Changes in lens fatty acid composition during galactose cataract formation

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A high level of fat in the diet of galactose-fed weanling rats has been reported to delay the onset of mature cataracts. Groups of rats fed lab chow, sucrose, and galactose-high-fat diets had essentially similar total fatty acid compositions in their lenses, but rats receiving a low level of corn oil in a galactose diet were observed to have differences in the long-chain unsaturated fatty acids when compared to the control groups. This observation suggests that the lenses of unsupplemented galactose-fed rats have a relative deficiency of certain long-chain unsaturated fatty acids and might account for the difference in the time of mature cataract formation which has been observed with the two diets.

Key words: cataracts, galactose, rats, fatty acids, lens, dietary fat.

It has been shown that the formation of galactose-induced cataracts in rats can be delayed by the addition of various supplements such as casein, corn oil, and fructose to the high-galactose diets. In the case of fat supplementation, the mechanism for this delay in cataract formation has been proposed to be an increase in the supply of available acetylcoenzyme-A and, therefore, of energy. We proposed to feed rats various diets and study by means of gas-liquid chromatography the composition of fatty acids in the lenses of control animals as well as animals on a high-galactose and a high-galactose-high-fat diet. By this method we hoped to determine any significant changes in the fatty acid composition in those lenses undergoing cataract formation due to the high-galactose diet.

Methods

Forty weanling rats* with initial weights of 44 to 54 grams were used in the experiment. They were randomly selected to be fed one of our diets (see Table 1) and were placed in separate cages, also by random selection. They were weighed every other day for the first ten days of the dietary regimen and on the day they were put to death. The diets were fed ad libitum, and water was always available. The animals were fed for ten days and then one animal from each of the four groups was killed on each of the following ten days.

The animals were decapitated and their lenses were removed and frozen immediately. Lenses from each animal were pooled. The paired, frozen lenses were homogenized with 2 c.c. of 2:1 CHCl₃:MeOH, the homogenizers were rinsed with

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Table I. Composition of purified diets

<table>
<thead>
<tr>
<th>Component</th>
<th>Galactose diet (%)</th>
<th>High fat and galactose diet (%)</th>
<th>Sucrose control diet (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin mixture</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Mineral mixture</td>
<td>5.0</td>
<td>5.0</td>
<td>5.0</td>
</tr>
<tr>
<td>Casein</td>
<td>20.0</td>
<td>25.0</td>
<td>25.0</td>
</tr>
<tr>
<td>Corn oil</td>
<td>5.0</td>
<td>20.0</td>
<td>20.0</td>
</tr>
<tr>
<td>Sucrose</td>
<td>20.0</td>
<td>—</td>
<td>49.5</td>
</tr>
<tr>
<td>Galactose</td>
<td>49.5</td>
<td>49.5</td>
<td>—</td>
</tr>
</tbody>
</table>

a small amount of the same solvent mixture, and the homogenate was centrifuged. The supernatant fluid was removed, transferred to a screw-top tube with a Teflon-lined cap, and taken to dryness under \( \text{N}_2 \). After adding 1 c.c. of 14 per cent KOH in MeOH, the residue was saponified at 100° C. for 90 minutes. When cool, 3 c.c. of water were added to the solution which was then extracted three times with 3 c.c. of petroleum ether; the extracts were discarded. The residue was then acidified with 0.5 c.c. of 6N HCl and extracted three more times with 3 c.c. of petroleum ether. These extracts were pooled and the lower layer discarded after the third extraction. The combined extracts were taken to dryness under \( \text{N}_2 \). The residue was dissolved in 1 c.c. of benzene, after which 1 c.c. of 10 per cent \( \text{BF}_3\text{-MeOH} \) (w/v) was added the mixture was refluxed at 100° C. for six minutes.° After cooling, 1 c.c. of water was added and the mixture centrifuged for five minutes at 5,000 r.p.m. The benzene layer containing the methyl esters was then transferred to a silicone–septum–stopped sample tube. It was taken to dryness under \( \text{N}_2 \) and then redissolved with 30 µl of chloroform. Aliquots of approximately 2 µl were analyzed by gas-liquid chromatography on a 6 foot column of 3 per cent EGSS-X.° Identification of fatty acid methyl esters was based on retention times observed with this column only.

Results

The ten rats which had consumed lab chow gained an average of 84.8 grams, the rats on a sucrose control diet, an average of 65.6 grams, those on the high-fat–galactose diet, an average of 68.9 grams, and those on the galactose diet, an average of 45.8 grams. Only the animals on the low-fat–galactose diet which were killed on days 19 and 20 had visible nuclear opacifications.

In the group of lenses from the chow-fed group, there was an increase on days 15 and 17 in the amount of fatty acids of more than \( C_{20} \) carbon chain length (Fig. 1). There was no significant change in the amount of unsaturated fatty acids less than \( C_{20} \).° There was a definite decrease in the saturated fatty acids less than \( C_{20} \) at day 17.

The lenses of the rats which had received the sucrose control diet showed an increase in fatty acids greater than \( C_{20} \) at day 15. This was also true in the case of the high-fat–galactose diet as well as the galactose diet with the peak increase coming at day 15, as opposed to day 17 in the group fed the chow diet. In all groups which consumed purified diets, there was a greater amount of fluctuation and variability in the relative amounts of fatty acid groups when compared with the rats which received chow.

The high-fat–galactose group had an initial high level of unsaturated fatty acids shorter in chain length than \( C_{20} \). Although dietary influences might be suspected, the lenses of the group consuming the sucrose diet showed no such effect.

In the galactose-fed group, there was a trend toward a decrease in the amount of saturated fatty acids shorter than \( C_{20} \). The increase in fatty acids longer than \( C_{20} \) at day 15 was due principally to one unidentified fatty acid and accounted for over 50 per cent of the increase in this group of fatty acids.

On day 19, although the relative amounts of fatty acids appeared comparable (see Fig. 1), the galactose-fed groups had a slightly different fatty acid composition. In the galactose-fed group, the amounts of \( C_{22} \) and \( C_{20} \) were more than twice the amounts of these fatty acids in each of the other groups. Although the changes

°Subscript refers to the number of carbons in the fatty acid and superscript refers to the number of double bonds present in the molecule.
observed were small, it should be noted that these two fatty acids along with C_{24}^2= and C_{22}^1= were the principal representatives of the long-chain group in all dietary groups for the last five or six days of the experiment.

With the finding of differences in the fatty acid composition on day 19, the data were more closely examined and the percentages of C_{20}^2= and C_{20}^5= were plotted (Fig. 2). The percentages of C_{22}^1= and C_{20}^5= show similar results for the chow control, sucrose control, and galactose-fat diets. With each of these diets, there was a peak on day 15 in the amounts of these fatty acids and then a decline to day 19. Therefore, the average values for all three control diets were used to plot the control curves, but the individual values are indicated. In the case of the galactose diet, there was a much smaller peak on day 15 and then a leveling off until day 19, at which time there was an abrupt increase in the amounts of these fatty acids corresponding with a decline of C_{22}^1= and C_{24}^2= (Table II).

Discussion

Although it has been proposed that most of the lipid in the mammalian lens is associated with the intercellular membrane structure, there is only histochemical evidence directly supporting this hypothesis. In most mammalian organs which
Fig. 2. Concentration of C_{22\text{:1}} (solid line) and C_{20\text{:5}} (dashed line) fatty acids in the lenses of rats consuming different diets. Solid symbols represent values for C_{20\text{:5}} and open symbols are the values for C_{22\text{:1}}. Individual diets are: circles, chow; triangles, high-fat-galactose; and squares, sucrose.

Table II. Comparative levels of selected lens fatty acids at termination of experiment (per cent of total fatty acids)

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Galactose diet (%)</th>
<th>High fat and galactose diet (%)</th>
<th>Sucrose control diet (%)</th>
<th>Lab chow control diet (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C_{22\text{:1}}</td>
<td>1.92</td>
<td>1.43</td>
<td>0.49</td>
<td>0.83</td>
</tr>
<tr>
<td>C_{20\text{:5}}</td>
<td>1.42</td>
<td>0.56</td>
<td>0.22</td>
<td>0.46</td>
</tr>
<tr>
<td>C_{22\text{:4}}</td>
<td>0.77</td>
<td>1.43</td>
<td>1.58</td>
<td>1.44</td>
</tr>
<tr>
<td>C_{24\text{:2}}</td>
<td>0.49</td>
<td>1.01</td>
<td>1.21</td>
<td>1.27</td>
</tr>
<tr>
<td>Total</td>
<td>4.60</td>
<td>3.05</td>
<td>3.50</td>
<td>4.00</td>
</tr>
</tbody>
</table>

have been investigated with the objective of membrane characterization, the results have demonstrated an association between cholesterol and phospholipids for the lipid fraction. The proteolipid fraction described by Feldman and Feldman in human lens is probably the most direct evidence available on the composition of the lipid component of lens fiber membranes. The lipids of this fraction are reported to comprise 66% of the total lens lipid but, due to structural considerations, contain a much lower percentage of the total fatty acids. Thus, it seemed apparent that, al-
though the fatty acids of intermediate length, such as palmitate and stearate, are important phospholipid constituents, changes in the amounts of these components of the phospholipid fraction might be "swamped out" by changes in the non-proteolipid fraction.

Accordingly, although the concentration of all tentatively identified fatty acids was calculated, we were not surprised by the lack of consistent differences between the galactose alone and the control diets insofar as the concentrations of the dominating fatty acid species are concerned. If it were possible to separate these two fatty acid sources there might well be significant differences between them. On the other hand, a trend toward declining levels of these intermediate-length fatty acids was noted in the lenses of the galactose-fed animals in contrast to the lenses of the rats fed the control diets—including the galactose-high fat diet in the latter category. This observation is of interest in view of the results reported by Radin's group, that in the brain, the C_{20} and longer fatty acids are synthesized by chain elongation of the C_{14} and C_{16} moieties. Thus, our observation of consistent differences between the experimental and the control groups in the longer chain fatty acid concentrations are given some support, and these differences, although apparently occurring in a minor group of fatty acids, may be of major significance in maintaining lens fiber membrane integrity.

The unidentified fatty acid appearing at day 15 in the lens of a galactose-fed rat represents a single observation and may, of course, be an artifact. The absence of a similar observation in that group of animals or in any of the other dietary groups suggests, however, that it is not artifactual. The fact that the identities of the specific fatty acids described in this experiment are only tentative should be emphasized. The differences in fatty acid composition measured between dietary groups, although consistent, were based on corresponding peaks on the chromatograph recordings and on the retention times of these peaks. The retention times were based on those of authentic fatty acid methyl esters, but the lens methyl esters were not chemically characterized and thus their identity can only be regarded as tentative.

The consistency between the three control diets is of interest and indicates that galactose and its metabolic products do not interfere with the development of a normal fatty acid pattern within the lens during the period investigated when a high level of fat supplementation is utilized. The data do not discriminate, however, between in situ biosynthesis and selective retention from the fluids surrounding the lens. Thus, although the biological pathways leading to the appearance of C_{20} in the lens lipid are not known at present, these data demonstrate that a galactose diet does not prevent its appearance 15 days after weaning, but it does reduce the amount present to less than 50 per cent of the values found in control lenses. The same lowering effect of the unsupplemented galactose diet was found for the C_{18} fatty acid.

Although caloric intake was uncontrolled in this experiment, Patterson and his colleagues demonstrated a delaying of mature cataract formation in weanling rats fed a commercial diet with galactose mixed in to the extent of 35% by weight, when the animals were isocalorically supplemented with casein and corn oil. The appearance of the C_{20} fatty acid in measurable quantities, under the assay conditions, in the lenses of unsupplemented galactose-fed rats at 14 days suggests that these lenses are indeed at the same developmental stage as the lenses of the rats consuming the supplemented galactose diet. Therefore, it would seem that variation of caloric intakes is not an interfering factor in this investigation. Furthermore, it is apparent that corn oil supplies a necessary factor for the appearance of normal levels of C_{20} at 15 days after weaning. The terminal rise of the level of lens C_{20} fatty acid could be the result of cataract
formation—partial breakdown of the lens fiber membrane with concomitant losses of selected fatty acids such as C24:2 and C22:4 (Table II).

Thus, feeding a high level of galactose reduces the amounts of at least two fatty acids, presumably in lens fiber membranes, and the deficiency of these fatty acids may contribute to the rapidity of the formation of mature cataracts.

REFERENCES


