). Plates were sprayed with 0.1% 2,7′-dichlorofluorescein solution and viewed under UV light. Lipid classes appeared as distinct separated bands, and PL, nonesterified fatty acids (NEFA), TG, and CE were scraped into glass tubes with Teflon caps. The lipids were transesterified with 2 ml of 5% (wt/vol) HCl in methanol at

Abbreviations: FA, fatty acid(s); EFA, essential FA; NEFA, nonesterified FA; PUFA, polyunsaturated FA; CE, cholesteryl ester(s); PL, phospholipid(s); TG, triacylglycerol(s); MS, multiple sclerosis; DBI, double-bond index; NR, normalcy ratio; n.s., no significance.

†To whom reprint requests should be addressed.

Table 1. Description of MS patients included in this study

Patient	Sex	Age, yr	MS type*	Stage†	Duration, yr	Kurtzke score
1	F	39	CD	AR	1.3	1
2	F	58	CD	C	20	5
3	F	24	CD	C	7	4
4	F	31	CD	C	10	7
5	F	49	CD	C	10	3
6	F	25	CD	C	15	6
7	F	19	CD	AR	3	1
8	F	55	CD	C	16	6
9	M	22	CP	AR	3	1
10	F	52	LSD	C	3	4
11	M	38	CD	C	5	4
12	F	46	CD	C	18	6
13	M	58	CD	C	15	6
14	F	37	CD	CAA	12	7

*CD, clinically definite; CP, clinically probable; LSD, laboratory-supported definite.
†AR, acute in remission; C, chronic; CAA, chronic in acute attack.

75°C. PL and CE were heated for 1.5 hr, and FA and TG for 0.5 hr. After esterification, the esters were extracted with petroleum ether (30–60°C), dried under N_2, and redissolved in heptane for GC analysis.

GC analysis was performed on a 50 m × 0.25 mm bonded, fused silica capillary 007 FFAP column (Quadrex, New Haven, CT) with a split ratio of 1:66, programmed from an initial 13-min hold at 180°C to 220°C at 2°C/min, with a final hold of 8 min. Identification of esters was made by comparison with authentic standards. Peak areas were calculated by a dedicated microprocessor. Because 17:0 was used as internal standard for calculating concentrations of FA in plasma and for quantification of the four major lipid classes in the control group only, calculation of 17:0 in control lipids was not possible, and the only odd-chain FA measured for comparison of the MS to control was 15:0. Trace components occurring near the retention times for branched FA may include their isomers, and in PL samples only, fatty aldehydes derived from plasmalogens.

Presentation of Data. Data are expressed as relative percentage of total FA because FA occur in vesicles and membranes that are domains of mixed lipids. The kinetics of their metabolism are governed by their concentrations at two-dimensional surfaces rather than by three-dimensional concentrations in surrounding aqueous medium, in which they are not soluble. The percentage of FA within a lipid class better expresses the concentration of substrate available to an interfacial enzyme than does the concentration within the aqueous space. The ratio of experimental to normal values (normalcy ratio, NR) indicates relative concentration change in disease, and this is plotted on a logarithmic scale so that increases and decreases of the same proportion are the same length. In the graphic profiles used here, the vertical axis is normal, open bars indicate no significance (n.s.); bars with wide striations, $P < 0.05$; bars with close striations, $P < 0.01$; and black bars, $P < 0.001$. Three profiles show individual PUFA, non-essential FA, and PUFA grouped by reaction or family. Triene/tetraene (Tri/tetra) ratio is 20:3ω9/20:4ω6, the first index of nutritional EFA deficiency (19), and double-bond index (DBI) is the average number of double bonds per FA.

RESULTS AND DISCUSSION

FA Profile of Plasma PL. PL are major structural components of membranes—the major locus of PUFA in tissue—and display the greatest changes in PUFA content induced by disease. Plasma PL reflect changes in PUFA that occur in tissue PL (20). The group profile of the 14 MS patients compared with 100 healthy controls is shown in Fig. 1. The 18:2ω6 content of PL in MS did not differ from normal, and 18:3ω6 was only slightly increased (n.s.). The elongation products, 20:2ω6 and 20:3ω6 and subsequent products, were significantly suppressed. The dietary precursor of the ω3 family, 18:3ω3, and its product 20:5ω3 were subnormal (n.s.), and 22:5ω3 and 22:6ω3 were significantly low. Mead's acid, 20:3ω9, often elevated in nutritional EFA deficiencies, was also low. MS patients appeared to desaturate at Δ6 but were unable to perform the first chain elongation, for all PUFA products subsequent to elongation to C_{20} were deficient in PL. This striking deficiency occurred with a normal triene/tetraene ratio because 20:3ω9 and 20:4ω6, both products of Δ5 desaturation, were suppressed. This is one more evidence that the triene/tetraene ratio is too simplistic a measure to be used alone, without considering the entire pattern of PUFA. In MS the total ω6 FA, total ω3 FA, total PUFA, and DBI all indicated that polyunsaturation was less than normal. Within total saturated FA of PL, whose increase was about equal to the loss of PUFA, replacement of the PUFA was preferen-

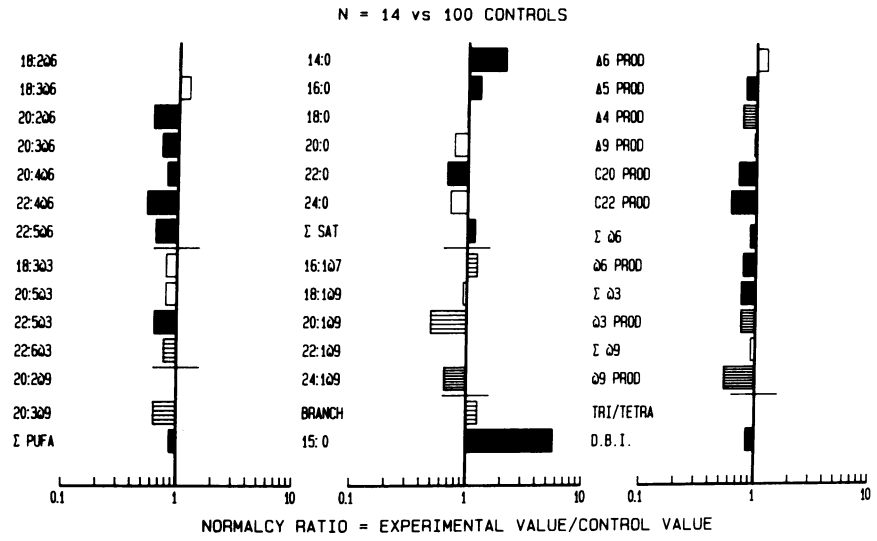

FIG. 1. FA profile of plasma PL in MS patients. SAT, saturated; PROD, products. For further explanation, see *Presentation of Data* under *Subjects and Methods*.

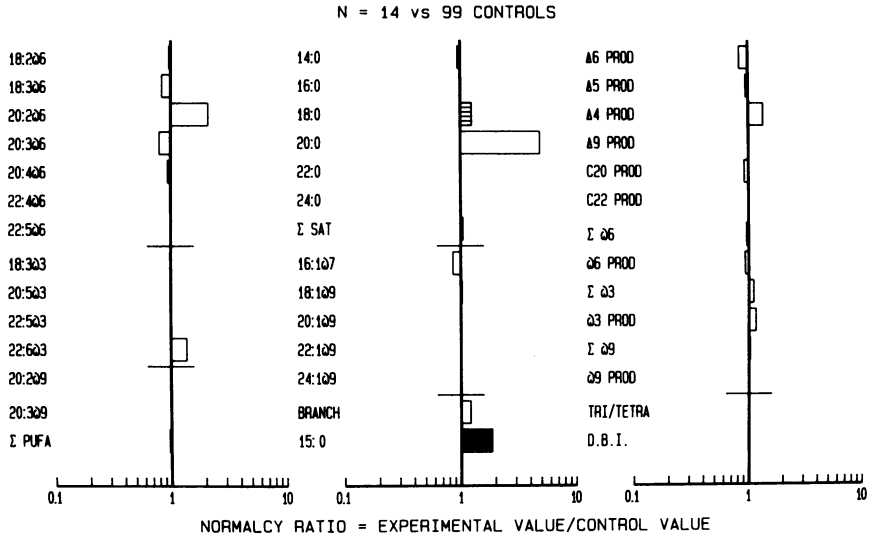

FIG. 2. FA profile of plasma CE in MS patients.

tially by shorter homologs, and longer-chain saturated FA were suppressed in MS. The greatest mass increase was due to 16:0. A similar disproportionation occurred with monounsaturated FA. Branched-chain FA and 15:0 increased significantly.

FA Profiles of Plasma CE and TG. The plasma CE profile (Fig. 2) was rather close to normal. None of the PUFA differed significantly from normal. Of saturated FA, only 18:0 (stearic acid; NR = 1.26, $P < 0.05$) and 15:0 (NR = 1.85, $P < 0.001$) differed significantly from normal. The plasma TG profile is shown in Fig. 3. Among saturated FA, only 20:0 (NR = 3.5, $P < 0.01$) and 15:0 (NR = 2.58, $P < 0.001$) were significantly elevated, and among monoenoic FA, only 20:1 differed from normal (NR = 7.52, $P < 0.001$).

Profile of Plasma NEFA. The plasma NEFA profile (Fig. 4) showed 18:2ω6 (NR = 0.81, $P < 0.001$), 20:3ω6 (NR = 0.38, $P < 0.05$), and 20:4ω6 (NR = 0.53, $P < 0.01$) as subnormal. The saturated FA 15:0, 20:0 (NR = 9.45, $P < 0.001$), and 22:0 (NR = 3.3, $P < 0.01$) were increased in NEFA of MS patients. The level of 20:1ω9 was elevated (NR > 10, $P < 0.001$). The Δ9 products were found to be increased, largely due to the 20:1ω9. *In toto,* profiles on NEFA indicated that the circulating FA pool has a profile grossly different from normal, which may exert an effect upon the pattern of autacoids (physiologically active endogenous substances) produced.

Correlations with Age, Duration of MS, and Kurtzke Score. Linear regressions were constructed between age, duration of MS, or Kurtzke score and each of the FA in plasma PL of the MS group. Few significant correlations were found. For age versus 18:3ω6, the correlation coefficient $r = -0.612$ ($P < 0.05$), and for age versus 22:4ω6, $r = -0.738$ ($P < 0.005$). For age versus saturated FA, $r = -0.599$ ($P < 0.05$). For duration of MS versus total saturated FA, $r = -0.702$ ($P < 0.01$), and for 16:0 (palmitic acid), $r = -0.632$ ($P < 0.05$). For Kurtzke score versus total saturated FA, $r = -0.599$ ($P < 0.05$). For 16:0, $r = -0.544$ ($P < 0.05$). For 22:0, $r = -0.534$ ($P < 0.05$). For 24:0, $r = -0.584$ ($P < 0.05$). Kurtzke score varied directly with DBI ($r = +0.542$, $P < 0.05$), which was low in the MS group as a whole. These three variables appear not to correlate strongly with those PUFA that distinguish the MS profile from the normal profile. Duration and Kurtzke score varied inversely with saturated FA, whereas saturated FA were high in MS.

PUFA Deficiency. Relative deficiencies of all groups of PUFA in the structural lipids are indicated by the PUFA profile of PL in MS patients, except for 18:2ω6 and its Δ6 desaturation product, 18:3ω6. Chain-elongation products and

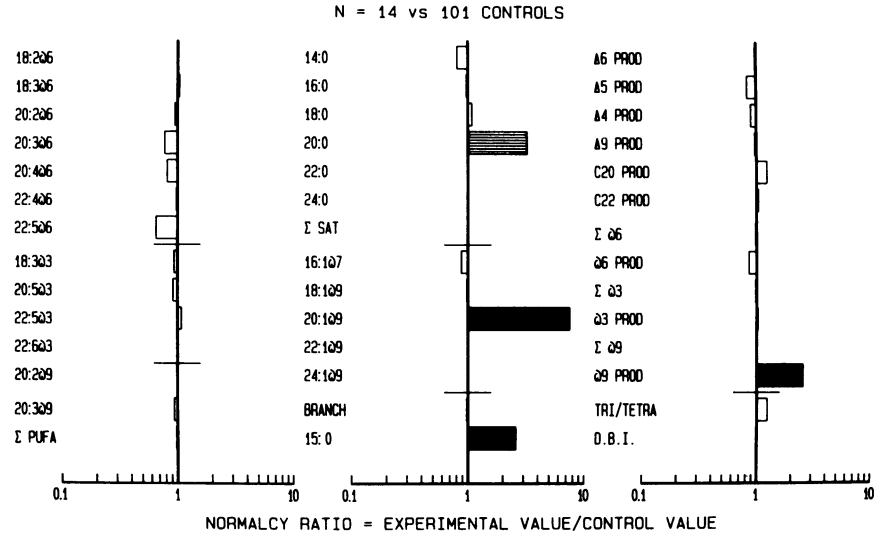

FIG. 3. FA profile of plasma TG of MS patients.

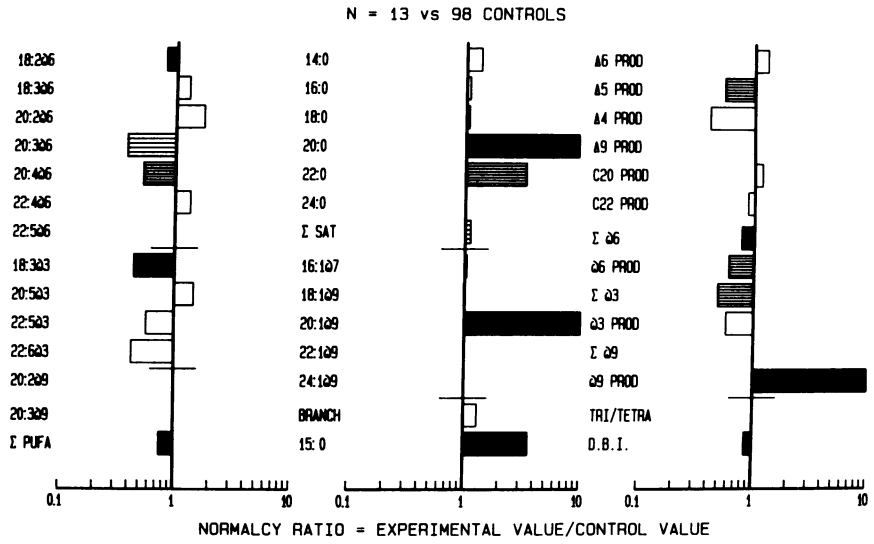

FIG. 4. FA profile of plasma NEFA in MS patients.

subsequent desaturation products in the ω6 family were consistently and significantly deficient in PL. Total ω3 PUFA were significantly subnormal. Despite deficiencies of ω6 and ω3 PUFA, 20:2ω9 and 20:3ω9 were not increased, indicating that elongation and subsequent desaturations in the ω6, ω3, and ω9 families of PUFA were impaired. The metabolic defect appears at the first elongation step, from C_{18} to C_{20}, but elongation to C_{22} is also impaired because 22:4ω6 is more severely suppressed than 20:4ω6, and because 22:5ω3 is more suppressed than 20:5ω3. Metabolic defects in MS caused a decrease of total PUFA from 47.7% ± 0.26% to 42.0% ± 0.88% (−5.7%), compensated by saturated FA increasing from 36.3% ± 0.22% to 42.2% ± 0.75% in MS (+5.9%). The DBI of serum PL was reduced to 85% of normal ($P < 0.001$) as a consequence of PUFA deficiencies in PL. Normal DBI, 1.55 ± 0.01, was reduced in MS to 1.32 ± 0.02 double bonds per FA (NR = 0.85). This is a very significant deficiency of unsaturation, reducing the "fluidity" of membrane lipids. In an early study of EFA deficiency and PUFA of liver lipids of rats, DBI dropped rapidly during the first 100 days of deficiency to about 0.9, and the lowest value observed in a rat surviving beyond 400 days was 0.83 (20), probably the minimum level of unsaturation compatible with life. Substitutions by endogenous, non-PUFA must occur if PUFA are decreased.

Consequences for Membrane Fluidity. The profile of replacement FA in PL (Fig. 1) shows distortion of the normal non-PUFA profile toward shorter-chain, more "fluid" FA having lower melting points. Among saturated FA, 14:0 increased from 0.02% ± 0.01% to 0.42% ± 0.04%, 16:0 increased from 21.1% ± 0.2% to 26.8% ± 0.5%, and 18:0 did not change perceptibly, whereas 20:0 decreased from 0.33% ± 0.02% to 0.25% ± 0.02%, 22:0 decreased from 1.11% ± 0.04% to 0.74% ± 0.04%, and 24:0 decreased from 0.88% ± 0.07% to 0.63% ± 0.04%. Disproportionation shortened the saturated FA chains, tending to lower melting point. When PUFA are adequate, long-chain saturated FA are accommodated in PL, but in PUFA deficiency short, more fluid, saturated FA are substituted. Monounsaturated FA revealed a similar disproportionation: 16:1ω7 rose from normal, 0.70% ± 0.02%, to 0.86% ± 0.06%, 18:1ω9 decreased from 8.87% ± 0.16% to 8.27% ± 0.22%, and 24:1ω9 decreased from 1.20% ± 0.04% to 0.78% ± 0.05%. This is a net decrease in total monounsaturated FA of 0.43% and a decrease in their average chain length. Branched-chain FA increased 0.27%, from 1.05% ± 0.05% to 1.32% ± 0.10%, in the plasma PL of MS patients. Melting points of branched FA are considerably lower than their normal-chain isomers, and branching should contribute strongly toward loosening the packing of acyl chains in membrane lipids (branched-chain FA serve a membrane function in bacteria similar to that of PUFA in animals). An increase of branched FA in MS could compensate partially for the decrease in fluidity caused by deficiency of PUFA. Slight increases in branched FA were observed in CE and NEFA, indicating an increase in the FA metabolic pool, perhaps the result of increased protein catabolism.

The odd-chain FA 15:0 was increased more than 5-fold in the plasma PL of MS patients, from 0.19% ± 0.01% in the normal group to 1.05% ± 0.04% in the MS group ($P < 0.001$), a net increase of 0.86%. Even more striking was the very significant increase of this acid in all four lipid classes, suggesting increased synthesis of odd-chain FA. In CE the increase was from 0.20% ± 0.01% to 0.36% ± 0.06%, in TG it was from 0.32% ± 0.01% to 0.82% ± 0.07%, and in NEFA it was from 0.25% ± 0.02% to 0.90% ± 0.10% (all $P < 0.001$). The increase in 15:0 may increase fluidity of acyl chains, because of alternation that occurs in melting points of saturated FA homologs. An odd-chain acid has a lower melting point than its next lower even-numbered homolog (21). Thus, 15:0 has a lower melting point than 14:0. Other properties of FA in the solid state, such as solubility, molecular heat, refraction, and rotation, show this alternation phenomenon. The presence of odd-chain FA in some PL molecules may thus be expected to increase the fluidity of membrane PL. Shortening chain length of saturated and monounsaturated FA, and increased proportions of branched and odd-chain FA in PL, partially correct the low fluidity caused by PUFA deficiency, extending the homeostatic range of composition of membranes compatible with function and life.

Mean chain length of FA. Substitutions for long-chain PUFA discussed above have a common feature: the replacement FA is of shorter chain length than the PUFA lost. Therefore, the mean chain length of the FA of each of the four lipid classes was calculated for MS and control groups to quantify the effect. For plasma PL, the value for MS patients was 17.358 ± 0.026 (SD), significantly lower than the normal value, 17.495 ± 0.037 ($P < 0.0001$). For NEFA, the values were 17.295 ± 0.082 and 17.337 ± 0.036, respectively ($P < 0.01$). For CE and TG, there was no significant difference between MS and the control. Although differences were small, they were significant because replicate values were very uniform.

Mean melting point of FA as a measure of membrane fluidity. Both loss of PUFA from PL and replacement by

shorter-chain FA have effects upon the melting point of the lipid mixture. The packing properties of PL in membranes (fluidity) are related to the associations between fatty acyl chains. Although mixed melting points for two FA generally fall below values expected by interpolation between melting points of pure FA, this error is small compared with the range of melting points of naturally occurring FA (22). As a first approximation, the mean melting point of FA in plasma PL was estimated for each population. For each individual, the mole fraction was calculated for each FA of the mixture and then multiplied by its melting point, all increments were summed, and mean and SD for the group were calculated.

The mean melting point for FA of PL from MS patients was found to be 6.5°C higher than normal (21.3 ± 2.3°C compared to 14.8 ± 2.5°C, $P < 0.001$) despite the shortened chain length in MS. For NEFA, MS patients had a value of 28.7 ± 4.6°C, 4.6°C higher than 24.1 ± 2.9°C for normals ($P < 0.001$). The values for CE and for TG were normal. Compensatory shortening of chain length was only partial, insufficient to balance the gross increase in mean melting point and the diminished fluidity caused by PUFA deficiency.

MS is accompanied by low PUFA in structural PL, indicated by a low DBI, one measure of fluidity. This shift has been shown here to be accompanied by significantly shortened mean chain length and elevated melting point. The mean melting point, which takes into consideration effects of unsaturation, chain length, and branching upon membrane fluidity, may be a helpful, more broadly based index of fluidity in future studies of disease, physiological function, or nutritional status.

Concluding Remarks. MS is an inflammatory disease with characteristic destruction of the myelin of the central nervous system, the mechanism of which is not understood. Several hypotheses, including persistent viral infection of oligodendrocytes, autoimmune destruction of myelin, and "bystander" demyelination, have been proposed. A recent hypothesis suggests that "immune cells recognize 'foreign' antigens on the surface of oligodendrocytes in the context of a major histocompatibility of gene products, and the final result of this process may be a 'dying back' gliopathy which is first noted in the most distal extension of the oligodendrocytes, that is, the myelin sheath" (23). In this hypothesis, a specific "foreign" antigen is not necessary, and different antigens in different patients can initiate the process. The proposed demyelination can occur under the influence of exogenous antigens, including viruses. Several viruses, including measles or canine distemper virus, have been suspected to have a role in the mechanism of MS. Most recently, human T-lymphotropic virus type I was detected as proviral DNA in the brains of MS patients (24).

A case of gay bowel syndrome (AIDS) was found to have a profile with some features of nutritional EFA deficiency, and both FA profile and clinical condition responded to supplements of 18:3ω3 [R.T.H., S.B.J., and J. Turner (1982), unpublished data]. A syndrome involving cytomegalovirus showed increased 18:2ω6, but subsequent PUFA products were subnormal (12). Reye syndrome, a sequel to a viral disease, involves loss of PUFA from PL and accumulations of PUFA in NEFA (11). Very similar effects were found in hepatitis (R.T.H., S. D. Phinney, and H. Sharp, unpublished data). Involvement of a virus that may release PUFA from PL (25) could be a cause of the PUFA deficiencies observed, and abnormalities of PUFA metabolism may be expected in other viral diseases.

PUFA deficiency in MS may be correctable by selective supplementation with PUFA, for tissue PUFA pattern responds to oral supplementation with single PUFA, whether by dietary precursors (26) or by their desaturation or elongation products (27). Whatever the cause of MS, accompanying PUFA deficiencies may respond beneficially to nutritional supplementation of ω6 and ω3 PUFA.

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