# Polyunsaturated fatty acids in plasma, red blood cells and mononuclear cell phospholipids of patients with atopic dermatitis

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Recent studies of polyunsaturated fatty acids (PUFA) in plasma and blood cell components of patients with atopic diseases have indicated disordered fat metabolism as linoleic acid (18:2n-6) tends to be increased while the more unsaturated fatty acids, such as gamma-linolenic acid (18:3n-6) and arachidonic acid (20:4n-6) are present in decreased amounts. To further clarify these abnormalities, we examined the PUFA content in phospholipids derived from plasma, red blood cells and mononuclear cells (MNC) in patients with atopic dermatitis. In plasma no significant differences were found between patients and controls. In red blood cells dihomo-gamma-linolenic acid (20:3n-6) was reduced in eczema patients, as compared with controls. The most significant findings in eczema patient MNC were reduced ratios of 20:4n-6/20:3n-6 in total lipids and in phosphatidyl ethanolamine (PE) and of 20:4n-6/18:2n-6 in both total lipids, phosphatidyl choline (PC) and PE. These findings indicate a disordered fatty acid metabolism in MNC of patients with atopic dermatitis. It is possible that these changes in the fatty acid profile of MNC may account for some of the immunological abnormalities seen in these patients.

Key words: atopic dermatitis; polyunsaturated fatty acids; mononuclear cells; phospholipids.

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In the 1930s and 1940s several papers reported that polyunsaturated fatty acid (PUFA) levels were low in the blood of patients with atopic dermatitis and that dietary supplementation with PUFA was of therapeutic value (6, 9, 12, 13).

Recent trials with large oral doses of evening primrose oil, which contains high levels of PUFA and is unique in containing gamma-linolenic acid (18:3n-6, GLA), administered to patients with atopic dermatitis (AD) have shown therapeutic effect in most cases (2, 19, 26, 28, 29). Analyses of PUFA in plasma and in blood cell phospholipids of patients with AD have indicated defects in the activity of desaturase enzymes, as linoleic acid (18:2n-6) tends to be increased, while the more unsaturated fatty acids, such as GLA and arachidonic acid (AA) are present in decreased amounts (17). To further clarify these abnormalities in atopics, we have examined the PUFA content in phospholipids derived from plasma, erythrocytes and mononuclear cells (MNC) in patients with atopic dermatitis.

## Material and methods

The study comprised 13 patients aged 20–47 years (median 23) with severe atopic dermatitis, as defined by Hanifen & Rajka (11), and 12 age- and sex-matched healthy non-atopic controls. At the time of investigation the eczema was in an active state and the patients were treated with topical steroids of various potencies. No patient had received systemic medication within 4 weeks prior to the study. Blood was drawn into EDTA-treated tubes, separated within 1 h of collection and stored at $-20\,^{\circ}C$ until analysis.

Quantitative determination of fatty acids of plasma and red blood cell phospholipids was performed as described by Manku et al. (17). Briefly, plasma samples were extracted with chloroform:methanol (2:1), filtered through sodium sulfate and evaporated to dryness under nitrogen before taken up in 0.5 ml chloroform. The lipid fractions were separated by thin-layer chromatography on silica gel

plates, and the phospholipid fraction was methylated with borontrifluoride-methanol. Methyl esters of the fatty acids were then separated by gas-chromatography under the conditions reported previously (17). Red blood cells were washed and lipids extracted according to the procedure of Dodge & Phillips (8). Total phospholipids were isolated by TLC (17). The phospholipid band was scraped off immediately and analysed for fatty acids as described for plasma.

To investigate phospholipid fatty acids in mononuclear cells (MNC) 50 ml blood was drawn into an equal amount of Hanks' balanced salt solution. MNC were isolated by gradient centrifugation in Ficoll-Hypaque (Pharmacia). The cell pellets were stored at −40°C until analysis. Total membrane lipids were isolated from the thawed cell pellet by 3 consecutive extractions with 4 ml of chloroform:methanol (2:1). The extracts, which were obtained after centrifugation at 1900 *g* for 10 min, were combined and washed with 0.9% sodium chloride. After recentrifugation, under the same conditions as above, the lower chloroform layer containing the total lipids, was isolated, evaporated to dryness and redissolved in 200 µl of chloroform:methanol (2:1) for further analysis. Verification by TLC showed only the presence of cholesterol and phospholipids, as would be expected for membrane lipids. The subfractionation of phospholipids was performed on TLC (Merck silica gel 60 Art. no. 5721) using chloroform:methanol:hexane:acetic acid (4:2:3:1 by volume) containing 1.8% boric acid as solvent system. Visualization was made by spraying with 0.2% ethanolic dichlorofluoresceine and identification by comparison with appropriate standards. Fatty acid methyl esters of phosphatidylcholine, phosphatidylethanolamine and of total lipids were prepared with boron-trifluoride and quantitated by GLC on SP 2330 as reported previously (14).

The Mann-Whitney rank sum test was used for statistical analysis of the data.

## Results

The fatty acid analysis of erythrocyte and plasma phospholipids is shown in Table 1. In plasma, no significant differences were found between patients and controls. In erythrocytes, dihomo-gamma-linolenic acid (20:3n-6) was reduced in eczema patients compared with controls.

Table 2 shows the fatty acid analysis of MNC. Total phospholipids, as well as the 2 major phospholipid classes, phosphatidylcholine (PC) and phosphatidylethanolamine (PE), were examined. In total, phospholipids, arachidonic acid (20:4n-6) were reduced in eczema patients. In the PC fraction, the deposition of both n-6 and n-3 fatty acids was equal for the 2 groups, whereas oleic acid (18:1n-9) and the main dietary essential fatty acid, linoleic acid (18:2n-6), were higher in the PE fraction from atopic eczema patients than in controls. Further, atopics showed a tendency to lower arachidonic acid values.

Table 1. Median fatty acid level in the total phospholipids of erythrocytes and plasma from patients with atopic dermatitis and controls

| Fatty acids | Erythrocytes | | Plasma | |
|---|---|---|---|---|
| | Eczema | Control | Eczema | Control |
| n–6 | | | | |
| 18:2 | 13.5 (10.9–17.3) | 13.1 (8.3–17.8) | 27.6 15.6–32.5) | 29.0 (20.9–35.6) |
| 18:3 | 0.1 (0.1–0.1) | 0.1 (0.1–0.1) | – | – |
| 20.3 | * 1.5 (1.1–1.7) | 1.8 (0.7–2.2) | 2.3 (1.5–4.3) | 2.5 (1.3–3.5) |
| 20.4 | 15.2 (7.9–19.0) | 15.7 (5.4–19.3) | 11.4 (9.5–21.5) | 11.7 (10.5–14.9) |
| 22:4 | 2.5 (0.9–3.2) | 2.5 (0.8–3.5) | 0.3 (0.2–0.5) | 0.2 (0.2–0.3) |
| 22:5 | 0.6 (0.1–0.7) | 0.4 (0.2–0.9) | 0.3 (0.1–0.7) | 0.3 (0.3) |
| n–3 | | | | |
| 18:3 | 0.5 (0.2–0.7) | 0.3 (0.3–0.6) | 0.4 (0.3–0.5) | 0.3 (0.1–0.4) |
| 20:5 | 0.7 (0.2–1.2) | 0.9 (0.5–1.7) | 1.1 (0.4–1.6) | 1.4 (0.8–2.3) |
| 22:5 | 1.9 (0.5–2.9) | 2.2 (0.7–2.5) | 0.9 (0.5–1.2) | 1.0 (0.5–1.2) |
| 22:6 | 5.0 (1.2–9.1) | 5.1 (2.7–6.9) | 4.6 (3.1–7.8) | 4.4 (2.6–6.4) |
| Others | | | | |
| 16:0 | 23.8 (19.4–33.9) | 23.1 (20.7–37.8) | 29.8 (26.5–36.2) | 30.2 (29.1–33.9) |
| 18:0 | 13.1 (10.7–17.9) | 14.7 (11.0–16.4) | 7.2 (6.3–12.8) | 6.8 (4.8–8.2) |
| 18:1 | 16.3 (13.9–20.6) | 16.1 (13.8–21.4) | 12.1 (9.8–14.4) | 11.7 (9.2–14.9) |

Results are expressed as median (range) in mg/100 mg total fatty acids present. Results were analysed by Mann–Whitney's rank sum test. Significant differences from control are indicated by * $p < 0.05$.

The ratio of (18:3n-6 + 20:3n-6)/18:2n-6 was equal in patients and controls, while the ratio of 20:4n-6/20:3n-6, was reduced in total phospholipids and in PE of MNC in patients, as compared with controls. The ratio of 20:4n-6/18:2n-6 was highly significantly reduced in both total phospholipids, PC and PE of MNC, as shown in Table 3. Such differences could not be detected in plasma and red blood cells.

## Discussion

In contrast to Manku et al. (17) and Strannegård et al. (27), who found increased levels of linoleic acid and decreased levels of the unsaturated metabolites of this fatty acid as well as the metabolites of the n-3 series, and to Muhlemann (21) who found decreased dihomo-gamma-linolenic acid and arachidonic acid in plasma of patients with AD, we did not detect

Table 2. Median fatty acid level in total phospholilpids, phosphatidylcholine (PC) and phosphatidyl ethanolamine (PE) of mononuclear cells from patients with atopic dermatitis and controls

| Fatty acids | Total phospholipids | | Phosphatidyl choline | | Phosphatidyl ethanolamine | |
|---|---|---|---|---|---|---|
| | Eczema | Control | Eczema | Control | Eczema | Control |
| n–6 | | | | | | |
| 18:2 | 9.5 (3.3–10.7) | 7.7 (1.6–9.4) | 9.6 (8.0–18.9) | 9.0 (6.0–11.9) | ** 3.7 (2.8–5.8) | 2.6 (1.0–4.2) |
| 18:3 | 0.4 (0.2–1.1) | 0.6 (0.2–1.9) | 0.3 (0.3) | 0.4 (0.1–2.2) | 0.2 (0.1–0.4) | 0.6 (0.3–1.0) |
| 20:3 | 1.7 (0.2–2.0) | 1.5 (1.3–2.0) | 1.6 (1.4–2.5) | 1.5 (0.8–2.1) | 1.4 (0.3–1.8) | 1.0 (0.5–2.4) |
| 20:4 | * 14.5 (8.1–16.5) | 17.8 (11.1–20.5) | 12.4 (6.0–20.5) | 12.9 (11.1–16.3) | 20.7 (17.2–24.0) | 23.3 (12.7–26.6) |
| 22:4 | 2.2 (0.7–4.0) | 2.4 (0.7–3.7) | 1.7 (1.3–2.5) | 1.4 (0.7–3.2) | 5.3 (1.9–13.5) | 5.4 (1.2–7.3) |
| n–3 | | | | | | |
| 22:5 | 1.7 (0.6–2.2) | 1.8 (1.1–2.6) | 0.8 (0.5–1.1) | 0.7 (0.2–1.2) | 2.9 (2.3–4.1) | 3.6 (1.6–7.0) |
| 22:6 | 2.2 (1.5–4.0) | 2.2 (1.7–4.1) | 0.9 (0.1–2.6) | 0.9 (0.8–1.7) | 3.8 (2.5–4.9) | 3.6 (2.0–11.2) |
| Others | | | | | | |
| 16:0 | 15.0 (11.2–17.6) | 17.0 (10.9–18.1) | 26.8 (15.7–33.0) | 31.0 (26.2–33.4) | 11.0 (8.7–18.5) | 10.4 (1.1–12.1) |
| 18.0 | 17.6 (11.5–22.9) | 20.4 (16.7–32.4) | 14.9 (7.0–18.4) | 14.8 (13.2–18.6) | 23.2 (15.4–33.8) | 22.5 (13.8–32.8) |
| 18:1 | 18.3 (9.4–26.4) | 15.3 (10.5–18.6) | 18.6 (17.4–22.1) | 19.0 (15.7–21.3) | * 9.9 (7.8–25.2) | 7.5 (4.7–11.6) |

Results are expressed as median (range) in mg/100 mg total fatty acids present. Results were analysed by Mann–Whitney's rank sum test. Significant differences from control are indicated by * $p<0.05$, ** $p<0.02$.

differences in the fatty acid profile in plasma from atopics and corresponding controls. However, in red blood cells, dihomo-gamma-linolenic acid was reduced in eczema patients, as compared with controls, while no change in 20:4(n-6) was observed. In contrast, Rocklin et al. (24) found increased arachidonic acid in red blood cells of patients with atopic rhinitis and/or asthma.

Plasma phospholipids are synthesized in the liver (1, 15), and their fatty acids reflect hepatic metabolism of the dietary fats. Blood cell fatty acids, which are mainly found in the bilayer phospholipid membranes, reflect intracellular metabolism as well as uptake from plasma. The results of the abovementioned studies (17, 21, 27) may be interpreted as defective hepatic desaturase activity in patients with atopic dermatitis. However, we did not find evidence of altered hepatic fat metabolism in our patients, as measured by the PUFA profile in plasma phospholipids. In a recent study Schäfer et al. (25) found elevated levels both of linoleic acid and total n-6 fatty acids in the phospholipids of neutrophils in AD patients. However, they could relate this to increased dietary intake of PUFA. In the present study we did not investigate the dietary habits of the patients or controls.

Table 3. Ratios between highly unsaturated n–6 fatty acids and less unsaturated n–6 fatty acids in total phospholipids, phosphatidyl choline (PC) and phosphatidyl ethanolamine (PE) in mononuclear cells from patients with atopic dermatitis and controls

| Fatty acid ratios | Total phospholipids | | Phosphatidyl choline | | Phosphatidyl ethanolamine | |
|---|---|---|---|---|---|---|
| | Eczema | Control | Eczema | Control | Eczema | Control |
| 18:3+20:3/18:2 | 0.2 (0.1–0.4) | 0.2 (0.2–0.3) | 18.8 (8.5—27.2) | 17.8 (10.9–61.7) | 0.4 (0.3–0.4) | 0.4 (0.4–0.4) |
| 20:4/20:3 | * 7.9 (4.6–9.5) | 11.2 (7.9–15.8) | 7.5 (4.3–13.7) | 9.8 (7.0–13.9) | * 15.7 (11.1–19.8) | 21.5 (11.1–34.1) |
| 20:4/18:2 | * 1.4 (0.8–4.6) | 2.0 (1.1–2.6) | *** 1.3 (0.6–2.6) | 1.7 (0.9–2.2) | *** 5.8 (3.6–8.5) | 8.2 (5.6–13.3) |

Results are expressed as median (range) of fatty acid fractions. Results were analysed by Mann–Whitney rank sum test. Significant differences from control are indicated by * $p<0.05$, ** $p<0.02$, *** $p<0.01$

A study (24) investigating phospholipids in white blood cells of atopics, showed increased levels of linoleic acid and decreased levels of arachidonic acid in monocytes, while the reverse pattern was present in lymphocytes of atopic patients. In partial accordance with these findings we observed that in MNC, which includes both lymphocytes and monocytes with the former cells predominating, arachidonic acid was reduced in total lipids of eczema patients, while in the PE fraction of phospholipids, linoleic acid was elevated. However, comparison between these studies is difficult, due to the different cell types investigated. Further, the patients of Rocklin's study were atopics with rhinitis and/or asthma, while our patients had eczema. While both groups might have disturbances in their PUFA profile, various cell systems might be differently affected. The reduced amount of MNC arachidonic acid which was found in the total lipids and, slightly in PE although not significantly, but which was not evident in PC, may be due to a distinct change in the fatty acid profiles of the phosphatidyl inositols (PI) which are present in detectable amounts, although not as abundantly as in PC and PE. The amounts did not allow a separate analysis of the PI fatty acid composition. The role of PI in receptor mediation and PG metabolism is well established, and may be the target phospholipid in signal transduction (16). It would certainly be of interest to study this phospholipid in MNC from atopics.

Ratios between fatty acids with different unsaturation were calculated for MNC (Table 3). We found significantly decreased values when arachidonic acid was related to dihomo-gamma-linolenic acid and to linoleic acid, respectively. The ratio between arachidonic acid and dihomo-gamma-linolenic acid reflects the activity of the enzyme delta-5 desaturase, while the ratio between arachidonic acid and linoleic acid reflects the combined activities of delta-6 and delta-5 desaturase and of the elongation enzyme. As the elongation enzymes are very rapid, the rate-limiting factors are the desaturase enzymes (4). These results suggest that the 2 saturases may be affected in MNC of patients with atopic dermatitis. Changes in the PUFA composition of the membrane lipids, especially of the fraction of polyunsaturated to less unsaturated PUFA, have profound effects on the membrane structure and on enzyme kinetics (5). It is well established that PUFA is involved in immunoregulation. Several studies have documented immunosuppressive effects of PUFA supplementation to various animal models of autoimmune disease (10, 20, 22). Matloff et al. (18) found that atopy was associated with a reduced ability of monocytes to produce $PGE_2$, which has been suggested to act as a suppressor factor in histamine-induced immune suppression (3, 23). Our finding of reduced arachidonic acid, the precursor of $PGE_2$, in the total lipid fraction of MNC, is in line with these results.

Although there are differences in the results of various studies of PUFA in atopics, there is a common tendency towards decreased levels of polyunsaturated fatty acids in atopics. Most studies, including the present have been of patients with active disease, making the results difficult to interpret, as the variations in the PUFA profile might be secondary to the disease state. One interesting study, however, suggests that disturbed PUFA metabolism might be primary in atopic dermatitis, as Strannegård et al. (27) found significantly higher levels of linoleic acid in umbilical cord sera derived from newborns with high serum IgE than in those with low or non-demonstrable serum IgE. This finding is of particular interest as elevated cord serum IgE is strongly associated with the development of atopic disease (7).

In conclusion, we have documented changes in the PUFA profile of phospholipids of MNC in patients with atopic dermatitis. One may speculate that the disordered fatty acid metabolism may account for some of the immunological abnormalities in patients with atopic dermatitis.

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