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Omega-3 fatty acids and multiple sclerosis: relationship to depression

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Abstract Depression is a common problem among patients with multiple sclerosis (MS). Previous research has shown differences between MS patients and controls in the levels of certain fatty acids, and differences in many of these same fatty acids have also been reported in psychiatric patients with major depression. The current study sought to determine whether fatty acid levels in MS patients might be associated with depression. Fatty acids were measured in red blood cells (RBCs) for 38 patients with relapsing-remitting MS and 33 healthy controls who also completed 3-day dietary records and depression questionnaires. Levels of certain omega-3 and omega-6 fatty acids were lower and levels of certain monounsaturated and saturated fatty acids were higher in the MS patients. These differences were generally of medium effect size and occurred despite the fact that no differences were found between the two groups in dietary intake of any fatty acids. However, neither RBC nor dietary fatty acid levels were related to depression in the MS sample.

Keywords Fatty acids · Omega-3 · Omega-6 · Multiple sclerosis · Depression

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Introduction

Interest in the relationship between multiple sclerosis (MS) and lipids originated largely from Swank et al. (1952) observation that Norwegians living in inland farming communities where diets contained higher levels of animal fats and dairy products had a greater incidence of MS than those living in coastal communities where higher amounts of fish were consumed. Subsequent epidemiological studies of other populations often indicated that the prevalence of MS is positively related to the consumption of animal fat and dairy products and negatively related to the consumption of fish and vegetables, although population-based case-controlled studies have been much less consistent in their support of these dietary relationships (Schwartz and Leweling 2005).

Direct comparisons between MS patients and healthy controls in terms of fatty acid levels in various tissues have also been reported. Table 1 presents the shorthand and trivial (or systemic) names of the specific fatty acids featured in this research. Amidst many instances of no differences between MS patients and controls, there is a general trend involving lower levels of both omega-3 (Cunnane et al. 1989; Holman et al. 1989; Nightingale et al. 1990; Nordvik et al. 2000) and omega-6 (Cherayil 1984; Cunnane et al. 1989; Fisher et al. 1987; Holman et al. 1989; Navarro and Segura 1988, 1989; Neu 1983; Nightingale et al. 1990) polyunsaturated fatty acids in MS patients, along with what are often characterized as "compensatory" increases in levels of monounsaturated (Cherayil 1984; Navarro and Segura 1989; Neu 1983) and saturated fatty acids (Cherayil 1984; Cunnane et al. 1989; Holman et al. 1989; Navarro and Segura 1988, 1989; Neu 1983; Nightingale et al. 1990). The studies cited utilized various methodologies to measure fatty acid levels in

Table 1 Names of fatty acids examined in this study

Shorthand name	Trivial or (systemic) name	Abbreviation
Polyunsaturated fatty acids (PUFA)		
Omega-3		
18:3n3	Alpha-linolenic	ALA
20:5n3	(Eicosapentaenoic)	EPA
22:5n3	(Docosapentaenoic)	DPA
22:6n3	(Docosahexaenoic)	DHA
Omega-6		
18:2n6	Linoleic	LA
18:3n6	Gamma-linolenic	GLA
20:2n6	(Eicosadienoic)	–
20:3n6	Dihomo-gamma-linolenic	dGLA
20:4n6	Arachidonic	AA
22:4n6	(Docosatetraenoic)	–
22:5n6	(Docosapentaenoic)	–
Monounsaturated fatty acids (MUFA)		
16:1n7	Palmitoleic	–
18:1n9	Oleic	–
20:1n11	Gadoleic	–
22:1n9	Erucic	–
24:1n9	Nervonic	–
Saturated fatty acids (SFA)		
14:0	Myristic	–
16:0	Palmitic	–
18:0	Stearic	–
20:0	Arachidic	–
22:0	Behenic	–
24:0	Lignoceric	–

plasma, erythrocytes, lymphocytes, or adipose tissue cells. However, inconsistencies are apparent even among studies using similar methods. This is illustrated by two studies using methods similar to those of the current investigation and evaluating fatty acids in red blood cells (RBCs). Nightingale et al. (1990) reported decreased levels of EPA (20:5n3) and increased levels of dGLA (20:3n6) and stearic acid (18:0) in MS patients. Koch et al. (2006) reported no differences. Thus no definitive conclusions can be drawn regarding the differences in the fatty acid profiles found among MS patients.

Epidemiological studies in the field of psychiatry have also indicated relationships between the prevalence of depression and dietary intake of saturated and polyunsaturated fats (Hibbeln and Salem 1995; Klerman and Weissman 1989; Silvers and Scott 2002). Furthermore, reduced levels of omega-3 fatty acids, lower ratios of omega-3 to omega-6, and higher levels of saturated fatty acids have consistently been reported for RBCs and plasma obtained from psychiatric patients with major depression compared to controls (Maes et al. 1996, 1999; Peet et al. 1998). Parker et al. (2006) have reviewed this literature. Also, a recent meta-analysis of controlled trials involving supplementation with omega-3 fatty acids has demonstrated significant improvement in cases of both unipolar and bipolar depression (Freeman et al. 2006).

Depression is a common problem accompanying MS, with a lifetime prevalence ranging from 27% to 54% and a point prevalence estimated at 15%. These figures often exceed those of the general population, and sometimes exceed those of other medically ill populations (Joffe et al. 1987; Patten and Metz 1997), though the latter finding is subject to question (Dalton and Heinrichs 2005). The differences in omega-3 fatty acids seen in conjunction with both MS and depression have led some to suggest that these fatty acids might account for the high prevalence of depression in MS patients (Hibbeln 1999; Horrobin and Bennett 1999). However, no empirical test of this hypothesis has been conducted.

The aim of the present study was to compare RBC fatty acid levels in MS patients and healthy controls and then to examine the relationship of these fatty acid levels to depression in the patient sample. Though the majority of previous studies have examined fatty acid levels in plasma or serum, the use of RBCs is preferable in that it yields a more stable assessment. Pronounced differences in dietary intake are required to alter fatty acid levels in RBC membranes (Smuts et al. 2003). Nevertheless, when assessing tissue differences in fatty acid levels, it is also important to evaluate subjects' dietary intake. In the only study to do so (Nightingale et al. 1990), no disparities were found between the dietary intake of fatty acids for MS patients and healthy controls, suggesting that the differences observed in RBC levels may be based more on metabolism than diet. This issue warranted further examination, and so dietary intake was evaluated along with RBC values in the present study.

Method

Subjects

Thirty-eight patients (32 females, 6 males) with clinically definite (McDonald et al. 2001) MS and 33 healthy controls (27 females, 6 males) were studied. A power analysis based on previous studies examining either fatty acid levels in MS patients and controls (Cunnane et al. 1989; Holman et al. 1989; Nightingale et al. 1990; Nordvik et al. 2000) or the relationship of RBC fatty acid levels to depression (Adams et al. 1996; Edwards et al. 1998; Garland et al. 2007) indicated that this sample size was adequate to

evaluate both outcomes. The patients were diagnosed with relapsing-remitting MS, had been under the care of their current neurologist (SGL) for at least 1 year, were receiving standard of care treatment, and were judged to be competent to complete the required procedures. Duration of MS ranged from 2 to 15 years (M = 7.6). Disability scores ranged from 1 to 8.5 (Mdn = 3.5) on the Expanded Disability Status Scale (EDSS: Kurtzke 1983) and from 0 to 35 (M = 11.4) on the Guys Neurological Disability Scale (GNDS: Sharrack and Hughes 1999). Standard of care treatment consisted of various combinations of medication, the most common ones being Copaxone (12 patients), Baclofen (7), Interferon Beta-1b (6), Neurontin (6), and Provigil (5). Medication regimens remained unchanged throughout the course of the study.

Controls were recruited through staff at the University of Kansas and its medical center. Prospective subjects were excluded from both groups if they reported diagnosis of a neurological disorder (other than MS for the patient group), active substance abuse, significant dietary practices, restrictions or supplementations (including omega-3 supplementation), or medications that may affect fatty acid levels. Sample characteristics for the patient and control groups are displayed Table 2.

Measures

The Chicago Multiscale Depression Inventory (CMDI: Chang et al. 2003) was developed to assess the severity of depression, especially in patients with MS. This scale was chosen because subscale scores are useful in distinguishing between MS symptoms (e.g., fatigue) and those attributable to depression. The questionnaire is composed of 50 items, each rated on a scale from 1 ("not at all") to 5 ("extremely"), according to the patient's experience during the past week. A total score is computed by summing the responses to 42 of the items, and three subscale scores are computed based upon responses to the 9 items pertaining to mood, 6 items pertaining to evaluative, and 9 items related to vegetative features of depression (Nyenhuis et al. 1998). Scores on this questionnaire were used as a continuous variable to measure severity of depression and also to distinguish between depressed and non-depressed subgroups of patients based upon cut off scores reported by Nyenhuis et al. 1998.

The 3-Day Food Record consisted of a booklet in which subjects recorded everything they ate or drank over a 3-day period that included two weekdays and one weekend day. Nutrient calculations were performed using the Nutrition Data System for Research software version 4.05 (Nutrition Coordinating Center 2000). Dietary levels of the individual fatty acids are reported in mg/100 g of absolute energy intake per day. Three day food records have been used in previous studies of dietary intake in various clinical and non-clinical populations (Bas et al. 2005; Brownbill et al. 2005) and have been shown to be suitable for estimating individuals' dietary intake through correlations with other self-report measures (Goulet et al. 2004), observational measures (Crawford et al. 1994), and biological markers, such as basal metabolic rate (Lührmann 1999). In the current study, subjects who reported significant recent changes in their diet were excluded from the study to decrease the likelihood that dietary intake at the time of report differed substantially from the months prior to the blood draw when the RBC membranes were being formed.

Procedures

This study was approved by the Human Subject Committee of the University of Kansas Medical Center. MS patients

Table 2 Baseline characteristics of MS and control groups

	MS Patients		Controls		Significance (*P*)*	Cohen's *d*
	Mean	(SD)	Mean	(SD)		
Age	44.7	(9.4)	43.9	(10.4)	.723	
Education (years)	14.8	(2.7)	17.21	(3.7)	.003	
CMDI Total	80.0	(30.0)	61.0	(12.6)	.001	.75
T-Score	55.2	(16.1)	45.0	(6.8)		
Mood	16.3	(8.5)	13.0	(4.0)	.045	.48
T-Score	53.0	(15.4)	47.0	(7.2)		
Evaluative	8.7	(4.9)	7.1	(2.8)	.106	.39
T-Score	54.6	(19.8)	48.2	(11.4)		
Vegetative	23.7	(9.6)	17.1	(5.9)	.001	.76
T-Score	57.6	(18.0)	45.2	(11.0)		

* Independent sample *t*-test with df = 69 and based upon raw data

Notes: CMDI—Chicago Multiscale Depression Inventory. *T*-Scores based upon normative data provided by Nyenhuis et al. (1998)

were recruited during their regularly scheduled outpatient appointments at the MS Clinic. After providing signed consent, subjects completed a demographic questionnaire and the CMDI. The subjects were given the 3-Day Food Record to complete and mail to the investigator, and the written instructions were carefully reviewed to ensure full understanding of how to complete the record. For MS patients, the investigator also completed the GNDS and the neurologist completed the EDSS during the course of the patient's clinic appointment.

All subjects also provided a 6 ml blood sample, collected in an EDTA-fortified blood collection tube using a 21-gauge needle. The blood was immediately mixed with the anticoagulant and placed on ice. The sample was centrifuged at 3,000 rpm for 10 min at room temperature, and the RBCs were immediately transferred to small glass vials, layered with nitrogen, and stored at –70°C.

Red blood cell analysis

Batches of RBC samples were later analyzed for phospholipid fatty acids. Lipids were extracted from a 250 µl suspension of RBCs with methanol/chloroform (1/2) (Dodge and Phillips 1967) and washed with 2 ml KCl (.15 M) to remove lipid contaminants (Folch et al. 1957). The lipid extracts were spotted on a thin layer chromatoplate (Uniplate Silica gel G, Fisher) and developed at room temperature in a mixture of hexane: diethyl ether: acetic acid (80:20:1 v/v/v). Following the migration of other lipids through the gel, the band at the origin containing the phospholipids was scraped from the plate. The phospholipid fatty acid methyl esters were synthesized by transmethylation with boron triflouride according to Morrison and Smith (1964), then extracted with pentane, followed by nitrogen to vaporize the pentane, then solubilized in 10 µl dichloromethane (chloroform: methanol; 2:1 v/v), and analyzed with capillary gas liquid chromatography according to Carlson et al. (1991). Identification of individual fatty acids was accomplished by comparing relative retention times with standard mixtures of fatty acids (Supelco 37 Component FAME mix, Supelco Park, Bellefonte, PA). The RBC level of individual fatty acids are reported as area percent of total identified fatty acids.

Statistical analysis

Independent sample *t*-tests were used to compare the MS patients versus controls or to compare subgroups of depressed versus non-depressed MS patients. Cohen's *d* was used to indicate the effect sizes for these comparisons. Pearson correlations and multiple regression analyses were used to examine the relationships between fatty acid levels and depression scores across the full sample of MS patients. Omega-3 fatty acids, such as EPA and DHA, were of primary interest based upon trends in both the MS and depression literature. However, because of inconsistencies in the MS literature, all fatty acids listed in Table 1 were included in analysis. Because numerous specific fatty acids were measured, the possibility of Type I error was substantially compounded in this study, as in virtually all the previous studies reviewed in the introduction. To maintain comparability with this previous work, uncorrected significance values are reported in relevant tables in the results. However, using Šidàk's (1967) procedure, the alpha level for the five general categories of fatty acids was adjusted to .01 and the alpha level for the 22 specific fatty acids was adjusted to .002. Significant differences based on these adjusted values are also indicated in these tables.

Results

Baseline characteristics of the MS patients and controls are presented in Table 2.

Patients and controls did not differ in age or gender, but level of education was significantly lower for the MS patients than for the controls ($t[69] = 3.10$, $P = .003$). Due to this difference, all statistical comparisons between patients and controls were conducted with and without education entered as a covariate. The results were essentially the same, and therefore the comparisons reported here are based on unadjusted data.

Depression scores

As shown in Table 2, the total score on the CMDI was significantly higher for the MS patients than for the controls ($t[69] = 3.39$, $P = .001$, $d = .75$). Separate *t*-tests applied to each subscale score indicated that MS patients had significantly higher scores on the vegetative ($t[69] = 3.44$, $P = .001$, $d = .76$) and mood subscales ($t[69] = 2.04$, $P = .045$, $d = .48$). Although analyses were conducted using raw scores, *T* scores based upon normative data provided by Nyenhuis et al. (1998) are also included in Table 2.

Red blood cell fatty acid levels

Table 3 presents the RBC fatty acid levels for the patients and controls. Compared to controls, the MS patients had significantly lower overall levels of polyunsaturated ($t[69] = 2.81$, $P = .007$, $d = .62$), omega-3 ($t[69] = 2.93$, $P = .005$, $d = .67$) and omega-6 fatty acids ($t[69] = 2.20$, $P = .031$, $d = .50$), along with significantly higher overall levels of monounsaturated fatty acids ($t[69] = 3.30$, $P = .002$, $d = .74$). The overall level of saturated fatty acids was also somewhat higher for MS patients, but this differ-

Table 3 Comparisons between MS and control groups: red blood cell fatty acid values

Fatty acid	MS patients		Controls		Significance (*P*)*	Cohen's *d*	MS vs. Control
	Mean	(SD)	Mean	(SD)			
PUFA	38.43	(4.54)	40.98	(3.07)	**.007****	.62	↓
Total omega-3	5.47	(1.52)	6.45	(1.24)	**.005****	.67	↓
18:3n3	.08	(.05)	.09	(.05)	.539	.20	↔
20:5n3	.33	(.14)	.41	(.13)	**.019**	.57	↓
22:5n3	1.98	(.48)	2.13	(.36)	.142	.35	↔
22:6n3	3.05	(1.07)	3.76	(1.07)	**.007**	.63	↓
Total omega-6	32.96	(3.48)	34.54	(2.56)	**.031**	.50	↓
18:2n6	11.14	(1.48)	11.40	(1.41)	.452	.18	↔
18:3n6	.02	(.03)	.04	(.12)	.204	.25	↔
20:2n6	.21	(.11)	.24	(.08)	.167	.30	↔
20:3n6	.72	(.31)	1.05	(.55)	**.003**	.70	↓
20:4n6	16.28	(2.46)	17.15	(1.58)	.077	.41	↔
22:4n6	4.06	(.74)	4.14	(.71)	.668	.11	↔
22:5n6	.74	(.22)	.76	(.16)	.736	.11	↔
MUFA	17.93	(1.76)	16.68	(1.35)	**.002****	.74	↑
16:1n7	.23	(.14)	.21	(.13)	.507	.15	↔
18:1n9	13.07	(1.37)	11.96	(1.06)	**<.001*****	.82	↑
20:1n11	.17	(.12)	.23	(.17)	.087	.40	↔
22:1n9	1.64	(.77)	1.20	(.77)	**.028**	.55	↑
24:1n9	1.36	(.62)	1.62	(.58)	.080	.43	↔
SFA	39.57	(4.40)	38.49	(3.10)	.242	.28	↔
14:0	.29	(.08)	.23	(.06)	**.003**	.75	↑
16:0	20.48	(4.10)	20.01	(1.86)	.616	.15	↔
18:0	17.16	(1.92)	16.19	(1.65)	**.027**	.52	↑
20:0	.17	(.09)	.20	(.06)	.086	.38	↔
22:0	.10	(.23)	.23	(.36)	.071	.43	↔
24:0	1.49	(.53)	1.68	(.51)	.117	.36	↔

* Independent sample *t*-test with df = 69; uncorrected *P*-value. Significant *P*-values appear in bold print

** Significant when the alpha level is adjusted for multiple comparisons across the five main groups of fatty acids: PUFA, MUFA, SFA, omega-3, and omega-6

*** Significant when the alpha level is adjusted for multiple comparisons for the number of specific fatty acids

Notes: PUFA—Polyunsaturated fatty acid; MUFA—Monounsaturated fatty acid; SFA—Saturated fatty acid

ence was not significant; nor was the overall difference in monounsaturated fatty acids after adjusting for multiple comparisons. With regard to specific fatty acids, the MS patients had significantly lower values on two omega-3 fatty acids, EPA (20:5n3) and DHA (22:6n3), and on one omega-6 fatty acid, d-GLA (20:3n6). They also had significantly higher values on two monounsaturated, oleic (18:1n9) and erucic (22:1n9), and two saturated fatty acids, myristic (14:0) and stearic (18:0). Only the difference in oleic acid remained significant after adjusting for multiple comparisons.

Dietary fatty acid levels

Twenty-five of the 38 MS patients (66%) and 27 of the 33 controls (82%) returned the 3-Day Food Record. Comparisons between patients and controls performed on overall dietary levels of the five main groups of fatty acids (i.e., polyunsaturated, omega-3, omega-6, monounsaturated, and saturated fatty acids) revealed no significant differences (all *P*'s > .24, all *d*'s < .28). Likewise, there were no significant differences between patients and control on any of the specific fatty acids detected through the Nutrition Data System software (all *P*'s > .12, all *d*'s < .43).

Fatty acids and depression in MS patients

Correlations were computed between the total CMDI score and each of the RBC fatty acid levels. None of the correlations between depression and the main groups of fatty acids was significant (all *P*'s > .38). With respect to the

specific fatty acids, only the correlation between CMDI total score and oleic acid (r[36] = –.34, P = .036) was significant. To further investigate the relationship between symptoms of depression and RBC fatty acids, correlations were examined using the mood and evaluative subscales of the CMDI. Although there was a trend for mood score to correlate with palmitoleic (r = .313, P = .056) and oleic (r = –.298, P = .07) acid, none of the correlations was significant (all other P's > .12). None of the correlations between individual or main groups of fatty acids and the evaluative subscale was significant (all P's > .12). However, the results pertaining to this third subscale were questionable because most of the MS patients had very low scores on this scale.

Previously reported cut off scores for depression on the CMDI (Nyenhuis et al. 1998) were used to identify "depressed" MS patients in the current study. Fatty acid levels for the 10 patients meeting this criterion were compared with those of the remaining 28 patients (Table 4). The depressed subgroup was found to have a significantly lower level of oleic acid (t[36] = 2.19, P = .035, d = .77). No other comparisons between "depressed" and "non-depressed" MS groups involving RBC levels were significant (all P's > .15).

Discussion

By examining the last column of Table 3, one can see that the results of the present study are consistent with many of the findings of past research concerning a general trend toward lower levels of polyunsaturated and higher levels of monounsaturated and saturated fatty acids in MS patients. The present sample of patients had significantly lower

Table 4 Comparisons between depressed and non-depressed MS patients: red blood cell fatty acid values

Fatty acid	Depressed (N = 10)		Non-depressed (N = 28)		Significance (P)*	Cohen's d
	Mean	(SD)	Mean	(SD)		
PUFA	39.34	(3.64)	38.11	(4.83)	.468	.27
Total omega-3	5.47	(1.05)	5.48	(1.67)	.993	.01
18:3n3	.08	(.06)	.08	(.05)	.837	.08
20:5n3	.33	(.09)	.34	(.15)	.880	.07
22:5n3	2.00	(.43)	1.97	(.50)	.855	.06
22:6n3	3.04	(.77)	3.05	(1.17)	.994	.01
Total omega-6	33.87	(3.20)	32.63	(3.57)	.341	.36
18:2n6	11.58	(.77)	10.98	(1.65)	.276	.41
18:3n6	.01	(.02)	.02	(.03)	.177	.33
20:2n6	.22	(.15)	.20	(.10)	.654	.18
20:3n6	.65	(.21)	.74	(.34)	.424	.29
20:4n6	16.73	(2.86)	16.12	(2.34)	.509	.25
22:4n6	4.15	(.78)	4.03	(.73)	.671	.16
22:5n6	.77	(.24)	.73	(.21)	.615	.18
MUFA	17.24	(1.34)	18.17	(1.85)	.156	.53
16:1n7	.27	(.17)	.22	(.12)	.279	.36
18:1n9	12.29	(0.82)	13.34	(1.43)	**.035**	.77
20:1n11	.13	(.09)	.18	(.13)	.255	.42
22:1n9	1.82	(.60)	1.57	(.83)	.383	.33
24:1n9	1.29	(.55)	1.39	(.65)	.697	.16
SFA	39.54	(3.34)	39.58	(4.78)	.980	.01
14:0	.28	(.06)	.29	(.09)	.634	.13
16:0	21.35	(2.07)	20.18	(4.60)	.445	.29
18:0	16.44	(1.50)	17.42	(2.01)	.170	.51
20:0	.15	(.08)	.18	(.09)	.292	.33
22:0	.03	(.05)	.12	(.27)	.287	.39
24:0	1.31	(.57)	1.55	(.52)	.230	.45

* Independent sample t-test with df = 36; uncorrected P-value. Significant P-values appear in bold print. None of these P-values is significant when the alpha level is adjusted for multiple comparisons

Notes: PUFA—Polyunsaturated fatty acid; MUFA—Monounsaturated fatty acid; SFA—Saturated fatty acid

overall levels of polyunsaturated, omega-3, and omega-6 fatty acids compared to controls. Their levels for two individual omega-3 fatty acids (EPA and DHA) and one omega-6 fatty acid (dGLA) were lower. On the other hand, the patients had significantly higher overall levels of monounsaturated fatty acids (including oleic and erucic acid) and higher levels of two specific saturated fatty acids (myristic and stearic acid). A strength of the current study is that it provides effect sizes for these differences, which are unavailable in previous research.

It should also be emphasized that the differences in the RBC levels of fatty acids occurred in conjunction with no differences between patients and controls in dietary intake of any of the fatty acids examined. One other study (Nightingale et al. 1990) has shown that differences in fatty acid levels between MS patients and controls cannot be attributed to differences in dietary intake. Together these studies indicate that the observed differences are more likely due to metabolic rather than dietary factors. Possible mechanisms might stem from the chronic inflammatory nature of MS itself. An astute reviewer pointed out that elevations in phospholipase A2 activity and increases in eicosanoid production might be conducive to depletion of certain omega-3 and omega-6 fatty acids from cell membrane pools. Studies showing differences in lipid peroxidation (Besler and Comoğlu 2003) and the production of antibodies that target specific fatty acids in MS patients (Boullerne et al. 1996) are also worth considering here.

However, dietary differences cannot be summarily dismissed on the basis of the present study. The assessment of dietary intake occurred at the time of the blood sample and not months earlier when the membranes of circulating RBCs were being formed. Subjects who reported significant recent changes in their diet were excluded from the study to insure that the 3-Day Food Record provided data representative of typical dietary intake over the prior months. Nevertheless disparities between subjects' intake during the three recorded days and that of the previous months could have occurred. Further studies are needed that involve larger samples of patients and controls, utilize more intensive dietary assessments such as interviews, and coordinate these assessments with the time when RBC membranes are being formed.

It is interesting to note that, although fatty acid intake levels reported by patients and controls in the current study are similar to those reported in the National Health and Nutrition Examination Survey for the U.S. (Ervin et al. 2004), the intake levels of EPA and DHA are considerably lower than those reported by individuals residing in either European (Astorg et al. 2004; Johansson et al. 1998) or Asian countries (Okita et al. 1995; Okuda et al. 2005). Although the present results suggest that differences in RBC fatty acid levels may not be due to differences in dietary intake, increased intake of omega-3 fatty acids could still play a protective role against autoimmune diseases. If MS patients do in fact differ in the metabolism of these fatty acids, decreased consumption in the American diet might act as a catalyst in the development of the disease. This account would be bolstered if the prevalence for MS were higher in the American population compared to these other regions of the world; however, it must be acknowledged that the epidemiological data do not indicate such differences (Noonan et al. 2002; Rosati 2001). Similarly, clinical studies conducted to examine the effects of omega-3 supplementation on disease progression in MS have been inconclusive because of varying results and trial quality (Farinotti et al. 2007).

One can also find many contradictions between the present results and the MS-related studies reviewed in the introduction, and indeed these studies alone are plagued by many inconsistencies. While the contradictions must be acknowledged, the more important focus of the present study concerns the question of whether the differences in patients' fatty acid levels that were observed bear any relationship to levels of depression. The answer appears to be negative. Among the 27 bivariate correlations between RBC fatty acid levels and depression scores, only one correlation (involving oleic acid) attained significance. Similarly, when the patients were divided into depressed and non-depressed subgroups, only a single comparison between the subgroups (again, involving oleic acid) was significant, and when an adjustment was made for multiple comparisons, this solitary finding failed to meet the revised criterion for significance. It should also be noted that oleic acid is a monounsaturated fatty acid, whereas most of the depression research has focused on polyunsaturated fatty acids. Finally, the level of oleic acid is *lower* for the depressed subgroup of MS patients, whereas it is significantly *higher* for MS patients overall relative to healthy controls.

There is some suggestion in the literature that the important factor relating to depression is the *ratio* of n3 to n6 fatty acids (Maes et al. 1996, 1999). We found no relationship between this ratio and depression in our present sample of MS patients. Both n3 and n6 fatty acid levels were lower in these patients, and thus the ratio of n3 to n6 was not altered.

It is also possible that fatty acids levels and depression are only linked when depression surpasses a certain level of severity. Depression scores were certainly high for the 10 patients singled out for comparison in the depressed subgroup. However, the greater part of these elevations was due to scores on the vegetative subscale, and the symptoms featured in this subscale are the most likely to be confounded with symptoms of MS. When we examined the relationship between fatty acids levels and just the mood subscale of CMDI, no significant relationships were found,

and when patients were selected on the basis of their mood subscale scores alone, virtually the same 10 patients emerged as the depressed subgroup. In sum, every attempt to address the issue of a critical level of depression severity in the present sample failed to produce compelling evidence of a linkage between depression and either RBC or dietary fatty acids.

References

Adams, P. B., Lawson, S., Sanigorski, A., & Sinclair, A. J. (1996). Arachidonic acid to eicosapentaenoic acid ratio in blood correlates positively with clinical symptoms of depression. *Lipids 31*(Suppl), 157–161.

Astorg, P., Arnault, N., Czernichow, S., Noisette, N., Galan, P., & Hercberg, S. (2004). Dietary intakes and food sources of n-6 and n-3 PUFA in French adult men and women. *Lipids 39,* 527–535.

Bas, M., Altan, T., Dincer, D., Aran, E., Gulper Kaya, H., & Yuksek, O. (2005). Determination of dietary habits as a risk factor of cardiovascular heart disease in Turkish adolescents. *European Journal of Nutrition 44*, 174–182.

Besler, H. T., & Comoğlu, S. (2003). Lipprotein oxidation, plasma total antioxidant capacity and homocysteine level in patients with multiple sclerosis. *Nutritional Neuroscience 6*, 189–196.

Boullerne, A., Petty, K. G., & Geffard, M. (1996). Circulating antibodies directed against conjugated fatty acids in sera of patients with multiple sclerosis. *Journal of Neuroimmunology 65*, 75–81.

Brownbill, R. A., Petrosian, M., & Ilich, J. Z. (2005). Association between dietary conjugated linoleic acid and bone mineral density in postmenopausal women. *Journal of the American College of Nutrition 24*, 177–181.

Carlson, S. E., Cooke, R. J., Rhodes, P. G., Peeples, J. M., Werkman, S. H., & Tolley, E. A. (1991). Long-term feeding of formulas high in linolenic acid and marine oil to very low birth weight infants: Phospholipid fatty acids. *Pediatric Research 30*, 404–412.

Chang, C. H., Nyenhuis, D. L., Cella, D., Luchetta, T., Dineen, K., & Reder, A. T. (2003). Psychometric evaluation of the Chicago Multiscale Depression Inventory in multiple sclerosis patients. *Multiple Sclerosis 9*, 160–170.

Cherayil, G. D. (1984). Sialic acid and fatty acid concentrations in lymphocytes, red blood cells and plasma from patients with multiple sclerosis. *Journal of the Neurological Sciences 63*, 1–10.

Crawford, P. B., Obarzanek, E., Morrison, J., & Sabry, Z. I. (1994). Comparative advantage of 3-day food records over 24-hour recall and 5-day food frequency validated by observation of 9- and 10-year-old girls. *Journal of the American Dietetic Association 94*, 626–630.

Cunnane, S. C, Ho, S., Dore-Duffy, P., Ells, K. R., & Horrobin, D. F. (1989). Essential fatty acid and lipid profiles in plasma and erythroytes in patients with multiple sclerosis. *The American Journal of Clinical Nutrition 50*, 801–806.

Dalton E. J., & Heinrichs, R. W. (2005). Depression in multiple sclerosis: A quantitative review of the evidence. *Neuropsychology 19*, 152–158.

Dodge, J. T., & Phillips, G. B. (1967). Composition of phospholipids and of phospholipids fatty acids and aldehydes in human red cells. *Journal of Lipid Research 8*, 667–675.

Edwards, R., Peet, M., Shay, J., & Horrobin, D. (1998). Omega-3 polyunsaturated fatty acid levels in the diet and in red blood cell membranes of depressed patients. *Journal of Affective Disorders 48*, 149–155.

Ervin, R. B., Wright, J. D., Wang, C. Y., & Kennedy-Stephenson, J. (2004). Dietary intake of fats and fatty acids for the United States population: 1999–2000. *Advance data 8*, 1–6.

Farinotti, M., Simi, S., Di Pietrantonj, C., McDowell, N., Brait, L., Lupo, D., et al. (2007). Dietary interventions for multiple sclerosis. *Cochrane Database of Systematic Reviews 24*, CD004192.

Fisher, M., Johnson, M. H., Natale, A. M., & Levine, P. H. (1987). Linoleic acid levels in white blood cells, platelets, and serum in multiple sclerosis patients. *Acta Neurologica Scandinavica 76*, 241–245.

Folch, J., Lees, M., & Sloane Stanley, G. H. (1957). A simple method for the isolation and purification of total lipids from animal tissues. *The Journal of Biological Chemistry 226*, 497–509.

Freeman, M. P., Hibbeln, J. R., Davis, J. M., Mischoulon, D., Peet, M., Keck, P. E., et al. (2006). Omega-3 fatty acids: Evidence basis for treatment and future research in psychiatry. *Journal of Clinical Psychiatry 67*, 1954–1967.

Garland, M. R, Hallahan, B., McNamara, M., Carney, P. A., Grimes, H., Hibbeln, J. R., et al. (2007). Lipids and essential fatty acids in patients presenting with self-harm. *The British Journal of Psychiatry 190*, 112–117.

Goulet, J., Nadeau, G., Lapointe, A., Lamarche, B., & Lemieux, S. (2004). Validity and reproducibility of an interview-administered food frequency questionnaire for healthy French–Canadian men and women. *Nutrition Journal 3*, 13.

Hibbeln, J. R. (1999). Long-chain polyunsaturated fatty acids in depression, related conditions. In M. Peet, I. Glen, & D. F. Horrobin (Eds.), *Phospholipid spectrum disorder in psychiatry* (pp. 195–210). Carnforth: Marius Press.

Hibbeln, J. R., & Salem, N. (1995). Dietary polyunsaturated fatty acids and depression: When cholesterol does not satisfy. *The American Journal of Clinical Nutrition 62*, 1–9.

Holman, R. T., Johnson, S. B., & Kokmen, E. (1989). Deficiencies of polyunsaturated fatty acids and replacement by nonessential fatty acids in plasma lipids in multiple sclerosis. *Proceedings of the National Academy of Sciences of the United States of America 86*, 4720–4724.

Horrobin, D. F., & Bennett, C. N. (1999). Depression and bipolar disorder: Relationships to impaired fatty acid and phospholipids metabolism and to diabetes, cardiovascular disease, immunological abnormalities, cancer, ageing, and osteoporosis. *Prostaglandins Leukotrienes, and Essential Fatty Acids 60*, 217–234.

Joffe, R. T., Lippert, G. P., Gray, T. A., Sawa, G., & Horvath, Z. (1987). Mood disorders and multiple sclerosis. *Archives of Neurology 44*, 376–378.

Johansson, L. R., Solvoll, K., Bjorneboe, G. E., & Drevon, C. A. (1998). Intake of very-long-chain n-3 fatty acids related to social status and lifestyle. *European Journal of Clinical Nutrition 52*, 716–721.

Klerman, G. L, & Weisman, N. W. (1989). Increasing rates of depression. *JAMA 261*, 229–235.

Koch, M., Ramsaransing, G. S. M., Fokkena, R., Heersema, D. J., & De Keyser, J. (2006). Erythrocyte membrane fatty acids in benign and progressive forms of multiple sclerosis. *Journal of the Neurological Sciences 244*, 123–126.

Kurtzke, J. F. (1983). Rating neurologic impairment in multiple sclerosis: An expanded disability status scale (EDSS). *Neurology 33*, 1444–1452.

Lührmann, P. M., Herbert, B. M., Gaster, C., & Neuhauser-Berthold, M. (1999). Validation of a self-administered dietary record for use in the elderly. *European Journal of Nutrition 38*, 235–240.

Maes, M., Smith, R., Christophe, A., Cosyns, P., Desnyder, R., & Meltzer, H. Y. (1996). Fatty acid composition in major

depression: Decreased ω3 fractions in cholesteryl esters and increased C20:4ω6/C20:5ω3 ration in cholesteryl esters and phospholipids. *Journal of Affective Disorders 38*, 35–46.

Maes, M., Christophe, A., Delanghe, J., Altamura, C., Neels, H., & Meltzer, H. Y. (1999). Lowered ω3 polyunsaturated fatty acids in serum phospholipids and cholestryl esters of depressed patients. *Psychiatry Research 85*, 275–291.

McDonald, W. I., Compston, A., Edan, G., Goodkin, D., Hartung, H. P., Lublin, F. D., et al. (2001). Recommended diagnostic criteria for multiple sclerosis: Guidelines from the International Panel on the Diagnosis of Multiple Scerosis. *Annals of Neurology 50*, 121–127.

Morrison, W. R., & Smith, L. M. (1964). Preparation of fatty acid methyl esters and dimethylacetals. *Journal of Lipid Research 5*, 606–608.

Navarro, X., & Segura, R. (1988). Plasma lipids and their fatty acid composition in multiple sclerosis. *Acta Neurologica Scandinavica 78*, 152–157.

Navarro, X., & Segura, R. (1989). Red blood cell fatty acids in multiple sclerosis. *Acta Neurologica Scandinavica 79*, 32–37.

Neu, I. S. (1983). Essential fatty acids in the serum and cerebrospinal fluid of multiple sclerosis patients. *Acta Neurologica Scandinavica 67*, 151–163.

Nightingale, S., Woo, E., Smith, A. D., French, J. M., Gale, M. M., Sinclair, H. M., et al. (1990). Red blood cell and adipose tissue fatty acids in mild inactive multiple sclerosis. *Acta Neurologica Scandinavica 82*, 43–50.

Noonan, C. W., Kathman, S. J., & White, M. C. (2002). Prevalence estimates for MS in the United States and evidence of an increasing trend for women. *Neurology 58*, 136–138.

Nordvik, I., Myhr, K. M., Nyland, H., & Bjerve, K. S. (2000). Effect of dietary advice and n-3 supplementation in newly diagnosed MS patients. *Acta Neurologica Scandinavica 102*, 143–149.

Nutrition Coordinating Center (2000). NDS-R food and nutrient database, version 31. Minneapolis: University of Minnesota, [CD-ROM].

Nyenhuis, D. L., Luchetta, T., Yamamoto, C., Terrien, A., Bernardin, L., Rao, S. M., et al. (1998). The development, standardization, and initial validation of the Chicago Multiscale Depression Inventory. *Journal of Personality Assessment 70*, 386–401.

Okita, M., Yoshida, S., Yamamoto, J., Suzuki, K., Kaneyuki, T., Kubota, M., et al. (1995). n-3 and n-6 fatty acid intake and serum phospholipid fatty acid composition in middle-aged women living in rural and urban areas in Okayama Prefecture. *Journal of Nutritional Science and Vitaminology 41*, 313–323.

Okuda, N., Ueshima, H., Okayama, A., Saitoh, S., Nakagawa, H., Rodriguez, B. L., et al. (2005). Relation of long chain n-3 polyunsaturated fatty acid intake to serum high density lipoprotein cholesterol among Japanese men in Japan and Japanese-American men in Hawaii: The INTERLIPID study. *Atherosclerosis 178*, 371–379.

Parker, G., Gibson, N. A., Brotchie, H., Heruc, G., Rees, A. M., Hadzi-Pavlovic, D. (2006). Omega-3 fatty acids and mood disorders. *American Journal of Psychiatry 163*, 969–978.

Patten, S. B., & Metz, L. M. (1997). Depression in multiple sclerosis. *Psychotherapy and Psychosomatics 66*, 286–292.

Peet, M., Murphy, B., Shay, J., & Horrobin, D. (1998). Depletion of omega-3 fatty acid levels in red blood cell membranes of depressive patients. *Biological Psychiatry 43*, 315–319.

Rosati, G. (2001). The prevalence of multiple sclerosis in the world: An update. *Neurological Sciences 22*, 117–139.

Schwartz, S., & Leweling, H. (2005). Multiple sclerosis and nutrition. *Multiple Sclerosis 11*, 24–32.

Sharrack, B., & Hughes, R. A. (1999). The Guys Neurological Disability Scale (GNDS): A new disability measure for multiple sclerosis. *Multiple Sclerosis 5*, 223–233.

Šidàk, Z. (1967). Rectangular confidence region for the means of multivariate normal distribution. *Journal of the American Statistical Association 62*, 626–633.

Silvers, K. M., & Scott K. M. (2002). Fish consumption and self-reported physical and mental health status. *Public Health Nutrition 5*, 427–431.

Smuts, C., Borod, E., Peeples, J., & Carlson, S. E. (2003). High-DHA eggs: Feasibility as a means to enhance circulating DHA in mother and infant. *Lipids 38*, 407–414.

Swank, R. L., Lerstad, O., Strom, P., & Barker, J. (1952). Multiple sclerosis in rural Norway; its geographic and occupational incidence in relation to nutrition. *The New England Journal of Medicine 246*, 721–728.