Platelet fatty acid composition in relation to fatty acid composition in plasma and to serum lipoprotein lipids in healthy subjects with special reference to the linoleic acid pathway

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Summary

1. The fatty acid composition in platelet phospholipids and in the plasma lipid esters as well as the serum lipoprotein lipid concentrations were determined in 67 healthy male subjects in order to establish the relationships between blood lipids and platelets.

2. A positive correlation was found between the concentrations of the triglyceride rich serum lipoprotein lipids and the relative percentage of saturated and monounsaturated fatty acids in plasma. The correlations were also positive between the serum high density lipoprotein-cholesterol concentration and the relative content of linoleic acid in the plasma cholesterol esters and phospholipids.

3. Negative correlations were found between the relative percentage of saturated and monounsaturated fatty acids in the plasma lipid esters versus linoleic acid in plasma and in the platelets. On the other hand there were positive correlations between linoleic acid in the plasma lipid esters and in the platelet phospholipids. These results indicate a direct dietary influence on the platelet phospholipid fatty acid composition.

4. The correlations between the fatty acids of the n-6 series within plasma and platelets as well as between plasma and platelets indicate that a high linoleic acid content is not associated with an increased arachidonic acid concentration. The results also indicate that the limiting metabolic step in the conversion of linoleic acid into arachidonic acid may be located at different levels in plasma and in the platelets.

Key words: arachidonic acid, fatty acids, linoleic acid, lipoproteins, platelets, prostaglandin metabolism.

Abbreviations: BHT, butylated hydroxytoluene; CE, cholesterol ester; DMA, dimethyl acetals; PL, phospholipids; PP, platelet phospholipids. PRP, platelet rich plasma; TG, triglycerides.

Introduction

Prostaglandin metabolism plays an important part in platelet function and reactivity [1]. The platelet prostaglandins are derived from C20 polyunsaturated fatty acids bound in phospholipids in the platelet membranes. How these fatty acids are related to fatty acid composition in plasma lipid esters and to the lipoproteins in serum is not fully understood. The amount and quality of dietary fat has a great influence on blood lipids [2, 3]. The influence on platelet fatty acid composition and reactivity is less well characterized, although dietary changes of fat intake have been reported to alter platelet fatty acid composition and reactivity [4, 5].

In order to understand changes of platelet fatty acid metabolism in patients with lipid disorders it is important first to define the relationships between plasma lipids and platelets in a healthy population.

The aim of this study was to characterize healthy subjects both with regard to the serum...
lipoprotein lipid composition and to the fatty acid pattern in plasma lipid esters and in platelets. The platelet fatty acid composition in healthy subjects has been described [6-8] but there is sparse information on the possible relationships between blood lipids and platelet fatty acid composition.

Materials and methods

Subjects

Male subjects (67) were studied. The mean age ($\bar{x} \pm sd$) was 49 years $\pm$ 8, range 33-61 years. The men were randomly chosen from two health surveys in the town of Uppsala, Sweden. Individuals with pronounced overweight or known elevation of serum lipids were excluded. All subjects were apparently healthy and on their habitual diets. They had a normal blood pressure and were not on any chronic medication. They were also asked to refrain from occasional aspirin medication or intake of other platelet active drugs at least 2 weeks before blood sampling, and they were not allowed to smoke in the morning of the investigation. A short medical history was obtained. Blood pressure was measured. Height, weight and smoking history were recorded. Venous blood was collected after 12 h overnight fast. Informed consent was obtained from all participants.

Serum lipoprotein analyses

Lipoprotein lipid concentrations were determined in serum after a 12-14 h overnight fast. EDTA was added to serum as a 5% solution to a final concentration of 0.05%. The samples were stored at $+4^\circ C$ for a maximum of 2 days. Separation of lipoprotein classes by preparative ultracentrifugation was carried out according to Havel et al. [9]. The very low density lipoproteins (VLDL) were isolated as the top fraction after centrifugation at $d = 1.006$ for 16 h at $15^\circ C$ at 105,000 g, in a Beckman L2-65B preparative ultracentrifuge with a 40,3 rotor. The low density lipoproteins (LDL) were precipitated from the bottom fraction at $d = 1.006$ by a heparin-Mn$^{2+}$ solution. The concentrations of heparin and Mn$^{2+}$ were 1.8 mg/ml and 0.046 mol/l respectively. After low speed centrifugation (1400 g) for 30 min, the high density lipoprotein (HDL) lipid levels were determined in the supernatant [10]. The concentrations of the lipids in the LDL were obtained indirectly by subtracting the HDL levels from the lipid concentrations of the bottom fraction after centrifugation at $d = 1.006$. The top and bottom fractions at $d = 1.006$

and the whole serum were analysed by agarose electrophoresis [11].

Cholesterol and triglyceride concentrations of serum and of the isolated lipoprotein classes were determined in propan-2-ol extracts by semi-automatic methods in a Technicon AutoAnalyzer II [12].

Fatty acid composition in plasma lipid esters

The plasma lipids were extracted into chloroform essentially as described by Carlson [13]. Methanol (5 ml) was added to 1 ml of plasma while the tube was swirled. Then 10 ml of chloroform, containing 0.005% butylated hydroxytoluene (BHT) as an antioxidant, was added, followed by 15 ml of sodium dihydrogen phosphate (0.2 mol/l).

After thorough mixing the extract was left at $+4^\circ C$ for 1-4 days. The chloroform phase was pipetted off and the solvent was evaporated to dryness at $+30^\circ C$ under a gentle stream of nitrogen. The lipid residue was dissolved in chloroform with BHT. The lipid esters (triglycerides, cholesterol esters and phospholipids) were separated by thin layer chromatography (t.l.c.) as previously described [14]. A portion (300-400 µl) of the chloroform solution was applied to the adsorbent containing POPOP as fluorescent agent. The t.l.c. plates were eluted at room temperature with the solvent system light petroleum/diethyl ether/acetic acid (81:18:1, by vol.). After at least 30 min drying at room temperature the lipid fractions were visualized in u.v. light and the spots were scraped off into vials.

The lipid esters were transmethylated at 60°C overnight after addition of 2 ml of 5% H$_2$SO$_4$ in methanol. The methyl esters were extracted into 3 ml of light petroleum (b.p. 40-60°C) containing 0.005% BHT after adding 1.5 ml of distilled water. The phases were separated after thorough mixing and centrifugation at 1500 g for 10 min. The light petroleum phase was pipetted off and the solvent was evaporated under nitrogen. The methyl esters were then redissolved in 1 ml of Uvasol, grade hexane.

The fatty acid methyl esters were separated by gas-liquid chromatography (g.l.c.) on a 25 m WCOT (wall coated open tubular) glass capillary column coated with SP 1000, with helium as carrier gas. A Hewlett-Packard system, consisting of GC 5830A, capillary injection system 18835B, operating terminal and integrator 18850A and auto-sampler 7671A, was used.

Plasma fatty acid methyl esters were separated with the temperature program: 180°C-215°C, 4°/min, after split injection (split ratio 1:15).
Platelet fatty acid methyl esters (see below) were separated with the temperature program: 70°C-180°C, 30°/min, then 4°/min to 215°C, after splitless injection. Injector and flame ionization detector temperatures were 230°C.

The fatty acids were identified by comparing retention times with those of Nu Check Prep (Elysian, MN, U.S.A.) fatty acid methyl ester standards and Supelco (Bellefonte, PA, U.S.A.) PUFa-mix no. 2 (animal source, 22:5 n-6, 22:5 n-3). The GC system was checked with Nu Check Prep g.l.c. reference standard GLC-68A.

The fatty acid composition was expressed as relative percentages. The following fatty acids are given in the Tables: 16:0, 16:1 n-7, 18:0, 18:1 n-9, 18:2 n-6, 18:3 n-6, 18:5 n-3, 20:3 n-6, 20:4 n-6, 20:5 n-3, 22:4 n-6, 22:5 n-3, 22:6 n-3. The relative concentrations of the individual fatty acids are expressed as percentages of the sum of these fatty acids.

Other identified long-chain fatty acids were present in small amounts and are not presented in the Tables. None of those amounted to more than 1%. Minor unidentified components were present, all together <1% in the triglycerides, <3% in the phospholipids and <8% in the cholesterol esters. The majority of the material in the cholesterol esters were unidentified short chain non-essential fatty acids.

### Preparation of platelets for lipid analyses

Blood was drawn with minimum damage using no or only minimal venous pressure. A 16 G butterfly needle cannula was used and the first drops of blood were discarded. Platelet rich plasma (PRP) was prepared from venous blood collected into plastic tubes, containing 1/10 trisodium citrate (0.13 mol/l), by centrifugation at low speed (120 g) for 15 min. PRP was pipetted off and the platelet sediment obtained after centrifugation at 1000g for 15 min was washed twice with Tris-HCl buffer (0.15 mol/l, pH 7.4).

### Fatty acid composition in platelets

From the washed platelet clot total platelet lipids were extracted with chloroform-methanol containing 0.005% BHT. The lipid esters were separated as outlined for plasma lipids. The phospholipid fatty acids were transmethylated and analysed by g.l.c. as described above. The concentrations of cholesterol esters and triglycerides in the platelets were too low to allow further detailed analysis. Among the identified fatty acids in platelet phospholipids, which are not presented in the Tables, the dimethyl acetics (DMA) were also included. DMA are mainly located in the phosphatidyethanolamine fraction in the platelets [6, 7]. The DMA fraction represents about 7% of the total platelet fatty acid content. Unidentified material in platelets was about 3%.

### Statistical methods

Means, standard deviations and Pearson product-moment correlation coefficients were calculated by ordinary methods. Relationships between dependent variables and explanatory variables were also estimated by the stepwise regression technique. We are indebted to Dr I. Selinus for performing the statistical calculations.

### Results and discussion

In the present study a group of 67 healthy male subjects have been investigated with regard to the serum lipoprotein lipids, plasma lipid ester fatty acids and the fatty acid composition in platelet phospholipids in order to establish the relationships between plasma and platelet lipids. The results of the serum lipoprotein analysis (Table 1) are well consistent with the findings from other health surveys in the same area [15, 16]. The results from the fatty acid analyses in plasma cholesterol esters (CE), triglycerides (TG) and phospholipids (PL), as well as in platelet phospholipids (PP) (Table 2), are essentially in accordance with earlier findings [6-8, 17-19]. Methodological and nutritional aspects may explain certain variations between the different studies.

<table>
<thead>
<tr>
<th>TABLE 1. Clinical characteristics and serum lipoprotein lipid concentrations (mmol/l) in 67 healthy male subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Results (except smoking habits) are given as mean values ± SD.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Weight index (Broca's)</th>
<th>0.99 ±0.09</th>
<th>weight (kg) height (cm) -100</th>
</tr>
</thead>
<tbody>
<tr>
<td>Platelet count</td>
<td>240 ±60</td>
<td>10³/mm³</td>
</tr>
<tr>
<td>Smokers</td>
<td>n = 19</td>
<td>(28%)</td>
</tr>
<tr>
<td>VLDL triglycerides</td>
<td>0.72 ±0.34</td>
<td>cholesterol 0.37 ±0.25</td>
</tr>
<tr>
<td>LDL triglycerides</td>
<td>0.44 ±0.14</td>
<td>cholesterol 3.88 ±0.77</td>
</tr>
<tr>
<td>HDL triglycerides</td>
<td>0.21 ±0.05</td>
<td>cholesterol 1.25 ±0.27</td>
</tr>
<tr>
<td>Serum triglycerides</td>
<td>1.40 ±0.43</td>
<td></td>
</tr>
<tr>
<td>Serum cholesterol</td>
<td>5.53 ±0.81</td>
<td></td>
</tr>
</tbody>
</table>
The serum lipids, as well as the plasma lipid ester fatty acid composition, are to a great extent influenced by the fat content of the diet [2-4, 20, 21]. In this study we found a positive relationship between the serum VLDL lipid concentrations as well as the HDL triglyceride concentrations on the one hand and the relative content of 18:0 and 18:1 n–9 in the plasma lipid esters on the other hand. This is compatible with the known association between a high intake of saturated fatty acids and increased lipoprotein triglyceride levels [22, 23]. Dietary studies have shown positive as well as negative associations between serum HDL-cholesterol and polyunsaturated fatty acids [2, 3, 24]. In our study we found a positive correlation (P < 0.05) between HDL cholesterol and the linoleic acid concentration in CE and PL but a negative correlation between the HDL-cholesterol content and 18:1 n–9 content in the CE. The well-known inverse relationship between VLDL triglycerides and HDL-cholesterol in serum [25] was also confirmed in this study (r = −0.36, P < 0.01). No significant correlations were found between LDL-cholesterol and the fatty acid concentrations in plasma.

With regard to the correlations between the lipoprotein lipid concentrations and the plasma long-chain polyunsaturated fatty acids some patterns were apparent. Thus the VLDL-lipids and the LDL- and HDL-triglycerides were positively correlated to the concentration of 20:3 n–6, but negatively to 20:4 n–6 in plasma triglycerides. Conversely, between HDL-cholesterol and the 20:3 n–6 content in the plasma triglycerides there was a negative correlation. These findings are not readily explained by dietary factors but might rather reflect different pathways in the metabolism of linoleic acid.

Between the serum lipoproteins and the platelet phospholipid fatty acids only weak correlations were found, with no consistent pattern indicating complex and indirect relationships.

Within all the lipid ester classes in plasma strong negative relationships were demonstrated between 16:0, 16:1 n–7 and 18:1 n–9, respectively, versus 18:2 n–6. This is compatible with earlier studies where inverse changes between these saturated and monounsaturated fatty acids and linoleic acid have been demonstrated during treatment with diets rich in polyunsaturated fatty acids (e.g. mainly linoleic acid [3, 21, 26, 27]). However, within the platelet lipids no such pronounced relationships were apparent.

Between the fatty acids in the plasma lipids and the platelets several strong correlations could be demonstrated. Despite the fact that the distribution of fatty acids in the plasma lipid esters and in the platelet phospholipids differ considerably (Table 2), the relationships between plasma cholesterol esters, triglycerides and phospholipids respectively, and the platelet phospholipid fatty acids, showed a similar pattern. There were strong positive correlations between the linoleic acid content in the plasma lipid esters and in platelet phospholipids. Conversely, a negative association was found between the saturated and monounsaturated fatty acids in plasma and the linoleic acid content in the platelets, as also demonstrated within the plasma lipid esters. This indicates a direct dietary influence of linoleic acid on the platelet phospholipid fatty acid composition, which also has been demonstrated by others [28, 29].

### Table 2. Fatty acid composition in platelet phospholipids (PP), plasma cholesterol esters (CE), plasma triglycerides (TG) and plasma phospholipids (PL) in healthy male subjects (n = 67)

<table>
<thead>
<tr>
<th></th>
<th>CE</th>
<th>TG</th>
<th>PL</th>
<th>PP</th>
</tr>
</thead>
<tbody>
<tr>
<td>16:0</td>
<td>11.0 ± 0.7</td>
<td>27.0 ± 2.6</td>
<td>31.2 ± 1.5</td>
<td>23.6 ± 2.0</td>
</tr>
<tr>
<td>16:1 n–7</td>
<td>3.1 ± 0.9</td>
<td>5.0 ± 0.9</td>
<td>0.9 ± 0.2</td>
<td>1.0 ± 0.2</td>
</tr>
<tr>
<td>18:0</td>
<td>1.0 ± 0.2</td>
<td>4.3 ± 0.7</td>
<td>14.3 ± 0.9</td>
<td>19.1 ± 1.4</td>
</tr>
<tr>
<td>18:1 n–9</td>
<td>18.0 ± 1.9</td>
<td>40.6 ± 2.8</td>
<td>11.5 ± 0.9</td>
<td>18.1 ± 0.9</td>
</tr>
<tr>
<td>18:2 n–6</td>
<td>57.9 ± 4.2</td>
<td>18.5 ± 4.0</td>
<td>24.8 ± 2.8</td>
<td>7.1 ± 1.1</td>
</tr>
<tr>
<td>18:3 n–6</td>
<td>0.6 ± 0.3</td>
<td>0.2 ± 0.2</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>18:3 n–3</td>
<td>0.7 ± 0.2</td>
<td>1.3 ± 0.4</td>
<td>0.1 ± 0.1</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>20:3 n–6</td>
<td>0.5 ± 0.2</td>
<td>0.1 ± 0.1</td>
<td>2.4 ± 0.5</td>
<td>1.5 ± 0.3</td>
</tr>
<tr>
<td>20:4 n–6</td>
<td>5.2 ± 1.2</td>
<td>1.2 ± 0.3</td>
<td>7.5 ± 1.3</td>
<td>22.8 ± 1.6</td>
</tr>
<tr>
<td>20:5 n–3</td>
<td>1.3 ± 0.5</td>
<td>0.3 ± 0.3</td>
<td>1.4 ± 0.6</td>
<td>1.0 ± 0.3</td>
</tr>
<tr>
<td>22:4 n–6</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
<td>0.1 ± 0.1</td>
<td>1.8 ± 0.3</td>
</tr>
<tr>
<td>22:5 n–3</td>
<td>&lt;0.1</td>
<td>0.4 ± 0.4</td>
<td>0.9 ± 0.2</td>
<td>1.7 ± 0.3</td>
</tr>
<tr>
<td>22:6 n–3</td>
<td>0.8 ± 0.3</td>
<td>1.3 ± 0.8</td>
<td>4.9 ± 1.0</td>
<td>2.3 ± 0.5</td>
</tr>
</tbody>
</table>

Results are given in relative percentages of fatty acids presented (mean ± SD).

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Fatty acids in plasma and platelets

18:2n-6
Linoleic acid

(6-desaturase)

18:3n-6
γ-Linolenic acid

20:3n-6
Dihomo-γ-linolenic acid

(5-desaturase)

20:4n-6
Arachidonic acid

22:4n-6
Docosatetraenoic acid

FIG. 1. Proposed metabolic pathway for linoleic acid.

Those studies also found a decreased platelet aggregability in subjects receiving a diet with a high linoleic acid content, confirming the findings in 1973 by Hornstra et al. [30]. However, the metabolism of linoleic acid in platelets and ultimately how this affects the prostaglandin metabolism is not fully understood.

The well-known negative association between the n-6 and the n-3 series of essential fatty acids [5] was also confirmed in our study with strong negative correlations between 20:4n-6 and 20:5n-3 both between plasma lipid esters and platelets as well as within platelet lipids.

The proposed metabolic pathway [31] for linoleic acid (Fig. 1) would theoretically result in arachidonic acid as a metabolite. Arachidonic acid is a substrate for the prostaglandin-2 series [1]. An increased arachidonic acid content of the platelets would consequently result in an increase in the pro-aggregatory thromboxane A2 levels and possibly an increased platelet reactivity. It has been demonstrated that oral administration of arachidonic acid in fact results in an increased platelet aggregability [32].

If an increased linoleic acid content in the diet results in an increased arachidonic acid production and consequently an increased thromboxane A2 production, the beneficial anti-atherogenic effect of lipid-lowering diets with a high content of linoleic acid seems less convincing.

In our study some interesting observations were made regarding the correlations between the lipid ester fatty acids of the n-6 series in plasma and platelets (Table 3). Thus it seems that the n-6 fatty acids from plasma CE and PL mainly do influence the fatty acid composition of the platelets, and the influence of the plasma TG is less evident. Also, when the results were subjected to a stepwise regression analysis technique similar results were obtained. A strong association was found between 18:2n-6 in CE and platelets

<table>
<thead>
<tr>
<th>Platelet fatty acid vs Fatty acid</th>
<th>Correlation coefficient</th>
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<tr>
<td></td>
<td>In PP</td>
</tr>
<tr>
<td>18:2n-6</td>
<td>18:2n-6</td>
</tr>
<tr>
<td>18:3n-6</td>
<td>18:3n-6</td>
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<tr>
<td></td>
<td>20:3n-6</td>
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<tr>
<td></td>
<td>20:4n-6</td>
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<tr>
<td>20:3n-6</td>
<td>18:2n-6</td>
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<td>22:4n-6</td>
<td>18:3n-6</td>
</tr>
<tr>
<td></td>
<td>20:4n-6</td>
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whereas the metabolites of \(18:2\ n-6\) in platelets mainly correlated to the corresponding fatty acids in plasma PL.

We found strong negative correlations \((P < 0.001)\) between the relative concentrations of linoleic acid and arachidonic acid within the plasma CE and PL whereas there were no significant correlations between these fatty acids in the platelets. McGregor et al. [33] have previously demonstrated an inverse correlation between 18:2 \(n-6\) and 20:4 \(n-6\) in rat platelets. The absence of an expected increase of the arachidonic acid level during linoleic acid feeding has been proposed [4] to be due to a relative competition for esterification sites on lipid esters or a limited desaturation capacity [34]. Between linoleic acid and 20:3 \(n-6\) within the platelet phospholipids there was a positive correlation (Table 3). Within the plasma CE, on the other hand, there was a significant negative correlation between linoleic acid and 18:3 \(n-6\) as well as 20:3 \(n-6\). Within the plasma PL the correlations were similar to those for the CE but weaker.

The present data may suggest (Fig. 2) that a negative correlation between linoleic acid and arachidonic acid in plasma is due to a limited capacity of desaturation between 18:2 \(n-6\) and 18:3 \(n-6\), whereas the corresponding limiting step in platelets may be located later in the metabolic pathway (between 20:3 \(n-6\) and 20:4 \(n-6\)). This means that linoleic acid feeding would cause in the platelets, in contrast to plasma, an increased level of 20:3 \(n-6\). 20:3 \(n-6\) is supposed to be the precursor of the prostaglandin-1 series, possibly less pro-aggregatory than the prostaglandin-2 compounds [35]. Diets rich in linoleic acid would subsequently result in an increased level of prostaglandin-1 precursors in the platelets and the arachidonic acid levels and the metabolites of the prostaglandin-2 series would show a relative decrease. Further detailed dietary studies are needed to elucidate the linoleic acid pathway and the possible beneficial effects of polyunsaturated diets rich in linoleic acid on platelets and prostaglandin metabolism.

**Deposited Tables**

An Appendix to this paper, containing detailed Tables on the correlations between serum lipoproteins, plasma lipid ester and platelet fatty acids, is deposited as *Clinical Science* Tables 85/2 with the Librarian, the Royal Society of Medicine, 1 Wimpole Street, London W1M 8AE, who will issue copies on request.

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**References**


