Study of plasma and red cell phospholipid fatty acids in extrahepatic cholestatic jaundice

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Abstract

The poor outcome in patients with extrahepatic cholestatic jaundice seems in some way related to reticuloendothelial dysfunction. Similar dysfunction can be caused by abnormal tissue phospholipid fatty acid patterns. Little is, however, known about such patterns in extrahepatic cholestatic jaundice. The phospholipid fatty acid patterns in 42 controls were compared with 42 patients with extrahepatic cholestatic jaundice. Many abnormalities were found. The general pattern was of a fall in polyunsaturated fatty acids and a rise in monounsaturated fatty acids, with a consequent fall in the double bond index (mean number of double bonds per fatty acid) showing an overall rise in saturation. All three major substrates for eicosanoid production were reduced in the jaundiced group. The changes seemed to be associated with jaundice itself, rather than the cause of the jaundice. The central roles of fatty acids in the determination of membrane function and in the provision of substrates of eicosanoid production, mean that these changes may explain some of the reticuloendothelial dysfunction found in extrahepatic cholestatic jaundice.

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For many years patients with extrahepatic cholestatic jaundice have suffered high surgical complication rates.^{1 2} Although recent reports suggest these may be falling,³ the explanation for the excess morbidity and mortality is unclear. The reticuloendothelial system seems important in these excess complications. Dysfunction of the fixed reticuloendothelial system seems to permit 'spillover' of endotoxins. The most important complications

have been associated with systemic endotoxaemia. Many abnormalities in reticuloendothelial system function have been shown in extrahepatic cholestatic jaundice.⁴⁻⁸

The essential fatty acids are polyunsaturated fatty acids of two families - the n-6 series derived from dietary linoleic acid (18:2n-6) and the n-3 series derived from dietary α linolenic acid (18:3n-3). The important roles of these fatty acids seem to be as the precursors of the oxygenated fatty acids, the eicosanoids, and as structural elements of lipid membranes.⁹ Their influence on membrane function and eicosanoid production, mean that abnormalities in tissue fatty acid patterns are possible causes of the reticuloendothelial system dysfunction that occurs in extrahepatic cholestatic jaundice.

Little is known, however, about tissue fatty acid patterns in extrahepatic cholestatic jaundice and this study aimed at establishing them.

Methods

Forty two patients with extrahepatic cholestatic jaundice (group 2) were compared with 42 matched controls (group 1). Group 1 comprised patients admitted for surgery for benign conditions; none had any history of hepatobiliary disease and all had normal liver function tests. All the jaundiced patients had large duct extrahepatic biliary obstruction. None of the patients in either group had any condition or propensity known to influence fatty acid concentrations such as diabetes mellitus or an atopic tendency.

Venous blood was drawn before operation, in group 1, and before any procedure to relieve jaundice, in group 2. The possible effects of diurnal variation and platelet aggregation on fatty acid values, were avoided by taking blood in the morning with large bore needles, without using a tourniquet. The patients did not fast before venesection. The blood was

TABLE I Study groups

	Sex	Age Median (range)	Weight (kg) Mean (95% CI)	Bilirubin (micromol/l) Mean (range)	ALP (IU/l) Mean (range)
Group 1: controls (n = 42)	23M:19F	63 (37-87)	70.8 (66.6 to 75.1)		
Group 2: jaundiced (n = 42)	23M:19F	69 (22-88)	67.8 (62.9 to 72.8)	247 (39-540)	823 (233-3060)
Group 2 subgroups					
Benign patients (n = 12)	5M:7F	71 (23-88)	66.7 (58.3 to 75.0)	136 (50-492)	625 (233-1480)
Malignant patients (n = 30)	18M:12F	68 (24-85)	68.2 (61.9 to 74.6)	292 (39-540)	902 (353-3060)

Using a two sample Student's *t* test no statistical difference was found between the weights or ages of the two groups. 95% CI - 95% confidence intervals. ALP - alkaline phosphatase (normal range 40-140 IU/l). Normal range for plasma bilirubin 5-17 micromol/l. None of the patients in group 1 had abnormal bilirubin or alkaline phosphatase values.

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TABLE II Results for plasma phospholipids

	Group 1: controls (n = 42)		Group 2: jaundiced (n = 42)		p	Ratio of jaundice: control
	Mean	95% Confidence intervals	Mean	95% Confidence intervals		
16:0	28.58	(27.69 to 29.47)	31.60	(30.75 to 32.44)	<0.001	1.11
18:0	9.92	(9.20 to 10.65)	8.35	(7.49 to 9.22)	<0.010	0.84
18:1n-9	12.32	(11.85 to 12.79)	16.47	(15.61 to 17.34)	<0.001	1.34
18:2n-6	24.62	(23.44 to 12.79)	21.74	(20.62 to 22.86)	<0.001	0.88
20:2n-6	0.26	(0.18 to 0.35)	0.21	(0.15 to 0.27)	NS	0.80
20:3n-6	3.09	(2.83 to 3.35)	2.01	(1.78 to 2.24)	<0.001	0.65
20:4n-6	10.63	(9.85 to 11.41)	8.99	(8.19 to 9.78)	<0.005	0.85
22:4n-6	0.23	(0.16 to 0.30)	0.17	(0.10 to 0.23)	NS	0.71
20:5n-3	1.60	(1.16 to 2.04)	0.84	(0.70 to 0.97)	<0.001	0.52
22:5n-3	0.99	(0.87 to 1.11)	0.57	(0.47 to 0.67)	<0.001	0.58
22:6n-3	4.84	(4.31 to 5.37)	4.07	(3.70 to 0.45)	0.020	0.84
SFA	38.86	(37.66 to 40.05)	40.32	(39.29 to 41.36)	NS	1.04
MUFA	13.51	(13.02 to 14.01)	19.31	(18.20 to 20.42)	<0.001	1.43
PUFA	46.90	(45.48 to 48.32)	39.45	(37.76 to 41.14)	<0.001	0.84
DBI	1.60	(1.54 to 1.66)	1.40	(1.35 to 1.46)	<0.001	0.88
Total (n-6)	39.03	(37.73 to 40.33)	33.34	(31.91 to 34.76)	<0.001	0.85
Total (n-3)	7.83	(6.90 to 8.77)	6.09	(5.56 to 6.61)	<0.005	0.78
18-carbon-PUFA	25.07	(23.90 to 26.25)	22.38	(21.24 to 23.52)	<0.001	0.89
20-carbon-PUFA	15.62	(14.60 to 16.65)	12.07	(11.10 to 13.05)	<0.001	0.77
22-carbon-PUFA	7.19	(6.55 to 7.83)	5.56	(5.03 to 6.09)	<0.001	0.77

All results shown are molar percentages, except double bond index (DBI), which has no units. The values of p result from two sample Student's *t* tests between groups 1 and 2. The ratios shown are the ratios of the means for group 2 to those for group 1. SFA = saturated fatty acids, MUFA = monounsaturated fatty acids, PUFA = polyunsaturated fatty acids, NS = *p* > 0.05.

separated by centrifugation, into plasma and red cells, and stored at -70°C until analysis. Under these conditions such samples are stable for many months (unpublished data).

Analysis was done in a standard fashion for the plasma and the red cells. Lipid was extracted, and the phospholipid fraction separated and extracted by thin layer chromatography. The fatty acids within the phospholipid fraction were methylated and assayed by fully automated packed column gas liquid chromatography. All these methods have been described previously.¹⁰ The results, expressed as molar percentages,⁹ were transferred to a microcomputer, on which data manipulation and analysis were done.

A number of variables, calculated from the fatty acid results, can be used to help with the interpretation of the fatty acid states.⁹ These were calculated from the results and analysed statistically. The double bond index is the mean number of double bonds per fatty acid. The other variables are totals for various groups of fatty acids, for instance total n-6 polyunsaturated fatty acids and

total 20-carbon polyunsaturated fatty acids. It should be noted that in humans, there are negligible quantities of non-n-6, non-n-3 polyunsaturated fatty acids in these fractions. Essential fatty acids and polyunsaturated fatty acids are thus, in effect, equivalent.

Two sample Student *t* tests were used to compare the two groups, producing a large number of comparisons. This is the only satisfactory way of comparing fatty acid data. Fatty acid states cannot be measured by a single variable – many variables must be considered together. To our knowledge, no simple method around this problem has been devised.

Results

In each group there were 23 men and 19 women. The groups were well matched for age and weight (see Table I). Group 2 consisted of 12 patients with gall stones and 30 with tumour compressing the extrahepatic biliary tree. Six of these tumours were metastatic –

TABLE III Results for red cell phospholipids

	Group 1: controls (n = 42)		Group 2: jaundiced (n = 42)		p	Ratio of jaundice: control
	Mean	95% Confidence intervals	Mean	95% Confidence intervals		
16:0	28.03	(26.05 to 30.00)	36.70	(34.26 to 39.14)	<0.001	1.31
18:0	12.85	(11.80 to 13.90)	11.02	(10.21 to 11.83)	<0.010	0.86
18:1n-9	19.36	(18.13 to 20.58)	20.95	(19.78 to 22.11)	NS	1.08
18:2n-6	12.00	(11.06 to 12.94)	11.69	(10.54 to 12.84)	NS	0.97
20:2n-6	0.43	(0.28 to 0.59)	0.57	(0.42 to 0.71)	NS	1.32
20:3n-6	1.35	(1.16 to 1.54)	0.86	(0.69 to 1.04)	<0.001	0.64
20:4n-6	12.41	(10.64 to 14.17)	7.18	(5.63 to 8.73)	<0.001	0.58
22:4n-6	1.66	(1.40 to 1.92)	0.91	(0.68 to 1.15)	<0.001	0.55
20:5n-3	0.98	(0.71 to 1.26)	0.47	(0.30 to 0.64)	<0.002	0.48
22:5n-3	1.74	(1.40 to 2.07)	0.85	(0.55 to 1.14)	<0.001	0.49
22:6n-3	4.91	(3.87 to 5.94)	2.47	(1.67 to 3.27)	<0.001	0.50
SFA	41.29	(38.71 to 43.87)	48.40	(45.81 to 50.99)	<0.001	1.17
MUFA	20.32	(19.06 to 21.59)	23.73	(22.38 to 25.08)	<0.001	1.17
PUFA	36.41	(32.47 to 40.35)	25.81	(22.16 to 29.46)	<0.001	0.71
DBI	1.52	(1.36 to 1.68)	1.08	(0.94 to 1.22)	<0.001	0.71
Total (n-6)	28.33	(25.69 to 30.96)	21.53	(18.98 to 24.08)	<0.001	0.76
Total (n-3)	8.07	(6.51 to 9.62)	4.27	(3.02 to 5.51)	<0.001	0.53
18-carbon-PUFA	12.44	(11.53 to 13.35)	12.19	(11.07 to 13.30)	NS	0.98
20-carbon-PUFA	15.19	(13.14 to 17.23)	9.10	(7.33 to 10.86)	<0.001	0.60
22-carbon-PUFA	10.52	(8.75 to 12.29)	5.37	(3.80 to 6.95)	<0.001	0.51

Footnotes and abbreviations as in Table II.

Figure 1: Ratios of mean results from group 2 against group 1 plasma phospholipid fatty acids. The ratios shown are from Tables I and II. They are the ratio of the means for the jaundiced patients (group 2) to the controls (group 1). Tables I and II show the degree of statistical significance of these changes. SFA = saturated fatty acids, MUFA = monounsaturated fatty acids, PUFA = polyunsaturated fatty acids.

from stomach (n=2), breast (n=1), oesophagus (n=1), rectum (n=1), and from an unknown primary (n=1). The site of the primary tumours, in the other 24 patients in group 2, were pancreas (n=13), bile duct (n=8), ampulla (n=2), and gall bladder (n=1). Table I shows the liver function tests for the jaundiced patients. As stated previously, the liver function tests of the patients in group 1 were normal.

Tables II and III show the results as molar percentages, except the double bond index, which has no units. There are many highly significant abnormalities, and the general pattern of these can be seen graphically in Figures 1 and 2, where the ratios of group 2 and group 1 are shown.

Separate, and similar, statistical analysis was done comparing the group 2 patients with malignant disease and those with benign disease. No statistically significant differences were found (analysis not shown).

Figure 2: Ratios of mean results for group 2 against group 1 red cell phospholipid fatty acids. The ratios shown are from Tables I and II. They are the ratio of the means for the jaundiced patients (group 2) to the controls (group 1). Tables I and II show the degree of statistical significance of these changes. Abbreviations as in Figure 1.

Discussion

Fatty acids are important for their influence on membrane function and as substrates for eicosanoid production. In each case the phospholipid fraction is crucial. Plasma and red cell phospholipid fatty acids, are known to reflect tissue levels¹¹ and are much less susceptible to short term dietary changes than, for instance, unesterified or triglyceride fatty acids. Despite their importance, it has been difficult to establish normal values, and different groups have reported different results.^{12 13} This may be because of geographical differences or possibly because of longterm dietary customs.¹⁴ Studies must thus use comparisons with local controls.

The shift in the pattern of fatty acids from polyunsaturated to more saturated ones, in the patients with jaundice, occurred in both red cell and plasma phospholipid. The low double bond index characterises this change. Such changes, if reflected in other tissues, are known to affect membrane fluidity, and thus physical behaviour and enzyme function.¹⁵ The more noticeable fall in longer polyunsaturated fatty acids – that is, 22-carbon – may further affect function by changing membrane thickness.

The eicosanoids are oxygenated polyunsaturated fatty acid metabolites, and include prostanoids and leukotrienes. The three important 20-carbon precursors – arachidonic acid (20:4n-6), dihomo γ linolenic acid (20:3n-6), and eicosapentaenoic acid (20:5n-3) – were all lower in the jaundiced group. Thus total 20-carbon polyunsaturated fatty acids were also lower.

Cells of the reticuloendothelial system are particularly sensitive to changes in membrane phospholipid fatty acid composition. Thus phagocytic function,^{16 17} cytotoxicity,¹⁸ and eicosanoid production¹⁹ are all influenced by changes in membrane fatty acid patterns. Furthermore, the cells of this system are the most important source of the powerful eicosanoids. Extrahepatic cholestatic jaundice has profound effects on reticuloendothelial system function, affecting clearance of carbon particles,⁴ colloid,⁶ bacteria,⁵ and micro-aggregated albumin,⁷ as well as monokine production.⁸ Similar changes occur when membrane phospholipid fatty acids become more saturated. The rise in fatty acid saturation that has been shown in this study, may thus explain some of the reticuloendothelial system dysfunction in extrahepatic cholestatic jaundice.

The absence of any significant differences between the malignant and benign jaundiced patients suggests that the effect results from biliary obstruction rather than the cause of the extrahepatic cholestatic jaundice.

The aetiology of these changes was not investigated in this study. Possibilities, however, must include a change in the function of hepatic fatty acid desaturase enzymes, and malabsorption. The desaturase enzymes are sensitive to many factors and seem to be the rate limiting steps in the metabolism of 18-carbon polyunsaturated fatty acids, through to longer more unsaturated fatty acids.²⁰ Changes

in diet only effect phospholipid fatty acids if dietary fat is severely restricted over a prolonged period. In fact, stores of polyunsaturated fatty acids in adipose tissue are very large compared with daily requirements.²¹ A further possible explanation of the changes is the damaging effect of high tissue bile acid concentrations on membrane phospholipid.²²

Considerable abnormalities in phospholipid fatty acids have been shown in extrahepatic cholestatic jaundice. They seem to result from biliary obstruction itself, and may explain some of the abnormalities in the reticuloendothelial system function known to occur in jaundice. Correction of these fatty acid abnormalities, provides a novel way of trying to improve the outcome of these patients. Further evaluation of this approach should be undertaken.

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