

## Polyunsaturated fatty acids in the pathogenesis and treatment of multiple sclerosis

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Epidemiological, biochemical, animal model and clinical trial data described in this overview strongly suggest that polyunsaturated fatty acids, particularly *n*-6 fatty acids, have a role in the pathogenesis and treatment of multiple sclerosis (MS). Data presented provides further evidence for a disturbance in *n*-6 fatty acid metabolism in MS. Disturbance of *n*-6 fatty acid metabolism and dysregulation of cytokines are shown to be linked and a “proof of concept clinical trial” further supports such a hypothesis. In a randomised double-blind, placebo controlled trial of a high dose and low dose selected GLA (18:3*n*-6)-rich oil and placebo control, the high dose had a marked clinical effect in relapsing-remitting MS, significantly decreasing the relapse rate and the progression of disease. Laboratory findings paralleled clinical changes in the placebo group in that production of mononuclear cell pro-inflammatory cytokines (TNF- $\alpha$ , IL-1 $\beta$ ) was increased and anti-inflammatory TGF- $\beta$  markedly decreased with loss of membrane *n*-6 fatty acids linoleic (18:2*n*-6) and arachidonic acids (20:4*n*-6). In contrast there were no such changes in the high dose group. The improvement in disability (Expanded Disability Status Scale) in the high dose suggests there maybe a beneficial effect on neuronal lipids and neural function in MS. Thus disturbed *n*-6 fatty acid metabolism in MS gives rise to loss of membrane long chain *n*-6 fatty acids and loss of the anti-inflammatory regulatory cytokine TGF- $\beta$ , particularly during the relapse phase, as well as loss of these important neural fatty acids for CNS structure and function and consequent long term neurological deficit in MS.

**Multiple sclerosis: Linoleic acid: Gamma-linolenic acid: Arachidonic acid: Clinical trials: Cytokines**

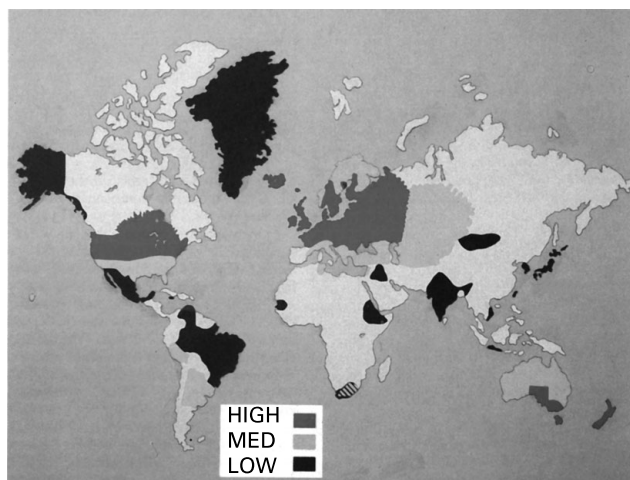
### Background to multiple sclerosis and its pathogenesis

Multiple sclerosis (MS) is a CNS-specific demyelinating disease, and is the most common neurological disorder that occurs in young adults<sup>1,2</sup>. The majority of patients with MS have the relapsing-remitting form of the disease, characterised by attacks (relapses) interspersed with periods of recovery (remission). The disease is most prevalent (30–100+ cases per 100 000 people) in Western Europe, Southern Canada, Northern United States, Southern Australia and New Zealand and of low frequency (0–19 per 100,000) in Asia, Central America, Africa and Greenland (See Fig. 1). Between 2 and 3 million people Worldwide are thought to live with MS. Although the aetiology of MS remains unknown there is strong evidence for the presence of autoimmune mechanisms in the disease pathogenesis<sup>3,4</sup>. Studies have shown that MS patients have a much higher number of neuroantigen e.g. myelin basic protein (MBP) and myelin oligodendrocyte glycoprotein (MOG) autoreactive T-cells, which are in an increased state of activation compared with healthy controls, and which increase during exacerbation<sup>4–13</sup>.

Cytokines from activated T cells and macrophages have been strongly implicated in the pathogenesis of MS<sup>14</sup>. For example, the up-regulation of adhesion molecules on endothelial cells and the subsequent infiltration of activated T cells into the CNS are immunopathogenic events controlled

by pro-inflammatory cytokines such as tumour necrosis factor- $\alpha$  (TNF- $\alpha$ ), interleukin-1 $\beta$  (IL-1 $\beta$ ) and interferon- $\gamma$  (IFN- $\gamma$ )<sup>15</sup>. Furthermore studies have shown that these cytokines exert direct myelinotoxic properties<sup>16,17</sup> and prolong the disease process in experimental autoimmune encephalomyelitis (EAE), an animal model of MS<sup>18,19,20</sup>. TNF- $\alpha$ , IL-1 $\beta$  and IFN- $\gamma$  have all been shown to be present in CNS active lesions in MS and elevated amounts of these cytokines are secreted from MS peripheral blood mononuclear cells (PBMC)<sup>21–25</sup>. Many studies, including our own, have also shown that an increase in these inflammatory cytokines coincides with the relapse phase of the disease<sup>25–33</sup>. Furthermore some studies have shown that transforming growth factor-beta1 (TGF- $\beta$ 1), a potent anti-inflammatory and immunosuppressive cytokine, is reduced during the relapse phase and increases as the patient enters remission<sup>25,34,35</sup>. In addition we have demonstrated that the balance between biologically active TGF- $\beta$ 1 and the pro-inflammatory TNF- $\alpha$ , IL-1 $\beta$  and IFN- $\gamma$  is dysregulated during MS relapse-remission<sup>25</sup>. The actual processes of axonal damage e.g. chronic inflammation, demyelination and astrogliosis in MS is complex but white matter inflammation and demyelination are considered to determine disease severity, whilst recent studies suggest that axonal damage in MS begins in the early stages of the disease and contributes to disability<sup>36,37</sup>. Furthermore some have

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**Fig. 1.** Geographical distribution of multiple sclerosis (from Adams C (1989) colour atlas of multiple sclerosis and other myelin disorders, Wolfe Medical Publications Ltd, with permission).

considered metabolic disturbances in some MS patients to be behind primary oligodendrocyte damage with secondary autoimmune-demyelination<sup>38,39</sup>.

#### Nutritional epidemiology of multiple sclerosis

Over half a century ago Roy Swank<sup>40</sup> found a positive relationship between fat intake as well as annual milk production and MS in Scandinavian countries. Furthermore studies by Alter *et al.*<sup>41</sup> implicated animal fat rich in saturated fatty acids as a causal factor in MS and Wolfgram<sup>42</sup> in an analysis of World Health Organisation (WHO) annual mortality statistics found a similar geographical distribution between MS coronary heart disease and cancer of the colon. In the multivariate analysis (inclusive of socioeconomic and medical services) of 20 countries Arganoff and Goldberg<sup>43</sup> not only implicated meat and dairy fats in positive correlations with MS, as noted previously, but also vegetable, seed, nut and fish, foods rich in both the *n*-6 and *n*-3 polyunsaturated fatty acids, in negative correlations with MS. Several other studies also confirmed strong MS associations with dairy and other animal fats<sup>44–46</sup>. Similar observations have also been made more recently by Esparsa *et al.*<sup>47</sup> in a large study (36 countries) assessing the impact of diet on MS mortality. They found that the higher the saturated fatty acid intake the higher the MS mortality and the higher the polyunsaturated to saturated fatty acid ratio the lower the MS mortality. However the large single country study, the Nurses' Health Study in the USA failed to show any relationship between MS and fat intake in women<sup>48</sup>. Thus the majority of epidemiological studies indicate that foods rich in saturated fatty acids are detrimentally associated with MS, whilst polyunsaturated fatty acid rich foods are beneficially associated with MS.

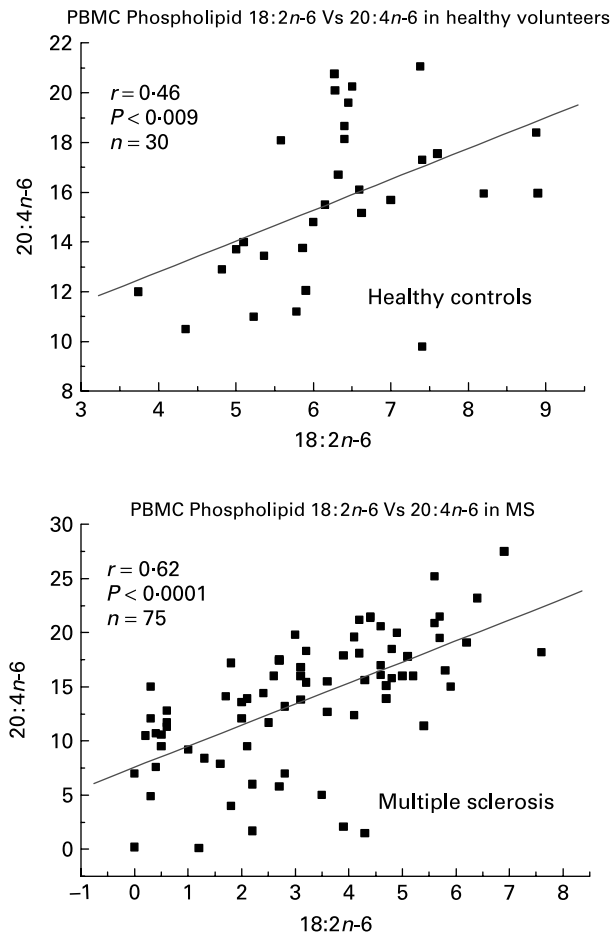
#### Biochemical and metabolic studies of fatty acids in multiple sclerosis

There is much evidence that the *n*-6 fatty acids particularly linoleic (18:2*n*-6) and arachidonic acids (20:4*n*-6) are

reduced in the plasma, platelets, erythrocytes, leucocytes and cerebrospinal fluid with changes in the unsaturated fatty acid composition of brain white matter in MS patients, much of this early work being undertaken at the National Hospital, Queen Square in London<sup>49–60</sup>. But there are also inconsistent reports<sup>61–64</sup> and Love *et al.*<sup>65</sup> observed that reduced linoleic acid was not specific to MS and occurred in patients with acute non-neurological illness. However many of these differences between studies are perhaps not surprising given cultural and ethnic differences, dietary variability (particularly when someone is ill), possible desaturase gene polymorphism, disease variability, serum versus cellular fatty acids and methodological differences for example total lipid fatty acids versus phospholipids fatty acid analysis.

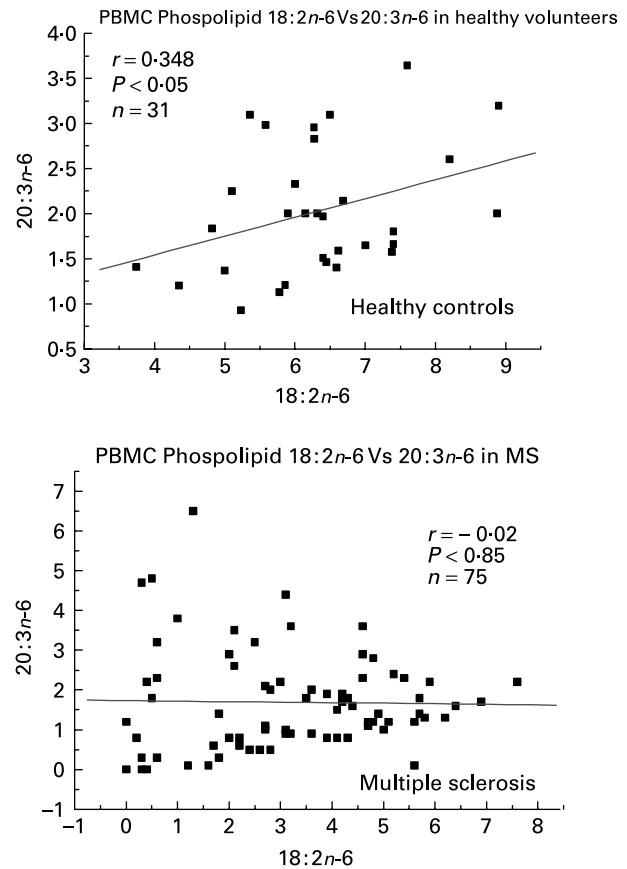
Previously we proposed nervonic acid as a marker of CNS myelin damage in MS<sup>66</sup> and found that MS patients consuming a diet rich in polyunsaturated fatty acids particularly linoleic acid had an inverse relationship between erythrocyte membrane linoleic acid and nervonic acid (24:1)<sup>67</sup>. A similar finding was described by Homa *et al.*<sup>68</sup>, showing a decrease in erythrocyte lignoceric acid (24:0) in sunflower oil (rich in linoleic acid) supplemented MS patients. In an open, uncontrolled 2 year fish oil intervention study by Nordvik *et al.*<sup>69</sup> in MS, they observed significant reductions in plasma total phospholipid nervonic and lignoceric acids with time and clinical improvement. Taken together the above indicate that nervonic and lignoceric acids could be useful pathogenic biomarkers of myelin damage and/or biomarkers for monitoring fatty acid treatments. We also found that the atypical erythrocyte electrophoretic response of MS patients was positively correlated with membrane linoleic acid and could be corrected by a diet rich in polyunsaturated fatty acids particularly linoleic acid<sup>67</sup>. This is in agreement with Field and Joyce<sup>71</sup> who found an increase in erythrocyte electrophoretic response in MS patients supplemented with evening primrose oil (EPO). However, Field *et al.*<sup>71</sup>, Field and Joyce<sup>70</sup> interpreted their electrophoretic results, without an analysis of membrane fatty acids, as an effect of the gamma-linolenic (GLA, 18:3*n*-6) component of the oil. EPO contains about 70% linoleic acid and 8–10% GLA, therefore it is more likely that the effect observed by Field *et al.* was due to the linoleic acid component of the oil rather than the GLA.

We have also investigated the metabolic relationships between the *n*-6 fatty acids in both healthy controls and MS PBMC total phospholipids (Figs. 2, 3 and 4). Both controls and MS patients (remission phase) demonstrate a positive correlation between linoleic acid (18:2*n*-6) and arachidonic acid (20:4*n*-6) as expected, although these *n*-6 fatty acids were low in a proportion of the MS patients studied (Fig. 2). Moreover, the relationship between linoleic acid (18:2*n*-6) and dihomo- $\gamma$ -linolenic acid (DGLA), and also between DGLA (20:3*n*-6) and arachidonic acid (20:4*n*-6) is clearly disturbed in MS compared with healthy controls (Figs. 3 and 4). This may indicate a problem with  $\Delta 6$  and  $\Delta 5$  desaturation and / or a greater requirement for these *n*-6 fatty acids in many of the MS patients studied, about 20–30 percent of the patients showed lower than normal PBMC phospholipid DGLA and arachidonic acid. In agreement with our findings Homa *et al.*<sup>55</sup> has also reported a similar disturbance in the relationship between linoleic acid and arachidonic acid in MS erythrocyte membrane lipids compared to healthy controls.



**Fig. 2.** Relationship between linoleic acid (18:2n-6) and arachidonic acid (20:4n-6) in peripheral blood mononuclear cell total phospholipids of healthy controls and multiple sclerosis.

Furthermore when we compared MS and healthy control PBMC total phospholipid 20:2n-6 we found a significant 2 fold higher 20:2n-6 in MS patients in remission compared to healthy controls and a significant 4 fold higher 20:2n-6 in the relapse phase of the disease. It appears that in MS there is a very active elongation of 18:2n-6 to 20:2n-6 in PBMCs and that this is even higher in the relapse phase (accounting for the low 18:2n-6) indicating a disturbance in the normal metabolism or a higher requirement for DGLA and arachidonic acid (20:2n-6 maybe further  $\Delta 8$  desaturated to DGLA?) by these n-6 fatty acid (20:4n-6) rich cells<sup>72</sup>, or both. This may also be reflective of the demand of cells and myelin in the brain which are also n-6 fatty acid-rich (20:4n-6 and 22:4n-6), significantly Stanley Rapoport of the NIH has shown that the human brain requires 4 times the amount of arachidonic acid (20:4n-6) than docosahexaenoic acid (22:6n-3) on a daily bases (ISSFAL 2006). In relation to 20:2n-6, although not discussed by the authors, the Nordvik *et al.*<sup>69</sup> study demonstrated a reduction with time of 20:2n-6 running parallel with clinical improvement, a similar finding to the nervonic and lignoceric acids mentioned earlier. Therefore 20:2n-6 may also be a useful marker of disease progression and/or monitoring fatty acid treatments in MS.



**Fig. 3.** Relationship between linoleic acid (18:2n-6) and dihomo- $\gamma$ -linolenic acid (20:3n-6) in peripheral blood mononuclear cell total phospholipids of healthy controls and multiple sclerosis.

### Fatty acids and animal models of multiple sclerosis

Experimental autoimmune encephalomyelitis (EAE) is an experimentally induced CD4<sup>+</sup>T cell mediated autoimmune-inflammatory and deyelinating disease in rodents often used as an animal model of MS. Studies in guinea pig and rat EAE treated with linoleic acid alone or a high linoleic and low  $\gamma$ -linolenic (GLA) acid rich oil (ratio 7:1) respectively, have shown partial suppression of the incidence and severity of EAE<sup>73,74</sup>. In a series of experiments we demonstrated important disease modifying effects of linoleic acid-rich oil (containing no GLA) and GLA-rich oil on clinical and histopathological manifestations of EAE. Depending on dose GLA was completely protective in EAE, whereas linoleic acid had a dose dependent action on the clinical severity of EAE, although not abolishing it<sup>75,76</sup>. Natural recovery in EAE is mediated by expansion of suppressor lymphoid cells<sup>77</sup> some of which have been characterised as TGF- $\beta$  producing CD4<sup>+</sup>T cells by Karpus and Swanborg<sup>78</sup>. Furthermore administration of TGF- $\beta$  protects in acute and relapsing EAE<sup>79,80</sup> and prostaglandin inhibitors such as indomethacin augment EAE<sup>81</sup>. In-addition during the natural recovery phase from EAE TGF- $\beta$  secreting T cells can inhibit EAE effector cells and TGF- $\beta$  is expressed in the CNS<sup>78,82,83</sup>. Consistent with these findings the protective effect of GLA-rich oil in EAE is linked to increased T cell TGF- $\beta$  transcription and increased production of PGE<sub>2</sub><sup>76</sup>.

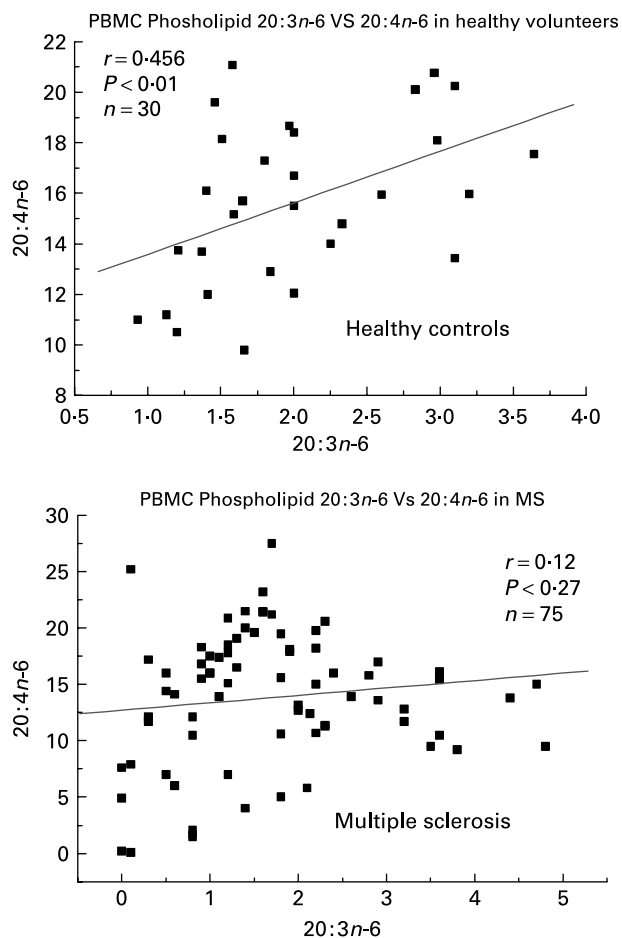


Fig. 4. Relationship between dihomogamma-linolenic acid (20:3n-6) and arachidonic acid (20:4n-6) in peripheral blood mononuclear cell total phospholipids of healthy controls and multiple sclerosis.

### Clinical trials and intervention studies in multiple sclerosis with fatty acids

Clinical trials to test the efficacy of linoleic acid-rich sunflower oil in MS patients by Miller *et al.*<sup>84</sup> and Bates *et al.*<sup>85</sup> over 2 years showed a reduction in the relapse rate and severity of the disease relapse, but Paty *et al.*<sup>86</sup> found no such effect. Nevertheless, Dworkin *et al.*<sup>87</sup> in a statistical reevaluation of the combined data of all three trials revealed significantly reduced relapse rate and severity, and in mildly affected a decrease in the long term progression of the disease. The Millar *et al.*<sup>84</sup> study based in two centres London and Belfast is particularly interesting as they observed that "the severity of the relapses, differed markedly between the treated and the control groups at both centres" relapses being twice as severe in the control group. Compared with current  $\beta$ -interferon treatment of MS the efficacy of linoleic acid-rich sunflower oil in the Miller *et al.* study is quite remarkable. Fish oil rich in long chain  $n$ -3 fatty acids has also been studied in MS<sup>88</sup>, no significant differences between fish oil treated and untreated MS patients was observed, there was, however, a trend for less deterioration in the fish oil treated group. In a 2 year open intervention study MS patients given fish oil and advised to lower their saturated fat intake had significant

reductions in the relapse rate and disability progression as measured by the Expanded Disability Status Scale (EDSS)<sup>69</sup> which quantifies disability in MS in eight functional systems (pyramidal, cerebellar, brainstem, sensory, bowel and bladder, visual, cerebral, other). It also appears based on open studies by us and by Roy Swank that long term low saturated fat diets containing both  $n$ -6 and  $n$ -3 fatty acids improve the course of the disease<sup>89-93</sup>.

Based on our MS fatty acid metabolic data and experimental animal model work, described above, we undertook a randomised double-blind placebo controlled trial to determine the effects of supplementation with a selected GLA (18:3n-6)-rich borage oil. This oil, BGC20-884 was high in sn-2 GLA, low in monoenes and contained only natural levels of vitamin E. This study evaluated two doses of BGC20-884 (low dose - 5 gram and high dose - 14 gram per day) and a placebo control (polyethylene glycol 400) on the clinical course and PBMC cytokine and membrane fatty acid profiles of 36 patients with active MS over 18 months<sup>94</sup>. Patients were diagnosed and assessed using international criteria for MS. Relapse rate and EDSS (Expanded Disability Status Scale) were assessed every three months and blood taken and PBMCs isolated for cytokine studies and membrane fatty acids. High dose BGC20-884 treatment markedly and significantly reduced the relapse rate (Fig. 5) and disability progression as measured by EDSS (Fig. 6) compared with the placebo control and low dose BGC20-884 treatment. In patients where we had follow up samples available PBMC cytokine changes were found to run parallel with the clinical findings e.g. the placebo control group showed significant decreases in the TGF- $\beta$ /TNF- $\alpha$  and TGF- $\beta$ /IL-1 $\beta$  ratios and associated loss of  $n$ -6 fatty acids particularly linoleic (18:2n-6) and arachidonic acid (20:4n-6) over time. Consistent with our findings Navarro and Segura<sup>59</sup> also found significant loss of linoleic and arachidonic acids over time in MS erythrocyte phospholipids. In contrast high dose BGC20-884 treatment showed no changes in TGF- $\beta$ /TNF- $\alpha$  and TGF- $\beta$ /IL-1 $\beta$  ratios and no changes in membrane  $n$ -6 fatty acids compared with the placebo group. We also found positive correlations between PBMC phospholipid arachidonic acid composition and TGF- $\beta$ 1 production ( $r = 0.26$ ,  $P < 0.02$ ,  $n = 73$ ) and DGLA and TGF- $\beta$ 1 production ( $r = 0.36$ ,  $P < 0.001$ ,  $n = 74$ ) *ex vivo* when all samples were included in the analysis. The EDSS improvement in the high dose group

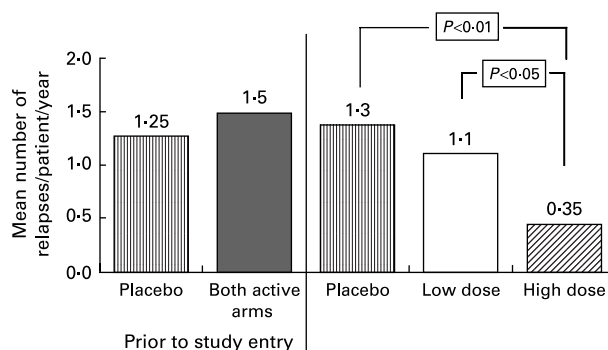
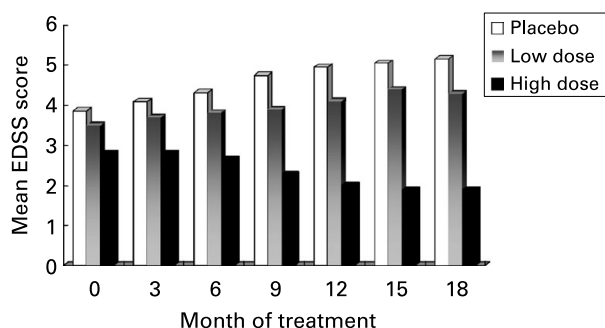


Fig. 5. Mean annualised relapse rate per patient for multiple sclerosis patients receiving high ( $n = 11$ ) and low dose ( $n = 7$ ) GLA-rich oil or placebo control ( $n = 10$ ) over 18 months.



**Fig. 6.** Disability progression as measured by the EDSS (Expanded Disability Status Scale) in multiple sclerosis patients receiving high ( $n = 11$ ) and low dose ( $n = 7$ ) GLA-rich oil or placebo control ( $n = 10$ ) over 18 months.

also suggests there maybe a beneficial effect on neuronal lipids and neural function in MS. The study thus further supports our hypothesis of dysregulation of fatty acid metabolism and cytokines in MS<sup>25,76</sup>.

To summarise and extend this section on clinical trials there is evidence to show a beneficial effect of  $n-6$  and possibly  $n-3$  fatty acids in MS. The mechanisms by which the  $n-3$  or the  $n-6$  fatty acids influence the immune-inflammatory response in MS are however likely to be different<sup>72</sup>. Both the  $n-6$  (arachidonic acid) and  $n-3$  (docosahexaenoic acid) fatty acids are important for neural structure and function<sup>95–101</sup> and this aspect may explain studies where improvements in EDSS have also been reported. Furthermore requirements for essential polyunsaturated fatty acids increase as a function of the amount of saturated fat in the diet<sup>102</sup> and we have recently found significant positive correlations between dietary total saturated and total monounsaturated fatty acids and delta-6 and delta-5 desaturase gene expression in human PBMC<sup>103</sup>. The level of dietary saturated and monounsaturated fatty acids should not therefore be ignored and may be important factors in some of the trials discussed above and relate to the MS epidemiological correlations, mentioned earlier in this overview, in relation to an increased requirement for polyunsaturated fatty acids in MS.

### Conclusions and perspectives

Taken overall the epidemiological, biochemical, experimental animal model and clinical trial data described in this overview show that polyunsaturated fatty acids, particularly the  $n-6$  fatty acids, do have a role in the pathogenesis and treatment of multiple sclerosis. We have demonstrated dysregulation of  $n-6$  fatty acid metabolism and cytokines in MS and have been able to show in a small “proof of concept” clinical trial a marked therapeutic benefit. Thus we suggest that dysregulation of  $n-6$  fatty acid metabolism and cytokines is one mechanism that is important in disease progression, which is modifiable by specific supplementation. Thus metabolic disturbance of the production of the long chain  $n-6$  fatty acids DGLA and AA affects the physiological integrity of immune cells, in that they have a limited ability to produce TGF- $\beta$ , under relapse conditions, which is important for the regulation of pro-inflammatory cytokine production e.g. TNF- $\alpha$ , IL-1 $\beta$ , IFN- $\gamma$  as well as other cellular biological functions. It is also known that TGF- $\beta$  and fatty acids such

as arachidonic acid are important in the growth and differentiation of oligodendrocytes and in myelination<sup>104–108</sup> which would therefore be of importance in the stimulation, growth and recovery of these cells in MS. These findings provide a link between, dietary, metabolic, immunological and neurobiological aspects of MS and therefore for the first time we can begin to make sense of the wealth of apparently unconnected aspects of MS, particularly in relation to dietary fats. More basic research is still required such as characterisation of possible desaturase gene polymorphisms, lymphocyte desaturase gene expression and analysis of specific lymphocyte phospholipid classes and their fatty acid composition in relation to cytokine and chemokine gene expression and production. This should be undertaken in well defined MS patient groups e.g. active MS and primary and secondary chronic progressive forms of the disease and over an extended period of time. Furthermore large well controlled clinical trials with different doses of well characterised and safe fatty acid formulations as well as manipulation of dietary saturated fatty acids could be undertaken. Clinical trials should include MRI, MR spectroscopy and analysis of lesion burden and cortical gray matter density in order to investigate any possible effects of polyunsaturated fatty acids on myelination, neuronal, dendritic, glial and neurite packing densities. In addition biochemical monitoring of peripheral blood cell membrane phospholipid fatty acids, particularly lymphocytes, should be undertaken as well as immunological studies such as T-cell and macrophage pro- and anti-inflammatory cytokine gene expression and production, T regulatory cells and anti-myelin antibodies. In this way a more complete picture will emerge of the clinical and therapeutic significance and the metabolic, immunological and neurological bases to the role of polyunsaturated fatty acids in the pathogenesis and treatment of MS.

### Conflict of interest statement

BGC20-884 and related intellectual property are patented by BTG International Ltd with LSH and MKS as named inventors. LH and MKS co-wrote the manuscript. At the time of the trial there were no conflicts of interests. Subsequently to the trial findings BGC20-884 and related intellectual property is now the subject of patents held by BTG International Ltd with LSH and MKS as named inventors. LH wrote the text and MKS was the lead trial neurologist.

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