Levels of linoleate and arachidonate in red blood cells of healthy individuals and patients with multiple sclerosis

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SUMMARY The major fatty acids were measured in total lipid extracts of red blood cells from 23 control subjects and 31 patients with multiple sclerosis. In the healthy control subjects an inverse correlation ($r = -0.83$) was found between the percentages of linoleate and arachidonate. In the patients such an inverse correlation was not found. The results suggest an abnormality in the red blood cells of patients with multiple sclerosis specifically with regard to the regulation of the relative amounts of unsaturated fatty acids, and this has implications for the possible treatment of multiple sclerosis with dietary supplements.

Abnormalities in the red blood cells of patients with multiple sclerosis have been reported on a number of occasions. The mean erythrocyte diameter has been found to be increased;^{1,2} the cells show greater osmotic fragility than normal;^{3–5} and the levels of linoleate in red blood cell phospholipids have been found to be lower in patients than in healthy control subjects.⁶ It has also been reported that erythrocyte glutathione peroxidase levels are low in multiple sclerosis.⁷

We have recently performed fatty acid analyses on red blood cell total lipids in a group of 31 patients and 23 control subjects. These analyses were carried out as a routine assay in support of another investigation. At the conclusion of the work, however, the results from the red cell lipid analyses were scrutinised, and the interesting finding emerged that, whereas in the normal subjects a highly significant inverse correlation was apparent between the linoleate and arachidonate levels, such a correlation could not be found in the cells from the patients.

Methods

The patients were chosen randomly from those attending the outpatient clinic or from the wards. They were not selected for stage of disease. A number were known to be taking sunflower seed oil, but in view of the publicity which has been given to the subject of dietary polyunsaturated fatty acids it is possible that others (both patients and healthy control subjects) were also supplementing their diet with unsaturated fat. Other drugs, particularly ACTH and dantrolene sodium, were prescribed for some of our patients, but with no obvious correlation with the results. The patients taking these two drugs are indicated on the scatter diagram in fig 1b. The control subjects were healthy individuals from the Middlesex Hospital Medical School, having a similar sex distribution and age range as the patients.

PREPARATION OF RED BLOOD CELLS

Venous blood was drawn into heparin, and the sedimented red cells were washed three times with 0.9% saline, the buffy layer being removed in the early washes.

LIPID EXTRACTION

Packed red cells (2 ml) were pipetted into 10 ml methanol and, after mixing, 20 ml chloroform

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Accepted 5 July 1979

Fig 1 Scatter diagrams for linoleate versus arachidonate in (a) control subjects and (b) patients. ■ patients on ACTH. ● patients on dantrolene sodium. ● other patients. Unit for both axes is per cent of major fatty acids.

	A Control subjects	B All patients	C Patients with linoleate levels 18% or less
Correlation coefficient	-0.831	-0.271	0.065
Regression coefficient	-1.585	-0.230	0.078
Residual variances	2.61	8.66	7.87
Comparison of variances	AvB F = 3.32		AvC F = 3.019
Comparison of regression coefficients	AvB t = 7.28		AvC t = 32.5

was added, followed by 6 ml 0.1M KCl.⁸ The chloroform layer was taken to dryness under oxygen-free nitrogen. The lipid extract was saponified, and fatty acid analyses carried out as previously described.⁹

PHOSPHOLIPID CLASS ANALYSIS

A part of the lipid extract was applied to thin-layer plates and fractionated according to the method of Skipiski *et al.*¹⁰ The spots were located by staining with iodine, scraped from the plate, and analysed for phosphorus essentially by the method of Rouser *et al.*¹¹

Results

The means of the percentage compositions of the fatty acids of the patients and controls are shown in table 1. These show little difference in linoleate level between the two groups. We have shown, however, that if the diet is supplemented with linoleate the difference previously found between patients and control subjects in the serum linoleate level is no longer found.⁹ Moreover, the level of linoleate in red cells reflects the linoleate content of the diet.⁹ These results therefore suggest that increased dietary levels of linoleate have brought the levels of red cell linoleate in the patients up to those found in the control subjects.

Figure 1 shows scatter diagrams of the individual values of linoleate plotted against those of arachidonate for each of the two groups. It is immediately apparent that an inverse correlation ($r = -0.831$) exists between the values for these two acids in the normal subjects, while in the patients we observed a much lower correlation ($r = -0.271$) which could be the result of sampling errors and is not significantly different from zero. Further statistical analysis reveals that the

Table 1 Erythrocyte total lipid fatty acid patterns in control subjects and patients

	Palmitate	Palmitoleate	Stearate	Oleate	Linoleate	Arachidonate
Control subjects	25.9 ± 0.35	1.9 ± 0.16	17.7 ± 0.59	19.0 ± 0.28	15.3 ± 0.31	20.5 ± 0.59
Patients	26.5 ± 0.30	1.8 ± 0.13	16.5 ± 0.42	19.3 ± 0.37	15.8 ± 0.58	19.6 ± 0.50

Values expressed as % (w/w) of total fatty acids determined ± s.e.m.

difference between the two groups is highly significant, both with regard to the variances of the deviations of the observed values from the slopes ($p < 0.005$) and also with regard to the regression coefficients ($p < 0.001$). We have similarly performed statistical analyses for other combinations of pairs of acids. In only one case, that of palmitate versus stearate, was there an inverse (or any) correlation ($r = -0.434$ for controls and $r = -0.283$ for patients).

Figure 1b shows that a number of patients have linoleate levels below the lower limit of the range of the control subjects (13%). In most of these patients with low linoleate levels, the arachidonate level is low by comparison with the value appropriate to the correlation shown in fig 1a, whereas a number of the patients with high linoleate levels have arachidonate levels inappropriately high by this correlation. These high values originate in patients taking sunflower seed oil for long periods. In studies with control subjects who took sunflower seed oil for five days the level of linoleate increased, but did not exceed 18%.⁶ It is not known how ingestion of sunflower seed oil over longer periods affects the correlation in normal subjects. However, the statistical significance of the results is not affected by the high levels in the patients, and if the analysis is carried out using only those patients whose linoleate level does not exceed 18%, the difference between the two groups shows the same significance ($p < 0.001$ for regression coefficients) as for the overall groups of subjects.

It is known that in the red blood cell lipids, phosphatidyl choline contains a relatively high proportion of linoleate, while phosphatidylethanolamine contains relatively more arachidonate.¹³ One possible explanation of the difference found between control subjects and patients could therefore be that the proportions of these two phospholipids were altered in the patients. However, phospholipid class analysis proved that there was no difference between control subjects and patients in this respect (table 2).

Table 2 Red blood cell phospholipid class analysis

	Control subjects (16)	Patients (22)
Phosphatidylcholine	30.16 $\pm$ 0.62	30.77 $\pm$ 0.67
Phosphatidylethanolamine	29.55 $\pm$ 0.61	27.94 $\pm$ 0.64
Phosphatidylserine plus phosphatidylinositol	10.50 $\pm$ 0.62	9.51 $\pm$ 0.90
Sphingomyelin	28.56 $\pm$ 0.46	29.53 $\pm$ 0.90
Lysolecithin	1.47 $\pm$ 0.38	2.20 $\pm$ 0.43

Results are expressed as a percentage of the total phospholipid phosphorus. Total phospholipid phosphorus was within the same range for the two groups.

Discussion

The inverse correlation which we have found in healthy subjects between red cell linoleate and arachidonate appears not to have been commented on previously, possibly because it emerges clearly only when an appreciable number of samples has been studied. Our levels of linoleate and arachidonate are somewhat higher than those previously reported¹³ because this study has concerned only the acids with chain lengths up to 20 carbons. This would not, however, affect the significance of correlation observed. The results in many of the patients studied bear out our earlier finding⁶ of a deficiency of polyunsaturated fatty acid (linoleate) in the red cells of patients with multiple sclerosis. Neither in our previous results nor in the present work does the mean arachidonate level differ significantly between the patients and control subjects. However, the present results show that, in a number of patients with low linoleate levels, the arachidonate level is lower than would appear to be appropriate from the correlation found in the healthy control subjects, suggesting that in these patients there is a relative deficiency of both these unsaturated fatty acids. Examination of the results from our earlier study⁶ appears to confirm this observation, but it is less apparent because of the smaller number of patients studied.

We have previously commented that in serum an inverse correlation exists between the percentages of linoleate and oleate.⁹ The levels of arachidonate in normal serum are low, and it does not seem that the correlation between linoleate and arachidonate in the normal red cell would arise simply from the relative amounts of these acids in the plasma. Indeed, much of the arachidonate in the red cell is found in the acidic phospholipids, phosphatidylserine and phosphatidylethanolamine,¹³ of which the levels are low in the serum lipoproteins.¹⁴ Thus, it does not appear that the difference between control and patient red cells arises simply from the low linoleate levels previously found in the plasma lipids, suggested by some to be merely a characteristic of severe illness (for review, see Smith and Thompson¹⁵). Indeed, in most disease states so far studied the red cell lipids have been reported to be normal.¹³ Moreover such an explanation would not apply to those patients with high linoleate levels and high arachidonate levels (as judged by the correlation shown in fig 1a and 1b).

In an earlier study, Farquhar and Ahrens¹⁶ found that, in hypercholesterolaemic patients

placed on liquid diets supplemented with corn oil emulsions, the red cell linoleate level rose at the expense of oleate. It is of interest that Van Gastel *et al*¹⁷ found an inverse relationship between linoleate and arachidonate when young and old red cells were compared, the younger red cells having lower linoleate and higher arachidonate than older cells. It seems unlikely that the proportions of young and old cells would vary in normal individuals sufficiently to explain the range of values shown in fig 1a, but the findings of Van Gastel *et al*¹⁷ show that there may be a mechanism in the red cell regulating linoleate and arachidonate levels in an inverse manner. It is also unlikely that those patients with low linoleate levels have an undue proportion of young cells, as then it would be expected that they would have correspondingly higher arachidonate levels.

Fig 2 Scatter diagrams for linoleate versus oleate in (a) control subjects and (b) patients. Correlation coefficients: for control subjects 0.096, for patients -0.314 . Unit for both axes is per cent of major fatty acids.

With regard to those patients with high levels of both linoleate and arachidonate, it could be argued that these are individuals who have been taking large doses of sunflower seed oil and thus, according to the results of Farquhar and Ahrens,¹⁸ the inverse correlation between linoleate and arachidonate would not be expected; rather should there be an inverse correlation between linoleate and oleate. Inspection of the scatter diagrams for linoleate versus oleate (fig 2) reveals, however, that neither for the control subjects nor for the patients is there an inverse correlation between these two acids, a finding that does not agree with the results of Farquhar and Ahrens. For the present we are unable to rationalise this discrepancy, and an explanation will be sought in further work.

Our present results suggest that there could be an abnormality in the red cells of patients with multiple sclerosis specifically with regard to the regulation of the relative amounts of unsaturated fatty acids. The findings are relevant to the possible treatment of multiple sclerosis with dietary supplements. Further work is needed both to explore the nature of the defect in the red cell, and to determine whether it may extend to other cell types.

We wish to thank the Multiple Sclerosis Society for financial support and Professor RHS Thompson for many helpful discussions and comments on the manuscript. The excellent technical assistance of Dolores Conroy is gratefully acknowledged. The help of Mr GB Newman with the statistical analyses was invaluable.

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