Erythrocyte membrane fatty acids in patients with multiple sclerosis

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Background
Reports on fatty acids levels in multiple sclerosis remain inconclusive.

Objective
To determine the erythrocyte membrane fatty acid levels in multiple sclerosis patients and correlate with Kurtzke Expanded Disability Status Scale.

Methods
Fatty acid composition of 31 multiple sclerosis and 30 control individuals were measured by gas chromatography.

Results
The membrane phosphatidylcholine C20:4\(^n–6\) concentration was lower in the multiple sclerosis patients when compared to that of the control group, \(P = 0.04\) and it correlated inversely with the EDSS and FSS.

Conclusion

Key words: multiple sclerosis; outcome measurement; relapsing/remitting

Introduction
In multiple sclerosis (MS) previous reports regarding the fatty acid (FA) composition in biological tissues have been inconclusive. Erythrocyte membrane FA composition reported by Koch, et al., [1] was not significantly different, whilst a significant decrease in C18:2\(^n–6\) and/or C20:4\(^n–6\) in the erythrocyte membranes of MS patients when compared to that of a healthy control group has been reported [2]. Cultural and ethnic differences, as well as dietary variability, especially in a diseased state have been implicated in the differences observed in these studies [3]. This study determined the erythrocyte membrane FA profile of MS patients and investigated a possible association between the erythrocyte membrane FA composition in MS patients and severity of neurological outcome as measured by the Kurtzke Expanded Disability Status Scale (EDSS) and its Functional System Scores (FSS) [4]. The exclusion criteria used in this study included the use of fatty acid supplements, interferon and cortisone or presence of a second disease for both MS patients and control subjects.

Materials and methods
Ethical approval for the study was obtained from the Health Sciences Research Ethics Committee.
(HSREC) of the Cape Peninsula University of Technology (CPUT). Thirty-one Caucasian females of which 28 were relapsing remitting MS, 1 with primary progressive MS and 2 with secondary progressive MS, and 30 age-, gender-, and race-matched control subjects were recruited through the MS Society, Western Cape Branch, South Africa. The patients recruited were diagnosed by a neurologist based on clinical, laboratory, and magnetic resonance imaging findings. Six of the patients were active disease cases, 11 had a relapse 5–12 months previously, and 14 had not relapsed for more than a year. The median (interquartile range) for years since diagnoses was 7 (11) years. Ten patients were using non-steroidal anti-inflammatory drugs (NSAIDs) and five patients were using immunosuppressive medication. Therefore, the MS patients were subdivided into two groups: Group A consisted of the total number of patients (N = 31) and Group B (N = 15) consisted of patients not on anti-inflammatory or immunosuppressive drugs. The categorization of cases was done to exclude the possible interference of medication on the eicosanoid pathway. The functional disability status (disease severity) of each patient was measured by a trained clinician using the Kurtzke EDSS and the median (interquartile range) for the EDSS was 5.5 (3.5).

Venous blood from both the patients and control subjects was collected into anti-coagulant ethylene-diaminetetraacetic acid (EDTA) tubes (Beckman Coulter, South Africa) and immediately separated using histopaque-1077 separation medium as per manufacturer’s instructions (Sigma-Aldrich, South Africa). Fatty acid composition of phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS), and sphingomyelin (SM) in the erythrocyte membranes were measured by gas chromatography (GC) as previously described [5,6] and results were quantified against an internal standard, C17:0. C-reactive protein (CRP) was determined on a Beckman nephelometer auto-analyzer using reagents from Beckman, South Africa.

Statistical analysis

STATISTICA (STATISTICA 7, StatSoft Inc 1984–2004) was used to perform all statistical analyses. Descriptive data are presented as median (interquartile range). For asymmetrical data, Mann–Whitney U-test was used to compare distributions between the cases and control subjects. Correlations were calculated using Spearman’s Rank correlation coefficient. Logistic regression was used to determine the adjusted odds ratio for FAs by adjusting for duration of symptoms. In view of the small sample size, P-values were corrected for multiple testing by Bonferroni. For comparison of FAs between MS and controls, the P-value < 0.006; for correlations between FAs and EDSS and FSS, the value of P < 0.003; for metabolic relationship between FAs, the value of P < 0.008 and for FAs and CRP, the value of P < 0.006 and they were considered as statistically significant.

Results

There were no significant differences in FA composition between the cases and the controls, but PC C20:4n−6 was lower in cases (quantified in μg/ml packed erythrocytes) 21.75 (6.4) and 24.38 (6.3), P = 0.04, respectively. Also the PE C22:4n−6 was lower in cases than in controls, respectively, 18.80 (4.9) and 21.06 (7.7), P = 0.06. PC C20:4n−6 demonstrated a significant inverse correlation with the EDSS (R = −0.73; P = 0.002) as well as with the Bowel and bladder FSS (R = −0.73; P = 0.002) (Figure 1). The effect of C20:4n−6 was studied after adjustments for the duration of symptoms and was shown to be significantly and independently associated with disease severity as measured by the EDSS (Beta = −0.72; R² = 0.48; P = 0.002). In MS, PC C20:3n−6 and C20:4n−6 demonstrated a more prominent disturbed relationship than that observed between C18:2n−6 and C20:3n−6 or C20:4n−6 (Table 1). No significant differences were observed in the CRP concentrations between the cases and the controls (MS Group B: 3.80 μg/ml/ml (5.2); controls: 3.70 μg/ml/ml (3.8); P = 0.86). However, non-significant inverse correlations were observed with PE C20:4n−6, C22:4n−6 and CRP (R = −0.45; P = 0.01; R = −0.36; P = 0.04, respectively).

Figure 1 Correlation between MS Group B erythrocyte membrane PC C20:4n−6 and the Kurtzke EDSS (N = 15): R = −0.727; P = 0.002.
Discussion and conclusion

In the present study, we provided evidence that membrane PC C20:4n-6 levels in MS patients who were not on FA supplements, interferon or cortisone treatment are lower, whilst C18:2n-6 levels are similar to that of control subjects. Furthermore, the decreased C20:4n-6 levels in MS correlated inversely with disease severity and inflammation as measured by the EDSS and CRP, respectively. Although a decrease in C20:4n-6 was only observed in the PC phospholipid fraction, PC is the most abundant phospholipid in animal cell membranes [7]. Erythrocyte membranes lack the desaturase enzymes and the membrane lipids are taken up from the plasma [5], but similar to previous reports [3,8], we observed a disturbed relationship between the FAs of the n-6 FA series in MS patients. Likewise, Harbige and Sharief [3] reported a disturbed relationship between C20:3n-6 and C20:4n-6 as well as between C18:2n-6 and C20:3n-6, whilst Homa, et al. showed the relationship between C18:2n-6 and C20:4n-6 to be disturbed. We, therefore, postulate that in MS, a decrease in C20:4n-6 in the erythrocyte membranes could be a result of insufficient incorporation due to depleted plasma stores. In MS plasma, decreased C18:2n-6 and subnormal subsequent n-6 FAs including C20:4n-6 levels have been reported [9,10]. C20:4n-6 and C22:6n-3 (DHA, docosahexaenoic acid) constitute 80–90% of the essential FAs in the brain [11]. Erythrocyte membrane FA composition has previously been used as an indicator of neural FA composition [12]. Neurons, like erythrocyte cannot synthesize C20:4n-6 ex novo, but depend on the supply from plasma and other brain cells [13].

The limitation of this study was that only female patients were used. The main strength of this study is that neither the cases nor the controls were on any FA supplements, and the patients were not on interferon or corticosteroid treatment; however, that resulted in a small samples size as MS patients not on any of these medications/supplementations are not easily available. In addition, MS cases on anti-inflammatory or immunosuppressive drugs were further excluded in MS patients subgroup B. In conclusion, our findings suggest that in MS patients, erythrocyte membrane FAs, particularly the decrease in C20:4n-6 in the erythrocyte membrane could be an indication of an increased demand of this FA elsewhere, as erythrocyte membranes lack the desaturase enzymes and the membrane lipids are taken up from the plasma, but a reflection of disease severity as demonstrated by the inverse correlation with the EDSS.

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References