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ORIGINAL PAPER

# Iron and the folate-vitamin B12-methylation pathway in multiple sclerosis

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**Abstract** Some subjects with multiple sclerosis (MS) present with low blood iron parameters. Anecdotal reports and a single patient study suggest that iron supplementation may be beneficial in these subjects. Myelin is regenerated continually, but prerequisites for this process are iron and a functional folate-vitamin B12-methylation pathway. The aim of this study was to determine iron status, folate and homocysteine in MS subjects, and to evaluate the effect on MS symptoms if deficiencies were addressed. Results: In relapsing-remitting MS subjects, serum iron concentration correlated significantly with age at diagnosis ($r = 0.49$; $p = 0.008$). In Caucasian female MS subjects, serum iron and ferritin concentrations were significantly lower than in matched controls. In a 6-month pilot study, 12 subjects taking a regimen of nutritional supplements designed to promote myelin regeneration, improved significantly neurologically as measured by the Kurzke EDSS (Total Score means 3.50 to 2.45, 29.9%; $p = 0.021$). These were significantly improved ($p = 0.002$) compared to 6 control group patients taking multivitamins (Kurzke Score increased by

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13.9% from 4.83 to 5.50). Both groups had significantly reduced homocysteine concentrations at 6 months, suggesting that methylation is necessary but not sufficient for myelin regeneration.

**Keywords** Multiple sclerosis · Myelin regeneration · Iron · EDSS · Nutrition · Homocysteine

## Introduction

Multiple sclerosis (MS) is a disorder in which autoreactive immune responses are involved in the attack on myelinated axons, thereby interfering with the conduction of signals to the periphery (Steinman, 1993). The disease process is often progressive, leading to chronic disability. The diagnosis of MS is extremely stressful for patients and care-givers alike because at diagnosis neurologists cannot deduce from the severity of the symptoms what the disease outcome will be. After an initial remission, some subjects experience few symptoms for long periods of time, while others deteriorate rapidly. The diagnosis of MS is mainly clinical (Poser and Brinar, 2004), assisted by cerebrospinal fluid analysis (Freedman *et al*., 2005), while magnetic resonance imaging (MRI) is gaining importance in diagnosis and follow-up (McDonald *et al*., 2001).

The aetiology of MS is complex and multi-factorial, involving genetic and environmental factors, while a viral component is also implicated (De Villiers *et al*., 2006). Furthermore, a combination of different factors may cause disease in different patients. Metals have previously been implicated in the aetiology of MS (Clausen, 1993). The role of excess iron has been investigated, but three clinical trials attempting to remove excess iron by chelation with desferrioxamine have been inconclusive (LeVine and Chakrabarty, 2004). A study by Grant *et al*. (2003) showed that low iron concentrations in food protected mice against developing experimental autoimmune encephalomyelitis (EAE), a mouse model for studying autoimmunity in MS. Contrary to these findings, a single patient study demonstrated stabilisation of the MS disease process with no further degeneration when a patient took iron supplements daily together with other nutrients (Rooney *et al*., 1999). The role of iron in the aetiology and pathology of MS has thus not been elucidated.

Since myelin regeneration is a prerequisite for remission, it is important to note that iron is indispensable for myelin synthesis. Iron deficiency during early postnatal life as well as in later life, results in a reduced amount of myelin in the spinal cord and white matter of rat pups (Yu *et al*., 1986; Beard *et al*., 2003). In mice, the disruption of iron availability, either by limiting dietary iron intake or by altering iron storage capacity, resulted in decreased myelin proteins and lipids (Ortiz *et al*., 2004). Proteolipid protein was most consistently affected, suggesting that limiting iron to oligodendrocytes results not only in hypomyelination but also in a decrease in myelin compaction, i.e. in the structure of the myelin. Electron microscopic studies have revealed high concentrations of iron in the cytoplasm of oligodendrocytes and within myelin (Connor and Menzies, 1996). Many of the enzymes involved in the biosynthetic pathways that produce myelin utilize iron as part of their catalytic center (LeVine and Chakrabarty, 2004). Myelinogenesis is a highly energy-intensive process resulting in a high metabolic demand for iron. Hulet *et al*. (1999) found ferritin receptors on oligodendrocytes and suggested that the significance of a cellular ferritin receptor was that ferritin was capable of delivering 2,000 times more iron per mole of protein than transferrin to the oligodendrocytes.

Iron enrichment within both oligodendrocytes and myelin raises the possibility that an imbalance in the management of iron during the disease process could lead to the production of iron-catalyzed free radicals that would cause oxidative damage. Toshniwal and Zarling (1992) found that the inflammation during an acute exacerbation causes so much lipid peroxidation that pentane can be measured in the breath of the patients, but during remission exhaled pentane was similar to values recorded for control subjects.

Vascular damage in MS is implicated in the finding of higher homocysteine levels in MS subjects (Vrethem *et al*., 2003). Interestingly, iron deficiency may also be linked to increased homocysteine levels. Prolonged iron deficiency anaemia is associated with gastritis and atrophy of glands producing intrinsic factor in the stomach (Davidson and Markson, 1955, Badenoch *et al*., 1959). This in turn leads to inhibition of vitamin B12 uptake (Cox *et al*., 1959). Optimal functioning of the folate-vitamin B12-methyl transfer cycle continuously providing activated methyl groups, is a prerequisite for myelin production and maintenance, and long-term deficiencies of this pathway cause demyelinating diseases of the brain and spinal cord (Selzer *et al*., 2003). Inborn errors of metabolism involving the genes of the methyl transfer pathway are known to cause inadequate myelination and serious disability from childhood, but supplementation with the chemical metabolite following each metabolic block was found to restore the myelin as well as some of the functional deficiencies (Surtees *et al*., 1991).

Polymorphisms in the genes of the methyl transfer pathway may occur without having an effect on the phenotype under normal circumstances, but when the substrates or co-factors of this pathway are depleted, demyelination may follow (Selzer *et al*., 2003). The latter case study illustrates this principle. The authors describe extensive demyelination of the brain and subsequent death of a child after anesthesia with nitrous oxide, which irreversibly oxidizes the cobalt in cobalamine (vitamin B12). The activity of methionine synthase (the enzyme which utilises cobalamine) is reduced to zero after 2 h of anaesthesia (Selzer *et al*., 2003). This adverse effect was amplified in the patient by the fact that he had two polymorphisms in 5,10-methylenetetrahydrofolate reductase (MTHFR) which reduced the activity of this enzyme as well, effectively blocking the production of active methyl groups. Importantly, in the deceased patient *normal* vitamin B12 levels were measured (403 pg/ml) even though the activity of the enzyme was zero, i.e. in this study the vitamin B12 assay did not distinguish between active and inactive (oxidized) vitamin B12. This may explain the results of Vrethem *et al*. (2003) who found increased homocysteine levels in MS subjects, but without decreased vitamin B12. Other researchers have found decreased levels of vitamin B12 and folic acid in serum and CSF of MS subjects (Reynolds *et al*., 1992; Frequin *et al*., 1993; Kolesar, 2000).

The aims of the present open-lable pilot study were to determine iron parameters, as well as folate and homocysteine concentrations in a group of MS subjects, and to correct deficiencies while observing possible effects on MS symptoms.

## Materials and methods

Ethical approval was obtained from the Ethics Committee of the University of Stellenbosch Faculty of Health Science. Written informed consent was obtained from participating subjects.

41 MS subjects recruited by the MS Society of the Western Cape, South Africa (MSSWC), were screened for participation in the study. The screening visit included demographic details, completing a dietary questionnaire, laboratory tests (see below), medical history and

diagnosis according to the criteria of McDonald *et al*. (2001). Ethnically, 29 females and 3 males were Caucasian, 6 females and 1 male were of mixed ancestry (the "coloured" population of South Africa) and 2 females were African (Xhosa). There were no Xhosa males. At screening, one patient failed to meet the criteria for diagnosis, and 5 subjects decided not to continue with the study.

Of the 35 remaining subjects, two Caucasian females and the two Xhosa females had secondary progressive MS (SPMS). 31 subjects had relapsing-remitting MS (RRMS). There were no primary progressive (PPMS) patients in the study group. Controls were healthy volunteers matched for age, gender, ethnicity and menstrual status in the females (27 females and 3 males).

Blood analyses were done in a routine diagnostic laboratory. Serum iron, transferrin (Tf) and C-reactive protein (CRP) were determined using a Scientific Group Vitros 250 Chemical analyser; hemoglobin (Hb) using a Scientific Group Pentra 60 Hematology analyser; ferritin and homocysteine with an Abbott AXSYM and folic acid with an Abbott Architect. Tf saturation was calculated.

In the first part of the study, the above-mentioned blood parameters were assessed in the MS subjects, and compared with controls. The results were analysed statistically to see whether any of the blood parameters could be linked to age at diagnosis, or severity of symptoms. The results of the SPMS subjects were analysed separately from the RRMS subjects.

In the second part of the study, a pilot study was conducted to evaluate a nutrient regimen which had anecdotally improved the symptoms of some MS subjects. The "Raphah Regimen" (Table 1), designed to promote myelin regeneration and endorsed by the MSSWC, was offered to all 35 participants. 18 of the RRMS subjects volunteered to take part in the study, which involved laboratory tests as well as neurological evaluation using the Expanded Disability Status Scale (the EDSS; Kurtzke, 1983) by a neurologist (PH) and a general practitioner (JB) at baseline and after 6 months. There were no SPMS volunteers.

A list detailing the composition of the Raphah Regimen (Table 1) was given to the subjects by the MSSWC. Iron supplements (15 mg/day) were prescribed for those who presented with lower iron status (which was determined by taking into account Hb, serum iron and ferritin concentrations, and Tf saturation values for each subject individually). Care was taken to prescribe an iron supplement that would not promote constipation. The subjects were contacted regularly to evaluate compliance. Of the 18 subjects, 12 had closely followed the regimen (the "compliant" group). Of the compliant subjects, 8 were on iron supplements in addition to the Raphah Regimen, while 4 (having had a higher iron status at baseline, Table 2) had been advised to follow the Raphah Regimen without iron. After 6 months, all 18 subjects, including the 6 who did not adhere to the Raphah Regimen, returned for follow-up EDSS and blood determinations. The latter (the "non-compliant" group) had taken some nutrients of their own choice, but not the full range as prescribed by the Raphah Regimen. Instead, one took homeopathic medicine, and the others took multivitamins containing folic acid and vitamin B12. One of the females took evening primrose oil in addition. None of the 6 non-compliant subjects took iron supplements.

None of the 18 subjects were on interferon therapy. One female in each group (compliant and non-compliant) had been taking azathioprine for more than a year, and continued to do so throughout the study. None of the other subjects were taking medication specifically for MS. Details of the two groups are given in Table 3. This was not a randomised clinical trial, but did investigate the clinical outcome in the two groups of people mentioned. All subjects had been in remission at baseline, the last relapse occurring more than 2 months before being included in the study.

**Table 1** The Raphah Regimen

| | | Vitamins and Minerals | | % RDA |
|---|---|---|---|---|
| Iron* | 15 mg/day | Beta Carotene | 3 mg | — |
| | | Vitamin C | 350 mg | 777 |
| Essential amino acids (e.g. diet milk shake) | | Vitamin E | 40 mg | 266 |
| | | Vitamin B1 | 3 mg | 200 |
| Leucine | 1235 mg | Vitamin B2 | 4 mg | 222 |
| Isoleucine | 890 mg | Nicotinamide | 20 mg | 100 |
| Lysine | 995 mg | Folic Acid | 5 mg | 1250 |
| Tryptophan | 175 mg | Vitamin B12** | 24 $\mu$g | 800 |
| Methionine | 315 mg | Vitamin B6 | 10 mg | 500 |
| Phenylalanine | 610 mg | Pantothenate | 10 mg | 130 |
| Threonine | 570 mg | Calcium | 28.5 mg | 2 |
| Valine | 840 mg | Magnesium | 150 mg | 37 |
| Histidine | 340 mg | Copper | 1 mg | 50 |
| | | Zinc | 15 mg | 100 |
| Lipids | | Manganese | 2.5 mg | — |
| Evening Primrose Oil | 500 mg | Chromium | 100 $\mu$g | — |
| Salmon Oil | 500 mg | Molybdenum | 100 $\mu$g | — |
| Lecithin | 300 mg | Selenium | 60 $\mu$g | — |

*Note.* *If patients do not present with excessively high iron parameters, 15 mg/day of an iron supplement (chosen for its ability not to promote constipation) is prescribed. **For the first three months the methylation metabolic pathway may be enhanced by additional weekly vitamin B12 injections, *or* a 1 mg/day vitamin B12 supplement that is dissolved under the tongue, *or* by taking S-adenosyl methionine (SAM), 200 mg/day.

In South Africa MRI scans are normally only done at the time of diagnosis, owing to financial constraint. For one of the compliant subjects (who was on the Raphah Regimen plus iron supplementation), however, serial MRI scans were available at baseline, after 6 months, 2 years and at 30 months (Fig. 1).

Statistical analyses of results were performed with Statistica software (StatSoft Inc. Tulsa OK, USA), version 7. The Wilcoxon matched pairs test (nonparametric) was used to compare two dependent variables. General ANOVA/MANOVA (one-way ANOVA) was used for the

**Table 2** Blood parameters of the MS patients on the Raphah Regimen at baseline and after 6 months, 8 taking iron, and 4 taking the other nutrients but not iron

| | Subjects taking iron ($n=8$) | | Subjects not taking iron ($n=4$) | |
|---|---|---|---|---|
| | Baseline | 6 months | Baseline | 6 months |
| Serum iron ($\mu$mol/L) | $14.04 \pm 0.95$ | $15.46 \pm 1.82$ | $19.23 \pm 1.43$ | $15.75 \pm 2.21$ |
| Transferrin (g/L) | $2.48 \pm 0.13$ | $2.14 \pm 0.11$ | $2.29 \pm 0.15$ | $2.00 \pm 0.24$ |
| % sat Transferrin | $23.52 \pm 2.80$ | $28.93 \pm 4.60$ | $32.67 \pm 1.29$ | $30.16 \pm 1.92$ |
| Ferritin (ng/ml) | $30.55 \pm 4.61$ | $34.26 \pm 6.41$ | $108.55 \pm 41.19$ | $112.02 \pm 37.51$ |
| Haemoglobin (g/dl) | $13.7 \pm 0.2$ | $13.9 \pm 0.3$ | $15.2 \pm 0.3$ | $14.9 \pm 0.4$ |
| Serum folate (nmol/L) | $25.6 \pm 6.8$ | $31.6 \pm 6.0$ | $28.6 \pm 9.6$ | $35.1 \pm 9.9$ |
| Homocysteine ($\mu$mol/L) | $9.1 \pm 2.1$ | $6.4 \pm 0.4$ | $6.7 \pm 1.4$ | $6.2 \pm 0.9$ |
| CRP (mg/L) | $5.4 \pm 3.6$ | $2.5 \pm 1.2$ | $1.9 \pm 0.7$ | $2.0 \pm 1.0$ |

*Note.* For the whole group of 12 patients, homocysteine ($p=0.018$) and transferrin ($p=0.002$) decreased significantly. Values are means $\pm$ 1 standard error.

**Table 3** Demographics of the "compliant" and "non-compliant" groups

| | | Compliant ($n = 12$) | Non-compliant ($n = 6$) |
|---|---|---|---|
| Age | | $44.3 \pm 8.4$ | $48.2 \pm 8.7$ |
| Menstruating females | | 9 (75%) | 2 (33%) |
| Hb (mean ± SE) | | $14.2 \pm 0.25$ | $13.5 \pm 0.28$ |
| Sex | | 12 females | 5 females and 1 male |
| Race | | 9 Caucasian, 3 mixed ancestry | 5 Caucasian, 1 mixed ancestry |
| EDSS TOTAL | Baseline | 3.50 | 4.83 |
| (mean) | Endpoint | 2.45 | 5.50 |

*Note*. Age and baseline EDSS Total Scores did not differ significantly. Hb values were lower in the non-compliant subjects although there were more menstruating women in the compliant group (see text).

analyses of single categorical independent variables. Data is given as mean ± standard error (SE), unless otherwise specified.

## Results

In the first part of the study blood parameters including iron status of the 31 RRMS subjects (including males and females, Caucasians and subjects of mixed race) were compared with disease outcome (retrospectively). It was found that the subjects could be placed into 4 groups according to their serum iron levels and age of MS diagnosis (Table 4). Group 1 (consisting of 9 females) had relatively lower serum iron values (5.70–15.20 $\mu$mol/L) and had been diagnosed at a young age (19–32 years). Group 2 (10 females and all 3 males) had higher serum iron values (15.90–27.30 $\mu$mol/L) and had been diagnosed at a later age (36–57 years). Group 3 (6 females) had lower serum iron (6.60–15.10 $\mu$mol/L), but were diagnosed at a later age (35–51 years). Group 4 (3 Caucasian females) had higher serum iron 20.30–29.30 $\mu$mol/L) and were diagnosed at a younger age (26–28 years). In the 28 subjects in Groups 1–3 there was a significant correlation ($r = 0.49$, $p = 0.008$) between serum iron concentration and age at diagnosis (Fig. 2). On the graph, Group 3 is highlighted (numbers 23–28) to show the clustering of these results. Group 4 is also shown on the graph (numbers 29–31), but the results were treated as outliers and were not included in the statistical analysis. Fig. 2 indicates a significant effect of serum iron concentration on disease outcome in these MS subjects, even though the values were within the normal range (6.60–30.40 $\mu$mol/L); only one of the subjects had a serum iron below the reference range and none were above.

The blood results of the subjects were analysed further to see whether any other parameters could explain the differences between the groups. It was found that Group 3 had significantly higher Hb concentrations ($13.9 \pm 0.1$ g/dl) than Group 1; ($13.3 \pm 0.2$ g/dl; $p = 0.042$), suggesting a protective effect of a higher Hb concentration against an early age of diagnosis in Group 3. Group 3 also had higher folic acid concentrations ($37.8 \pm 5.0$ nmol/L) than Group 1 ($23.5 \pm 4.9$ nmol/L; $p = 0.07$). Although there were only 3 subjects in Group 4, and hence statistical differences could not be calculated, they differed from the other groups by having lower ferritin, low folic acid and higher homocysteine concentrations than subjects in the other groups (Fig. 3A, B and C).

Table 5 shows a comparison of iron parameters in subjects and controls in the Caucasian female group. Serum iron concentrations were significantly lower in the MS subjects ($15.95 \pm 1.25$ $\mu$mol/l) compared to matched controls ($19.84 \pm 1.69$ $\mu$mol/l; $p = 0.045$).

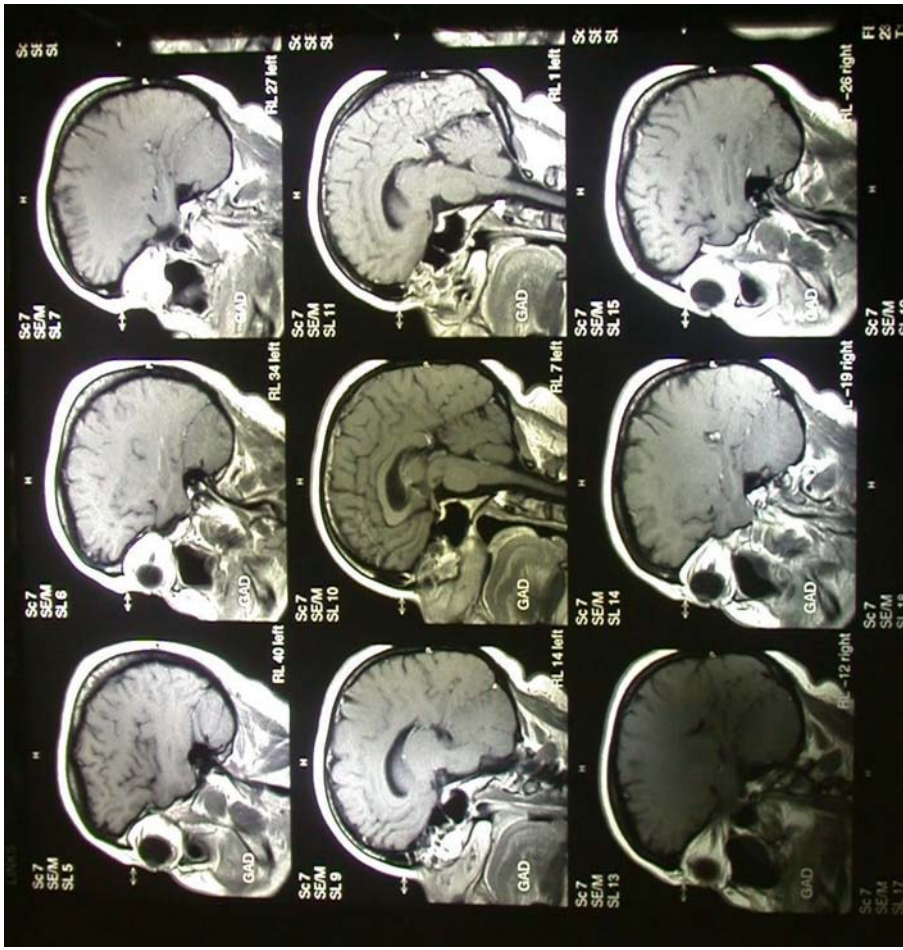


**Fig. 1** Gadolinium enhanced T1 weighted MRI image of an MS patient after taking the Raphah Regimen plus iron for 30 months. No contrast enhanced hyperintense lesions are visible, indicating that there is no disease activity.

**Table 4** Serum iron concentration versus age of diagnosis in 31 RRMS patients

| | | Serum iron concentration ($\mu$mol/L) | | Age at diagnosis (years) | |
|---|---|---|---|---|---|
| | *n* | range | Mean ± SE | range | mean ± SE |
| Group 1 | 9 | 5.70–15.20 | 12.10 ± 1.00 | 19–32 | 28.3 ± 1.4 |
| Group 2 | 13 | 15.90–27.30 | 20.22 ± 1.10 | 36–57 | 43.9 ± 1.5 |
| Group 3 | 6 | 6.60–15.10 | 10.95 ± 1.12 | 35–51 | 42.1 ± 2.2 |
| Group 4 | 3 | 20.30–29.30 | 25.00 ± 2.61 | 26–28 | 26.7 ± 0.7 |

*Note.* Group 3 had significantly higher Hb concentrations (13.9 ± 0.1 g/dl) than Group 1 (13.3 ± 0.2 g/dl; $p = 0.042$), suggesting a protective effect of a higher Hb concentration against an early age of onset in Group 3. Reference ranges: serum iron 6.60–30.40 $\mu$mol/L; and Hb: 13.0–18.0 g/dl.

Ferritin, given as median (lower, upper 95% confidence limit), was also significantly lower ($p = 0.046$) in the patients, 32.00 (28.72, 56.72) than control values, 54.22 (44.18, 104.47). Serum Tf did not differ and was somewhat lower in the MS subjects. Normally, Tf concentration increases when serum iron decreases. This may indicate a discrepancy of iron metabolism.

Menstrual status in the females was similar in the 4 groups, and did not differ from controls. In the control group, 18 females menstruated normally (Hb 13.3 ± 0.2 g/dl), while 7 had undergone hysterectomies or were not menstruating (Hb 13.9 ± 0.4 g/dl). In the RRMS subjects, Hb values in 18 who were menstruating (14.0 ± 0.2 g/dl) were paradoxically higher than in MS females who had hysterectomies (13.5 ± 0.3 g/dl). In Group 3 the increased Hb values were also not due to menstrual status: of the 6 females 3 were menstruating (Hb 14.0 ± 0.3 g/dl) while 3 had undergone hysterectomies (Hb 13.8 ± 0.1 g/dl). Here again the Hb's of the non-menstruating MS females were lower than of those who were regularly losing blood.

In the second part of the study, EDSS neurological determinations and laboratory tests were done on 18 volunteers at baseline and after 6 months. The "compliant" patient group, who stayed on the Raphah Regimen for 6 months, consisted of 12 females (9 Caucasian and 3 of mixed race). Table 2 shows a comparison of blood parameters of the compliant patients at baseline and after 6 months. Of the compliant patients 8 took iron supplements in addition to the Raphah Regimen, while 4 took the Raphah Regimen without iron. Supplementing the MS subjects with iron did not increase iron parameters markedly: in the compliant patients taking iron, the mean serum iron increased from 14.04 to 15.46 $\mu$mol/L, the ferritin increased from 30.55 to 34.26 ng/ml, and the Hb stayed the same. This may indicate the presence of genetic discrepancies in iron uptake or metabolism. In the group taking iron, homocysteine decreased by 2.7 $\mu$mol/L, while in the 4 subjects not taking iron, it decreased by 0.5 $\mu$mol/L. P-values were calculated for the whole group of 12 subjects. Homocysteine ($p = 0.018$) and Tf ($p = 0.002$) decreased significantly, while ferritin concentrations increased in both the subjects taking iron, and those not taking iron, but not significantly.

The 6 "non-compliant" subjects, who were used as a control group, consisted of 5 females (4 Caucasians and one of mixed race) and one Caucasian male. These subjects did not take the Raphah Regimen, and none of them took iron. Instead, one took homeopathic medicine, and the others took multivitamins containing folic acid and vitamin B12. Table 6 shows the blood parameters of the non-compliant subjects at baseline and at 6 months. As was the case in the compliant group, homocysteine ($p = 0.046$) and Tf levels ($p = 0.028$) decreased significantly while ferritin levels increased, but not significantly.

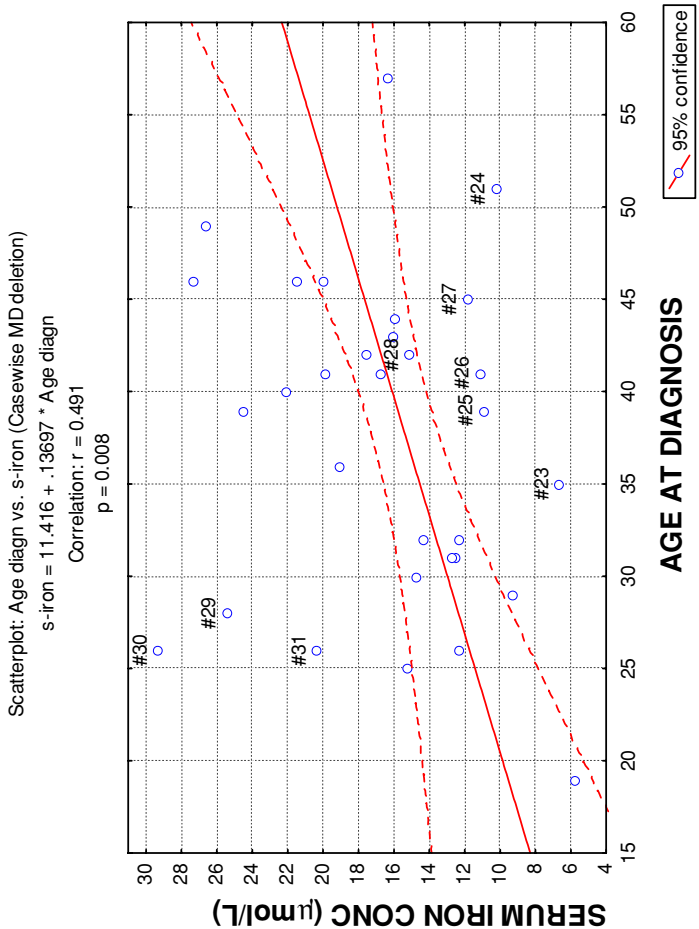


**Fig. 2** Significant correlation between serum iron concentration and age at diagnosis for 28 patients with RRMS. Group 3 (numbers 23–28) are highlighted. Group 4 (numbers 29–31) were treated as outliers, and were not included in the analysis (see Table 4)

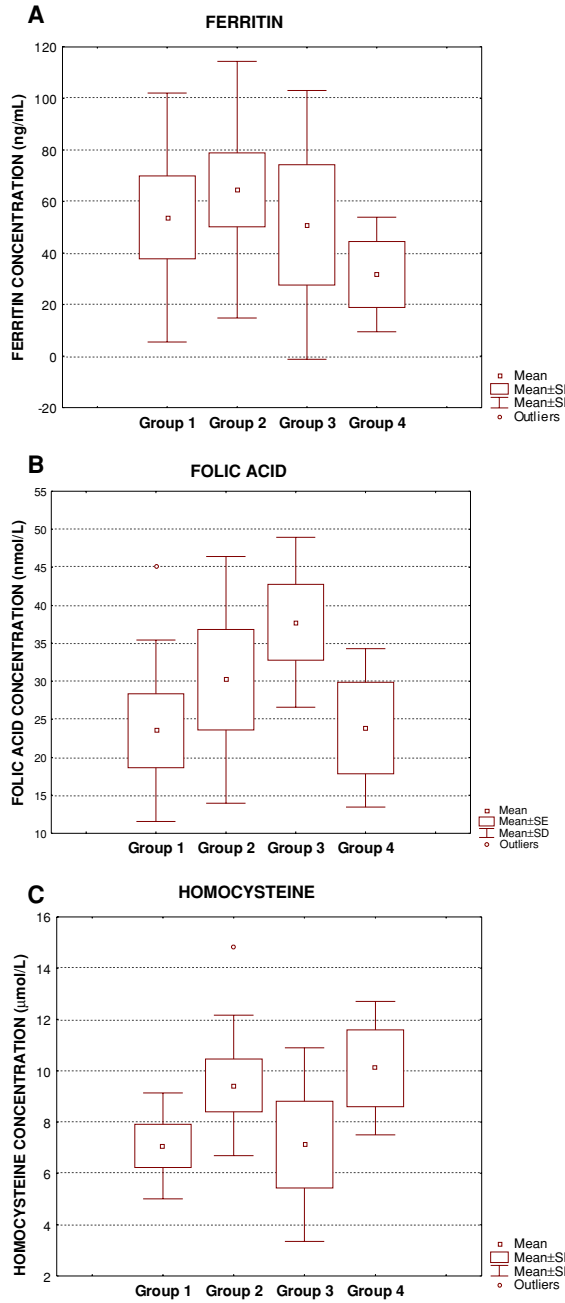


**Fig. 3** (**A, B and C**) Ferritin (A), folic acid (B) and homocysteine concentrations (C) in four groups of patients (See text). Group 4 had lower ferritin and higher homocysteine concentrations than patients in the other groups

**Table 5** Blood parameters in caucasian females: 26 MS patients and 22 controls

| | MS | Control | *p*-value | Reference values |
|---|---|---|---|---|
| Serum iron ($\mu$mol/L) | $15.59 \pm 1.25$ | $19.84 \pm 1.69$ | **0.045** | 6.60–30.40 |
| Transferrin (g/L) | $2.43 \pm 0.06$ | $2.62 \pm 0.10$ | 0.087 | 2.00–3.30 |
| % sat Transferrin | $25.21 \pm 1.99$ | $30.55 \pm 3.35$ | 0.162 | 15.00–50.00 |
| Ferritin (ng/ml), median (95% CI)* | 32.00 (28.72, 56.72) | 54.22 (44.18, 104.47) | **0.046** | 10.00–290.00 |
| Haemoglobin (g/dl) | $13.73 \pm 0.15$ | $13.43 \pm 0.22$ | 0.260 | 13.0–18.0 |
| Serum folate (nmol/L) | $27.48 \pm 3.02$ | $32.42 \pm 5.95$ | 0.460 | 5.9–45.4 |
| Homocysteine ($\mu$mol/L) | $8.07 \pm 0.70$ | $8.36 \pm 1.60$ | 0.858 | 5.0–15.0 |
| CRP (mg/L) | $3.74 \pm 1.01$ | $3.56 \pm 0.97$ | 0.911 | 0.0–10.0 |

*Note*. Values are means $\pm$ 1 standard error; *for ferritin median (lower, upper 95% confidence limit). Significant differences are in bold.

Table 7 shows the results of the EDSS neurological examination of the 12 compliant subjects at baseline and after 6 months. The mean Total EDSS score improved significantly from $3.50 \pm 0.57$ at baseline to $2.45 \pm 0.50$ at endpoint (29.9%; $p = 0.022$; Fig. 4). The most dramatic improvement was recorded in the cerebral sub-scale of the EDSS (which measures mood and "mentation"); all patients recorded zero deficit after 6 months ($p = 0.028$; Fig. 5). Two patients had a score of 0 on the Total EDSS after 6 months, indicating no neurological disability. For one of the compliant patients on the Raphah Regimen plus iron, MRI scans done after 6 months, at 2 years and at 30 months showed no additional hyperintense lesions, and no disease activity on gadolinium enhancement. Fig. 1 is a gadolinium-enhanced T1 weighted image of the brain of the patient at 30 months.

None of the 12 compliant patients were admitted to hospital for intravenous prednisone during the 6 months that they were on the Raphah Regimen, while one of the non-compliant patients received intravenous prednisone. The neurological outcome in the group of 6 non-compliant patients was deterioration from a mean Total EDSS score of $4.83 \pm 0.84$ to $5.50 \pm 0.55$ at endpoint (13.87%). There was also a significant difference in neurological outcome ($p = 0.002$) when the compliant and non-compliant groups were compared (Fig. 4).

**Table 6** Blood parameters of the non-compliant MS patients at baseline and after 6 months. None were taking iron

| | Baseline | 6 months |
|---|---|---|
| Serum iron ($\mu$mol/L) | $17.13 \pm 3.00$ | $16.62 \pm 1.90$ |
| Transferrin (g/L) | $2.22 \pm 0.05$ | $1.96 \pm 0.09$ |
| % sat Transferrin | $30.02 \pm 5.62$ | $33.49 \pm 4.68$ |
| Ferritin (ng/ml) | $66.02 \pm 20.41$ | $75.31 \pm 18.03$ |
| Haemoglobin (g/dl) | $13.6 \pm 0.3$ | $14.2 \pm 0.4$ |
| Serum folate (nmol/L) | $25.8 \pm 6.1$ | $35.6 \pm 4.6$ |
| Homocysteine ($\mu$mol/L) | $8.7 \pm 0.5$ | $6.6 \pm 0.7$ |
| CRP (mg/L) | $1.1 \pm 0.4$ | $2.7 \pm 1.1$ |

*Note*. For the group of 6 patients, homocysteine ($p = 0.046$) and transferrin ($p = 0.028$) decreased significantly. Values are means $\pm$ 1 standard error.

**Table 7** Mean values of the Kurtzke EDSS at baseline and after six months for patients taking the Raphah Regimen

| EDSS SYMPTOMS | Baseline (means) | Follow up (means) | *p* values |
|---|---|---|---|
| Pyramidal | $2.00 \pm 0.58$ | $1.70 \pm 0.42$ | 0.500 |
| Cerebellar | $1.50 \pm 0.48$ | $0.50 \pm 0.22$ | 0.091 |
| Brainstem | $0.20 \pm 0.13$ | $0.20 \pm 0.20$ | 1.000 |
| Sensory | $2.11 \pm 0.54$ | $1.22 \pm 0.40$ | 0.068 |
| Bowel and bladder | $0.80 \pm 0.33$ | $0.60 \pm 0.31$ | 0.500 |
| Visual | $1.56 \pm 0.55$ | $1.56 \pm 0.58$ | 1.000 |
| Cerebral | $1.40 \pm 0.43$ | $0.00 \pm 0.00$ | **0.028** |
| EDSS TOTAL | $3.50 \pm 0.57$ | $2.45 \pm 0.50$ | **0.022** |

*Note*. Improvements were recorded in 5 of the sub-scales of the EDSS. All subjects had a score of 0 on the cerebral subscale after 6 months. The difference between baseline and follow up values for the Total EDSS ($p = 0.022$) and the cerebral subscale ($p = 0.028$) were significant. Values are mean $\pm$ 1 standard error.

## Discussion

A significant correlation was found between serum iron concentration and age at diagnosis in patients with RRMS (Fig. 2), i.e. in this group, higher serum iron was apparently protective against an early age of onset, even though all but one patient had serum iron concentrations within the normal range. Six females who had a later onset of disease, but relatively low serum iron values, had higher Hb concentrations, while 3 Caucasian females, who had higher serum iron concentrations, but an early age of diagnosis, presented with lower ferritin concentrations (Fig. 3A). These findings suggest a continuum of iron dysregulation (that may be caused by different genetic and/or environmental factors) as an underlying cause of MS, as previously proposed by Kotze *et al*. (2001). These authors reported significantly lower serum ferritin levels in a group of patients with RRMS compared with PPMS, which correlated with younger age as well as earlier age of disease onset. The relationship between iron insufficiency and the aetiology of MS has hitherto remained unapparent because patients do not usually present with below-normal iron parameters. Menstrual status in the MS patients was not different from controls. The lowering of iron due to blood loss in females could strengthen the argument that low iron could precipitate MS, since fewer males than females are diagnosed with MS, and MS is often diagnosed after childbirth. Of the 41 MS subjects recruited for the present study, 37 were females and 4 were males, giving a ratio of 9:1 of females to males.

Another reason why a diagnosis of iron deficiency would normally not be made in MS subjects, is the fact that their Tf levels do not increase as is normally the case when iron status is low. The possibility exists that the Tf response may be blunted, or that the catabolism of Tf may be increased in MS (Van Rensburg *et al*., 2004). Together with ferritin, Tf is involved in myelin synthesis, Saleh *et al*. (2003) having demonstrated that myelination and motor coordination were increased in Tf transgenic mice.

The concentration of ferritin, which plays a role in delivering iron to oligodendrocytes (Connor and Menzies, 1996; Hulet *et al*., 1999) was significantly lower in Caucasian female RRMS subjects than in controls (Table 5). According to Fitzsimons and Brock (2001) reduced serum ferritin provides unequivocal evidence of diminished iron stores. Ferritin was also lower in 3 subjects who had adequate serum iron concentrations, but a young age at diagnosis (Fig. 3A). Valberg *et al*. (1989) reported high ferritin values in MS patients who required bilateral assistance to walk or were confined to a chair, while high ferritin

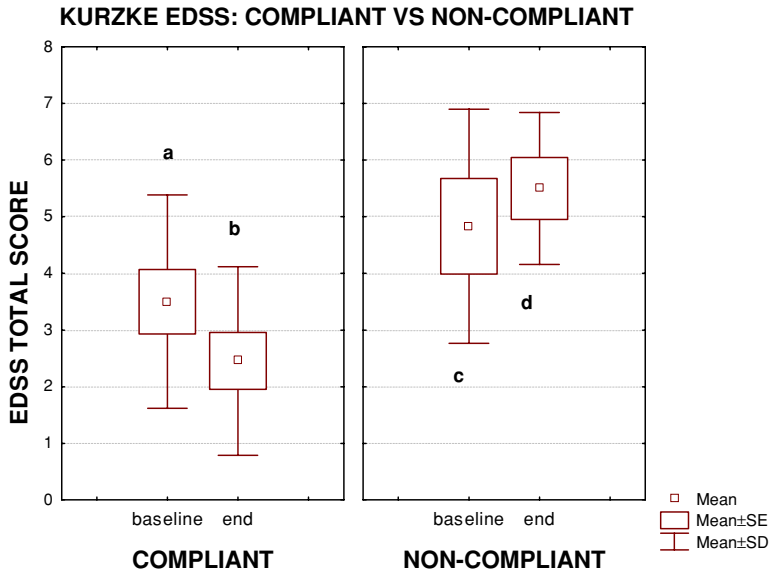


**Fig. 4** Total EDSS scores in patients taking all the nutrients of the Raphah Regimen (the compliant group) versus patients who were non-compliant. **b** significantly different from **a**: $p = 0.022$; **c** compared to **a**: $p = 0.197$; **d** compared to **c**: $p = 0.11$; **d** significantly different from **b**: $p = 0.002$

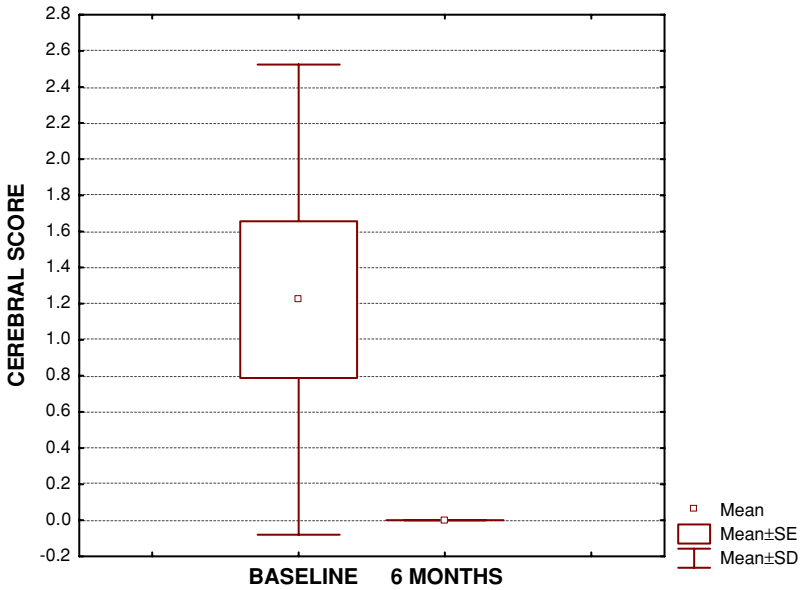


**Fig. 5** The cerebral sub-scale of the EDSS for 12 MS patients at baseline and after 6 months. All the patients recorded zero disability at 6 months, which was a significant improvement ($p = 0.028$)

appeared to be related to the severity of the disease. In our study population the three RRMS subjects with the highest EDSS values (6.5) had ferritin concentrations of 14.10, 33.00 and 101.94 ng/ml respectively, while their Hb values were 14.7, 13.6 and 13.0 g/dl. The Hb of the latter subject was at the lower level of the reference range (13.0–18.0 g/dl), showing that she was tending towards anaemia. Ferritin levels are raised in the anaemia of chronic disease (Fitzsimons and Brock, 2001).

Apart from its role in iron storage, and in delivering iron to oligodendrocytes, ferritin also plays a role as antioxidant by virtue of its iron-scavenging capabilities (Hulet *et al*., 1999). Free radical activity plays a pivotal role in MS. Syburra and Passi (1999) found significantly lower antioxidant levels in the blood of MS patients compared to controls. Studies have found lower glutathione peroxidase levels (Shukla *et al*., 1977; Szeinberg *et al*., 1979; Jensen and Clausen, 1984), and significantly lower selenium concentrations in subjects with MS than in controls (Wikstrom *et al*., 1976). MS prevalence is higher in areas where selenium concentration in soil is low (Wikstrom *et al*., 1976). Therapies aimed at neutralising oxidants in MS have also had some success (LeVine and Chakrabarty 2004), confirmed by the results of the present study.

In the second part of the study the authors collaborated with the MSSWC. From the practical experience of subjects and from the literature, valuable information was gleaned to formulate a hypothesis for the regeneration of myelin and unconventional treatment of MS. It was reasoned that, since one of the functions of the immune system is to remove dead or damaged tissue, macrophages would be attracted to myelin which was structurally not intact whether as a result of a deficiency, oxidative damage, a genetic defect, a viral attack, nitrous oxide or any other insult. A study by Barnett and Prineas (2004) demonstrated that 7 patients with RRMS who died during or shortly after the onset of a relapse, exhibited extensive oligodendrocyte apoptosis in myelinated tissue *before* the arrival of lymphocytes or myelin phagocytes. It may be postulated that, since myelin is regenerated continually (Surtees *et al*., 1991), the disease process could be slowed down, and relapses prevented, if myelin were enabled to regenerate to the correct structure by providing all the appropriate nutrients. Verifying this hypothesis, subjects taking the nutrients of the Raphah Regimen improved significantly over 6 months when compared with themselves ($p = 0.022$) as well as with a non-compliant control group ($p = 0.002$). Two of the subjects taking the Raphah Regimen plus iron had EDSS Total scores of 0 after 6 months, demonstrating no neurological disability, while the only set of MRI scans that were available demonstrated no gadolinium enhanced lesions (i.e. no disease activity) (Fig. 1). After 6 months on the Raphah Regimen, mental functioning returned to normal (zero deficit) in all the compliant subjects which was reflected in the significant improvement in the cerebral sub-scale of the EDSS ($p = 0.028$; Fig. 5). Another advantage obtained anecdotally by subjects on iron supplements was the relief of fatigue, which is one of the most distressing MS symptoms. However, subjects who had high iron parameters at baseline and who took the Raphah Regimen without iron, improved neurologically as well, indicating that additional iron was not necessary nor advisable.

Although homocysteine concentrations decreased significantly in the compliant group ($p = 0.018$), similar values were obtained for the non-compliant group ($p = 0.046$), some of whom had taken multivitamins containing vitamin B12 and folic acid. Reduction of homocysteine (and concomitant production of activated methyl groups) is thus a necessary but not sufficient parameter for the regeneration of myelin.

Although this was a small, non-randomised, open-lable pilot study, the significant outcomes may serve as indicators for the direction of future research into the management of MS. One of the problems experienced during the study was the recruitment of adequate

numbers of subjects. Compared to Europe and America the prevalence of MS in South Africa is relatively low among Caucasians and people of mixed ancestry, and even lower in the African population (Dean *et al*., 1994). The two Xhosa MS subjects in our cohort were the only two cases known to the MSSWC at the time of the study. Interestingly, one of the Xhosa patients informed the research team that her great-grandmother had been a German. Her parents and grandparents were Xhosa. The other Xhosa subject could not recall having any ancestors of European descent. More research is required to identify possible protective elements in the South African environment; for example, the adequate sunlight may provide raised vitamin D levels in plasma, thereby suppressing interferon-$\gamma$ mediated macrophage activation (Helming *et al*., 2005).

Another issue is that of the large number of nutrients administered in this study. From a biochemical point of view, a trial not involving iron, vitamin B12, folic acid and essential fatty acids among others would in all probability not be successful, because addressing vascular insufficiency and myelin regeneration needs all of these nutrients to be present simultaneously. For example, vitamin B12 is only one of the cogs in the wheel of the metabolic process of methylation. If myelin production is compared to a production line in a factory, it would be reasonable to provide all the raw materials in adequate quantities on a continuous basis rather than provide only one constituent at a high concentration.

Serum iron concentration therefore will not necessarily correlate with disease severity, because iron is not the only factor which influences myelin regeneration. Theoretically, however, iron deficiency may underlie a number of the prominent metabolic discrepancies involved in MS, such as the inhibition of vitamin B12 uptake (Cox *et al*., 1959), which would in turn inhibit methylation and lead to higher homocysteine concentrations implicated in the vascular aspect of the disease. Furthermore, both iron and methylation are essential for lipid metabolism, e.g. in the elongation and desaturation of fatty acids (Beard *et al*., 2003) which are pertinent to the lipid component in myelin. Dysfunction of iron-dependent $\Delta$6- and $\Delta$9- desaturases have been related to aberrant myelination (Kwik-Uribe *et al*., 2000). In rats, iron deficiency aggravated signs of essential fatty acid deficiency in the brain (Oloyede *et al*., 1992). Furthermore, choline, a constituent of lecithin and myelin, is synthesised from ethanolamine by three successive methylations. Lipids also have effects on the immune system and blood cell coagulation, both of which influence MS. In MS, differences in the lipid composition of blood cells have been demonstrated, and before the advent of medical treatments, supplementation with omega-6 (Field and Joyce, 1983) and omega-3 unsaturated fatty acids (Nordvik *et al*., 2000) were found to be beneficial in MS. However, even if alleviating iron deficiency could correct all these other discrepancies, it would take a long time to accomplish. For MS patients it is crucial to experience improvement of their symptoms as soon as possible, not only for physical rehabilitation, but also to alleviate stress and anxiety.

The Raphah Regimen (Table 1) is an endeavour to find the correct combination of nutrients that would facilitate the regeneration of myelin to its correct structure, thereby avoiding over-activation of the immune system. Of anecdotal interest is the following single case study: a low serum iron concentration (5.70 $\mu$moles/l) was measured in one of the patients at diagnosis, and iron and folic acid together with intravenous prednisone prescribed. (MS was confirmed by a relapse 2 months after diagnosis). While on the Raphah Regimen this person has experienced no further symptoms of MS for 4 years (no heat sensitivity, normal fatigue levels, while a skin rash produced by sunlight gradually disappeared over time). What is not known is whether the person's myelin has regenerated to the correct "compaction" (Ortiz *et al*., 2004).

## Conclusion

Preliminary data suggest that it may be possible to influence the course of MS by providing the nutrients necessary to regenerate myelin daily in adequate amounts. Though the number of patients in the present study is very small, the results are encouraging. Since the prevalence of MS in South Africa is relatively low it is envisaged to test the hypothesis in a large multicenter, blinded, randomized, placebo-controlled trial in collaboration with researchers in countries where MS is more prevalent.

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