Fatty Acid Pattern of Red Blood Cell Membranes and Risk of Ischemic Brain Infarction: A Case-Control Study

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The fatty acid composition of red blood cell membranes (which reflects dietary fat intake) was studied in 28 male patients with recent (<3 days) ischemic stroke and 56 matched controls. Fifteen fatty acids were measured by means of chromatographic analysis. Percentages of linoleic, 22:5, and 22:6 acids were significantly lower in red blood cell membranes of stroke patients than in those of matched controls. The results suggest that a low unsaturated fatty acid diet could be an independent risk factor for ischemic brain infarction. (Stroke 1987;18:575-578)

It is widely accepted that dietary fats have a role in the etiology of ischemic heart disease (IHD). Patients with IHD appear to have a low linoleic acid content in their plasma cholesterol esters as well as in adipose tissue. A low content of polyunsaturated fatty acids (PUFA) in serum phospholipids has been shown to be an independent risk factor for IHD. Diets with a high PUFA:saturated fatty acids (SFA) ratio might reduce the risk of IHD through their effect of lowering plasma cholesterol concentration or, possibly, through an antithrombotic property of PUFA. The limited role of hypercholesterolemia as a risk factor in cerebrovascular diseases (CVD) is well known. A low alimentary PUFA:SFA ratio was found in IHD patients, but, as far as we are aware, no data are available on this aspect in patients with CVD.

The composition of red blood cell membrane phospholipids reflects the type of fats eaten in the preceding weeks, and it is unlikely to change immediately after an acute event such as a stroke. Therefore, we studied the composition of red blood cell membrane phospholipids in patients with ischemic stroke and in matched controls to find out whether the type of fats consumed in these two groups was different.

Subjects and Methods

We studied 28 consecutive male patients, aged 40-75, who had suffered a first-event ischemic stroke, defined as an acute focal neurologic deficit lasting >24 hours that was attributed to ischemia on clinical grounds and confirmed by a computed tomography (CT) scan within 3 days. We did not study women due to organizational difficulties. We excluded patients with myocardial infarction within the previous 3 months and also patients with other possible cardiac sources of emboli and without other known risk factors for cerebral ischemia (e.g., mitral stenosis with atrial fibrillation in a young, normotensive, normoglycemic patient). Each patient was matched with 2 controls (56 men matched for age ±5 years, smoking habits, hypertension, and diabetes); these were male inpatients admitted to our hospital in the same period who were not suffering any acute vascular disease and had no stroke or myocardial infarction in their history. Men chosen as controls in this study suffered from a wide spectrum of diseases (tumors, epilepsy, infections, cardiac failure, arthritis, hepatic disorders, etc.).

After an overnight fast, 20 ml of blood were collected into EDTA tubes; after centrifugation (1800g for 15 minutes at room temperature) plasma was removed and the red blood cells were washed 3 times with saline then collected into 2 separate tubes and sent for biochemical studies. Lipid determinations were performed blindly with respect to case or control status. The membrane phospholipids extraction method is summarized in Appendix 1. The following fatty acids were measured: 14:0, 16:0, 16:1, 17:0, 18:0, 18:1, 18:2, 18:3, 20:1, 20:3, 20:4, 20:5, 22:4, 22:5, and 22:6. The unsaturation index (UI) formula, given in Appendix 1, was calculated.

Statistical analysis was as follows: a multivariate analysis (Hotelling-Lawley test) was performed preliminarily to find out whether the 2 groups differed in at least 1 variable. Thereafter, unpaired t tests were performed for each variable, having chosen a p value of 0.003 (Bonferroni correction, 16 variables measured) as significant.

Results

As shown in Table 1, patients and controls were well matched for risk factors (age, hypertension, dia-
The proportions of fatty acids (expressed as percent) in the phosphatidylcholine fraction of red blood cell membrane lipids are shown in Table 2. Linoleic acid (18:2), 22:5, 22:6, and UI were significantly lower in stroke patients than in controls (Figure 1). Stroke patients also had slightly higher levels of palmitic (14:0) and stearic acid (18:0) and lower levels of arachidonic acid, 18:2) suggest a diet with cholesterol-raising effects in stroke patients. This, however, was ruled out by the lack of any significant difference in serum cholesterol concentration between the 2 groups. It is also known from extensive epidemiologic studies that high serum cholesterol levels are not a significant risk factor for ischemic stroke. Even though a positive trend has been found in young men (<50 years old), the mean age of the stroke patients (62.5 ± 9.4 years) and controls (62.8 ± 9.4) in our study were well beyond the limit found in the earlier studies.

Recently it was suggested by several authors that PUFA might have an antithrombotic action. In vitro stearic acid (18:0) produces the highest activity of platelet Factor 3 when added to platelet-rich plasma, whereas linoleic acid (18:2) leads to the lowest activity. The antithrombotic effect hypothesis seems to be supported by our data; in fact, the long-chain saturated fats—which we found higher in stroke patients than in controls—have the highest clotting activity, and linoleic acid (18:2)—which was significantly lower in stroke patients—is the one with the lowest clotting activity.

We did not find any differences in 20:3 acid, whose reduced levels were reported to be strongly associated with IHD, but we found a significant reduction of linoleic acid (18:2), which is most probably the precursor of mammalian 20:3.

The possible relevance of the lower levels of 22:5 and 22:6 acids found in stroke patients deserves comment. Eicosapentaenoic acid (20:5) was shown to reduce thromboxane A2 production by platelets. Concen-

tration of this acid is high in plasma lipids of Eskimos, whose incidence of IHD is very low; moreover, the bleeding time in Eskimos is prolonged.

### Discussion

Our findings show that patients who have suffered an ischemic stroke within the last 3 days have a different fatty acid composition of red blood cell membranes when compared with controls matched for age, hypertension, diabetes, and smoking habits. Since the fatty acid composition of red blood cell membrane phospholipids reflects the long-term dietary fat intake, we can assume that our patients ate less PUFA and more SFA than controls in the weeks preceding their stroke. This is, as far as we know, the first evidence relating relative dietary PUFA (reflected in the fatty acid composition of red blood cell membranes) and cerebral ischemia. The reason for an association between a low PUFA: SFA ratio in diet and CVD as well as IHD is not completely understood. A few hypotheses are worth considering.

There is some clinical and experimental evidence that a diet high in PUFA reduces blood pressure. In our study we excluded any significant difference in long-term history of high blood pressure between patients and controls by matching; although it is possible that some stroke patients had unknown high blood pressure, this could be true for control patients as well. Furthermore, the nonsignificant difference in SFA (namely palmitic and stearic acid, 14:0 and 18:0) and the significant difference in PUFA (namely linoleic acid, 18:2) suggest a diet with cholesterol-raising effects in stroke patients. This, however, was ruled out by the lack of any significant difference in serum cholesterol concentration between the 2 groups. It is also known from extensive epidemiologic studies that high serum cholesterol levels are not a significant risk factor for ischemic stroke. Even though a positive trend has been found in young men (<50 years old), the mean age of the stroke patients (62.5 ± 9.4 years) and controls (62.8 ± 9.4) in our study were well beyond the limit found in the earlier studies.

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### Table 2. Fatty Acid Content in Phosphatidylcholine of Erythrocyte Membranes in Patients and Controls

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Patients (n = 28)</th>
<th>Controls (n = 56)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>14:0</td>
<td>0.43 (0.61)</td>
<td>0.15 (0.26)</td>
<td>0.03</td>
</tr>
<tr>
<td>16:0</td>
<td>45.81 (4.80)</td>
<td>44.65 (5.29)</td>
<td>0.33</td>
</tr>
<tr>
<td>16:1</td>
<td>0.58 (0.56)</td>
<td>0.47 (0.46)</td>
<td>0.31</td>
</tr>
<tr>
<td>17:0</td>
<td>0.28 (0.32)</td>
<td>0.23 (0.15)</td>
<td>0.48</td>
</tr>
<tr>
<td>18:0</td>
<td>13.13 (2.00)</td>
<td>11.40 (2.75)</td>
<td>0.004</td>
</tr>
<tr>
<td>18:1</td>
<td>24.68 (3.44)</td>
<td>24.08 (2.49)</td>
<td>0.42</td>
</tr>
<tr>
<td>18:2</td>
<td>9.46 (3.19)</td>
<td>12.24 (3.16)</td>
<td>0.0003*</td>
</tr>
<tr>
<td>18:3</td>
<td>0.03 (0.11)</td>
<td>0.07 (0.25)</td>
<td>0.28</td>
</tr>
<tr>
<td>20:1</td>
<td>0.63 (1.03)</td>
<td>0.31 (0.45)</td>
<td>0.12</td>
</tr>
<tr>
<td>20:3</td>
<td>0.89 (0.88)</td>
<td>1.18 (0.67)</td>
<td>0.10</td>
</tr>
<tr>
<td>20:4</td>
<td>2.41 (2.05)</td>
<td>3.79 (2.25)</td>
<td>0.008</td>
</tr>
<tr>
<td>20:5</td>
<td>0.25 (0.59)</td>
<td>0.15 (0.31)</td>
<td>0.42</td>
</tr>
<tr>
<td>22:4</td>
<td>0.41 (0.88)</td>
<td>0.39 (0.51)</td>
<td>0.92</td>
</tr>
<tr>
<td>22:5</td>
<td>0.07 (0.11)</td>
<td>1.19 (0.18)</td>
<td>0.0003*</td>
</tr>
<tr>
<td>22:6</td>
<td>0.09 (0.15)</td>
<td>0.39 (0.53)</td>
<td>0.0003*</td>
</tr>
<tr>
<td>UI</td>
<td>1.04 (0.31)</td>
<td>1.34 (0.47)</td>
<td>0.0008*</td>
</tr>
</tbody>
</table>

Fatty acid content expressed as mean percent (± SD). UI, unsaturation index.

*p value chosen as significant, 0.05/16 = 0.003.
possibly due to the \( \omega-3 \) structure of eicosapentaenoic acid. In our study we did not find any difference in eicosapentaenoic acid (20:5), but in stroke patients we found a significant reduction of 22:5 and 22:6 membrane content, and these acids share the \( \omega-3 \) structure with 20:5.

We detected low arachidonic acid content in erythrocyte membranes of stroke patients. This tallies with earlier observations in patients with myocardial infarction.\(^4\) In the last mentioned study, serum phospholipids were measured long before myocardial infarction, and the fatty acid pattern of serum phospholipids was considered to be an independent risk factor for coronary heart disease, a conclusion which is not far from our own suggestion about the role of dietary PUFA in ischemic CVD. The linoleic acid differences we found in our study are very similar to those shown in previous trials on IHD.\(^4\) The very high level of significance and the fact that similar results were obtained in 3 separate studies on 2 different diseases (IHD and CVD) make a fortuitous result rather unlikely.

The results of the present study suggest that a low dietary PUFA intake—reflected in the PUFA content of red blood cell membranes—could be an independent risk factor for ischemic brain infarction, at least in men. Only prospective clinical trials will prove a possible preventive effect of a high-PUFA diet on ischemic stroke.

**Appendix 1**

The red blood cell pellet was extracted with 20 volumes of methanol:chloroform (1:1 v:v) for 20 minutes at 37° C. After centrifugation, the organic phase was washed in 5 ml of chloroform:methanol (2:1 v:v), then evaporated to dryness under nitrogen. The sample was reconstituted with 5 ml of chloroform:methanol (2:1 v:v) and dried on Na\(_2\)SO\(_4\). The filtered material was then passed under nitrogen flow.

Samples of the lipidic solution were spotted on silica gel plates, and phosphatidylcholine was insulated by developing the plates in chloroform:methanol:water (65:25:4 v:v:v). Phosphatidylcholine was eluted with methanol, dried under nitrogen, and then transesterified with 1–2 ml of H\(_2\)SO\(_4\), 3% in methanol, for 3 hours at 65° C.

After refrigeration, the solution was extracted 3
times with 5 volumes of petrol ether, then dried under nitrogen; the residue was reconstituted with a few milliliters of petrol ether. This solution was eventually used for chromatographic analysis. Final results were expressed as a single fatty acid percent of the whole fatty acid composition of phosphatidylcholine.

Unsaturation index (UI) was calculated as

\[
UI = \frac{\text{Sum} \% \text{ unsaturated acids} \times \text{number of double bonds}}{\text{Sum} \% \text{ saturated fatty acids}}.
\]

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References

17. Willis AL: Dihomogammalnonic acid as the endogenous protective agent for myocardial infarction. Lancet 1984;2:697

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