Age-related changes in the retinal capillaries and the fatty acid composition of retinal tissue of normal and essential fatty acid-deficient rats

Gary L. Forrest and Sidney Futterman

Changes with time in the relative proportion of intramural pericytes and endothelial cells in capillaries of the retina were examined in essential fatty acid-deficient rats and their safflower oil-supplemented controls. The ratio of pericytes to endothelial cells rose, approaching a maximum value near unity only at about 9 months past weaning. The changes were identical in both groups. In the deficient animals' retinal tissue 5,8,11-epicosatrienoate was accumulated and arachidonate was reduced. In both groups 4,7,10,13,16-docosapentaenoate accumulated. It was concluded that altered fatty acid composition of the magnitude occurring in essential fatty acid-deficiency was insufficient to provoke pathologic changes in the capillaries within the times of observation covered by these experiments.

Key words: essential fatty acids, retinal capillaries, diabetes mellitus, docosapentaenoic acid.

In diabetes it is well established that secondary changes occur in the retina of various species and include microaneurysms and loss of intramural pericytes (mural cells) from the capillaries of the posterior pole of the retina. Studies to determine whether altered lipid metabolism might constitute an underlying mechanism for these secondary changes were encouraged by evidence that the synthesis of polyenoic fatty acids was depressed in retinal tissue of diabetic animals, and that this alteration was comparable to that occurring in essential fatty acid-deficient animals. A preliminary observation of preparations of the retinal capillaries from the essential fatty acid-deficient animals seemed to indicate a reduction in the relative number of intramural pericytes, making apparent the necessity for a detailed investigation of age-related changes in capillaries of the retina in essential fatty acid-deficient rats and their normal controls.

Methods

Essential fatty acid-deficiency was induced in weanling Sprague-Dawley male rats as described.
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Fig. 1. Storage of vitamin A in livers of essential fatty acid-deficient rats and safflower oil-supplemented controls. Three livers were analyzed for each data point; one μ mole of retinol = 954 I.U.

Table I. Changes with time in the relative abundance (mole per cent) of the principal fatty acids of retinal tissue from essential fatty acid-deficient rats and their safflower oil-supplemented controls

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Essential fatty acid-deficient diet (months on diet)</th>
<th>Safflower oil-supplemented diet (months on diet)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Palmitate</td>
<td>16:0</td>
<td>18:0</td>
</tr>
<tr>
<td>Stearate</td>
<td>16:0</td>
<td>18:0</td>
</tr>
<tr>
<td>Oleate</td>
<td>16:0</td>
<td>18:0</td>
</tr>
<tr>
<td>Eicosatrienoate</td>
<td>16:0</td>
<td>18:0</td>
</tr>
<tr>
<td>Arachidonate</td>
<td>16:0</td>
<td>18:0</td>
</tr>
<tr>
<td>Docosahexaenoate</td>
<td>16:0</td>
<td>18:0</td>
</tr>
</tbody>
</table>

*Position of first double bond counting from methyl carbon end of fatty acid molecule is given by number following ω and identifies polyenoic fatty acid as being derived from oleate (ω9), linoleate (ω6), or linolenate (ω3).

Controls were fed the deficient diet supplemented to contain 5 per cent safflower oil. The methods used for fixation and analysis of tissues were those previously employed. Retinas were digested in 3 per cent trypsin in 0.1 M phosphate buffer, pH 7.3, without agitation in a water bath at 37° C.; the vessel preparations were air dried on slides and stained with hematoxylin-PAS. Two hundred cells from each capillary preparation were enumerated as intramural pericytes or endothelial cells under 450 x magnification based upon density of staining, shape, and orientation of nuclei with respect to inner and outer surfaces of capillaries. Counts were distributed equally between the centrally and peripherally located capillaries.

Results

Cessation of weight gain essentially as reported previously,7 loss of muscle tone and development of rough dry skin and hair in the experimental group as compared to safflower oil-supplemented controls, indicated that essential fatty acid deficiency had been produced. Ample liver stores of retinyl esters were present in both groups (Fig. 1).

The only marked change occurring in the proportions of the major fatty acids of the retina was a decrease of approximately 50 per cent in the amount of arachidonate (Table 1) in the deficient animals. This was accompanied by the appearance of increasing concentrations of 3,5,8-eicosatrienoate.

When the polyunsaturated fatty acids of the retina were further examined, it be-
Fig. 2. Effect of essential fatty acid-deficient and safflower oil-supplemented diets on the ratio of 4,7,10,13,16-docosapentaenoic acid to 4,7,10,13,16-docosapentaenoate in retina.

Table II. Retinal capillary cell counts on essential fatty acid-deficient rats and their safflower oil-fed controls

<table>
<thead>
<tr>
<th>Months on diet</th>
<th>Essential fatty acid-deficient*</th>
<th>Safflower oil-supplemented*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Intramural pericytes</td>
<td>Endothelial cells</td>
</tr>
<tr>
<td>0</td>
<td>24.8 ± 1.1</td>
<td>74.2 ± 1.1</td>
</tr>
<tr>
<td>3</td>
<td>32.8 ± 1.1</td>
<td>67.2 ± 1.1</td>
</tr>
<tr>
<td>6</td>
<td>40.8 ± 0.5</td>
<td>59.2 ± 0.6</td>
</tr>
<tr>
<td>9</td>
<td>47.8 ± 0.5</td>
<td>52.2 ± 0.4</td>
</tr>
<tr>
<td>11</td>
<td>43.7 ± 1.1</td>
<td>56.3 ± 1.0</td>
</tr>
</tbody>
</table>

*One hundred cells were counted in each of two capillary preparations per rat; each value is the mean ± S.E.

came apparent that a minor polyenoic fatty acid, 4,7,10,13,16-docosapentaenoate, became much more abundant in essential fatty acid-deficient animals than rats fed a diet of commercial stock pellets (Fig. 2). An even greater increase in this fatty acid occurred in safflower-supplemented controls. Safflower oil contains approximately 75 per cent linoleate and increased dietary linoleate results in docosapentaenoate accumulation in tissues. By comparing analytic data for retina with other tissues where this fatty acid is known to occur, it was identified as 4,7,10,13,16-docosapentaenoate (22:5ω6) derived from linoleate metabolism. On the other hand there was no accumulation of linoleate in the retina in rats receiving the safflower oil-supplemented diet. The results indicated that both linoleate and arachidonate were metabolized to docosapentaenoate when exogenous linolenate was not available for desaturation and chain elongation.

Counts of endothelial cells and pericytes in preparations of the retinal capillaries could be made with little variation from one preparation to another (Table II). At no time were any microaneurysms or acellular changes detectable in capillary preparations from either group. The ratio of intramural pericytes to endothelial cells rose with time approaching a maximum value near unity only at about 9 months past weaning (Fig. 3). The age-related changes in the relative abundance of pericytes and endothelial cells were identical in the essential fatty acid-deficient animals enduring progressive depletion of linoleate and in the safflower oil-supplemented control group.
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Discussion

The retina shows no tendency to accumulate linoleate under conditions in which dietary safflower oil makes linoleate abundant systemically; the retina, instead, increases its conversion to docosapentaenoate. It appears that in essential fatty acid deficiency, the retina converts some of its arachidonate to docosapentaenoate while metabolizing olate to eicosatrienoate. At the same time the retina shows no dramatic loss in docosahexaenoate. The results indicate that docosahexaenoate turnover in the retina is slow and that the docosahexaenoate of photoreceptor membranes engulfed by cells of the pigment epithelium is re-utilized efficiently in the process of photoreceptor renewal.

The decrease in the concentration of arachidonate in the retina in essential fatty acid deficiency, though modest, is comparable in magnitude to that observed in experimental diabetes. Unlike diabetic rats, which in time show some minimal retinopathic changes, essential fatty acid-deficient animals show none. In addition, essential fatty acid deficiency is incapable of altering the normal course of changes with time in the relative abundance of pericytes and endothelial cells in the retinal capillaries. Although essential fatty acid deficiency has been reported to increase capillary fragility, no evidence of any pathologic changes in the retinal capillaries could be found. The findings support the view that altered fatty acid composition of the magnitude observed in either diabetic or essential fatty acid-deficient rats is insufficient to compromise membrane function and unlikely to provoke significant pathologic changes in capillaries within the times of observation covered by these experiments.

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REFERENCES