Tocopherol Distribution in Serum Lipoproteins with Respect to Red Blood Cell Tocopherol Levels in Children

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Summary The relation of lipoprotein tocopherol levels to red blood cell (RBC) tocopherol was investigated in 81 healthy children, comprising 44 males and 37 females, using a new technique for separation of individual lipoprotein fractions. 1. In children there were no age and sex differences in tocopherol contents among individual lipoprotein fractions. The tocopherol content of high density lipoprotein (HDL) was slightly higher than that of low density lipoprotein (LDL), but this difference was not statistically significant. 2. The tocopherol content of HDL fractions was closely correlated with RBC tocopherol concentration, but there was no relationship in tocopherol levels between RBC and LDL and between RBC and very low density lipoprotein (VLDL). 3. There were no age and sex differences in contents of total cholesterol (T-ch), triglycerides (TG), phospholipids (PL), total lipids, HDL-cholesterol, LDL or VLDL in children.

Key Words vitamin E, lipoproteins, lipids, erythrocytes, plasma, children

The mechanism of delivery of vitamin E (tocopherol) from plasma to biomembranes in tissue cells has not been clarified, since no specific carrier proteins for vitamin E have been found. Circulating tocopherol is well known to be distributed among all classes of lipoprotein fractions (1) and to be exchanged between different lipoproteins, and between lipoproteins and red blood cell membranes. Thus, tocopherol may be similar to cholesterol (2). Ogihara et al. (3) have reported that distribution of tocopherol is directly related to the total lipid content of the individual lipoprotein fractions. Traber and Kayden (4) and Thellman and Shireman (5) demonstrated in the LDL receptor mechanism that tocopherol is delivered to cells by the LDL receptor. However since red blood cells

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(RBCs) and platelets do not have LDL receptors, the tocopherol may be freely exchangeable between plasma and blood cells. For clinical study, it may be important to ascertain the tocopherol distribution among the lipoproteins, since tocopherol levels in RBCs (6-9) and platelets (10-12) are generally assumed to constitute indexes for nutritional assessment of vitamin E.

The authors investigated the relation of lipoprotein tocopherol content to RBC tocopherol levels in children by using a new simple method (13) of heparin-Ca precipitation technique (14).

MATERIALS AND METHODS

Subjects. The group studied consisted of 81 children ranging from 6 months to 15 years of age, comprising 44 males and 37 females, who visited our outpatient clinic for health check or with various complaints (headache, nausea, lassitude and/or motion sickness) possibly due to autonomic dysfunction, but who displayed no evidence of either organic, metabolic, or other pathology which could affect blood vitamin E concentrations. They received adequate diets but no vitamin E supplements. The study protocol was approved by the ethics committee of the hospital, and the study was performed after obtaining informed consent from children’s parents.

Sampling. After overnight fasting, heparinized blood was drawn and the RBCs were separated from the plasma by centrifuging at 3,000 rpm for 10 min.

Analysis. Plasma separated from RBCs was used for analysis of tocopherol, total cholesterol, triglycerides, phospholipids, and beta-lipoproteins. Total cholesterol, phospholipids, and triglycerides were measured by the methods of Allain et al. (15), Takayama et al. (16), and Bucolo and David (17), respectively, using an autoanalyzer. Total lipids were estimated by addition of the three major lipids. Tocopherol in plasma and RBCs were analyzed by high-performance liquid chromatography (HPLC) using Ishibashi’s modification (18), as described in a previous report (9). The tocopherol concentration in RBCs obtained by HPLC analysis were corrected by hematocrit values in each sample. In this study, only values for alpha-tocopherol are given unless otherwise stated.

Separation of lipoproteins: A heparin-Ca method (14) was used in this study, by virtue of its simplicity, convenience, and speed. Details of the procedure and its reliability have been described in another paper (19).

RESULTS

1. Distribution of tocopherol in plasma and RBCs, and individual lipoprotein fractions

Reliability of the results was first confirmed with reference to the distribution of plasma- and RBC-tocopherol levels, which displayed a normal distribution similar to that described in our previous report (9), as illustrated in Fig. 1. The
tocopherol content in LDL, HDL, and VLDL also displayed a normal distribution.

2. Age differences in plasma lipids, lipoproteins, and tocopherol contents in lipoproteins

As shown in Table 1, there were no significant age differences in plasma lipids including total cholesterol (T-ch), triglycerides (TG), phospholipids (PL), and total lipids, or in lipoproteins, including HDL-cholesterol (HDL-ch), LDL, and VLDL. Table 2 shows the relation of tocopherol content in RBC, plasma, HDL, LDL, and VLDL to age. There were no significant age differences in any of these categories. Among the lipoproteins, HDL was slightly higher than LDL in both the male and
Table 1. Age differences in distribution of lipids and lipoproteins levels.

<table>
<thead>
<tr>
<th>Lipids &amp; Lipoproteins</th>
<th>T-ch (mg/100 ml)</th>
<th>TG (mg/100 ml)</th>
<th>PL (mg/100 ml)</th>
<th>Total lipids (mg/100 ml)</th>
<th>HDL-ch (mg/100 ml)</th>
<th>LDL (mg/100 ml)</th>
<th>VLDL (mg/100 ml)</th>
</tr>
</thead>
</table>
| < 1 yr
n = 6                 | 153.2 ± 21.3    | 88.5 ± 19.9    | 194.5 ± 21.7   | 417.0 ± 49.2             | 42.8 ± 10.8       | 378.8 ± 48.1   | 80.0 ± 50.0    |
| 1 yr ≤ < 3 yrs
n = 15                | 158.6 ± 21.8    | 90.8 ± 31.5    | 170.1 ± 27.0   | 428.1 ± 83.3             | 42.0 ± 21.7       | 389.5 ± 76.5   | 75.5 ± 42.7    |
| 3 yrs ≤ < 6 yrs
n = 35                | 163.0 ± 28.6    | 80.6 ± 34.4    | 175.7 ± 31.9   | 425.1 ± 78.9             | 45.8 ± 14.4       | 388.8 ± 85.9   | 57.0 ± 43.7    |
| 6 yrs ≤ < 11 yrs
n = 11                | 162.3 ± 25.2    | 81.3 ± 30.9    | 177.5 ± 25.9   | 429.5 ± 75.6             | 54.7 ± 22.7       | 342.5 ± 68.3   | 58.8 ± 56.0    |
| 11 yrs ≤ < 12 yrs
n = 12                | 164.8 ± 20.1    | 77.7 ± 27.8    | 178.7 ± 17.9   | 425.3 ± 44.9             | 50.0 ± 8.6        | 326.7 ± 69.5   | 73.1 ± 41.9    |
| Total
n = 81                | 163.5 ± 25.9    | 83.0 ± 30.2    | 178.4 ± 29.6   | 435.4 ± 76.8             | 46.8 ± 16.4       | 374.0 ± 83.4   | 65.9 ± 47.5    |

Values represent M ± SD.
female groups, and therefore the HDL-tocopherol was also slightly higher than LDL-tocopherol, but these differences were not statistically significant.

3. Sex differences in plasma lipids, lipoproteins, and tocopherol content of lipoproteins

No sex differences were observed with respect to plasma lipids, lipoprotein fractions, and tocopherol content of individual lipoprotein fractions (Tables 3 and 4).

4. Relationship between RBC tocopherol and tocopherol content of individual lipoproteins

As shown in Fig. 2, a close correlation was found between RBC tocopherol concentrations and tocopherol content in HDL fractions, but there were no correlations between RBC tocopherol and LDL tocopherol (Fig. 3), and between RBC tocopherol and VLDL tocopherol (Fig. 4).

DISCUSSION

Among eight naturally occurring compounds of tocopherols, only the alpha-form was considered in this study, since alpha-tocopherol is biologically the most active form and exits in more than 80% of tocopherols involved in human tissues. The samples studies were standard for Japanese children, since the mean...
Table 3. Sex differences in distribution of lipids and lipoproteins.

<table>
<thead>
<tr>
<th>Lipids &amp; Lipoproteins (mg/100 ml)</th>
<th>All (n=81)</th>
<th>Males (n=44)</th>
<th>Females (n=37)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T-ch</td>
<td>163.5 ± 25.9</td>
<td>162.1 ± 26.5</td>
<td>165.2 ± 25.4</td>
</tr>
<tr>
<td>TG</td>
<td>83.0 ± 30.2</td>
<td>84.4 ± 30.3</td>
<td>80.0 ± 30.7</td>
</tr>
<tr>
<td>PL</td>
<td>178.4 ± 29.6</td>
<td>177.0 ± 31.7</td>
<td>180.1 ± 27.2</td>
</tr>
<tr>
<td>Total lipids</td>
<td>435.4 ± 76.8</td>
<td>436.2 ± 77.9</td>
<td>434.6 ± 76.7</td>
</tr>
<tr>
<td>HDL-ch</td>
<td>46.8 ± 16.4</td>
<td>45.6 ± 17.0</td>
<td>47.1 ± 16.7</td>
</tr>
<tr>
<td>LDL</td>
<td>374.0 ± 83.4</td>
<td>379.0 ± 74.0</td>
<td>364.0 ± 91.1</td>
</tr>
<tr>
<td>VLDL</td>
<td>65.9 ± 47.5</td>
<td>64.3 ± 48.9</td>
<td>64.3 ± 44.3</td>
</tr>
</tbody>
</table>

Values represent M ± SD.

Table 4. Sex differences of tocopherol contents in RBCs, plasma, and individual lipoprotein fractions.

<table>
<thead>
<tr>
<th>x-Toc. (µg/100 ml)</th>
<th>All (n=81)</th>
<th>Males (n=44)</th>
<th>Females (n=37)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBC</td>
<td>182 ± 44.5</td>
<td>187 ± 44.2</td>
<td>177 ± 44.7</td>
</tr>
<tr>
<td>Plasma</td>
<td>664 ± 168.8</td>
<td>673 ± 186.4</td>
<td>653 ± 149.7</td>
</tr>
<tr>
<td>HDL</td>
<td>306 ± 85.1</td>
<td>313 ± 88.1</td>
<td>297 ± 81.9</td>
</tr>
<tr>
<td>(47.0 ± 11.0%)</td>
<td>(48.0 ± 11.4%)</td>
<td>(46.0 ± 10.7%)</td>
<td></td>
</tr>
<tr>
<td>LDL</td>
<td>274 ± 109.1</td>
<td>277 ± 115.9</td>
<td>269 ± 101.9</td>
</tr>
<tr>
<td>(40.5 ± 10.4%)</td>
<td>(40.0 ± 10.0%)</td>
<td>(41.0 ± 11.2%)</td>
<td></td>
</tr>
<tr>
<td>VLDL</td>
<td>78 ± 44.6</td>
<td>77 ± 47.8</td>
<td>81 ± 43.5</td>
</tr>
<tr>
<td>(11.9 ± 5.8%)</td>
<td>(11.2 ± 6.0%)</td>
<td>(12.5 ± 6.2%)</td>
<td></td>
</tr>
</tbody>
</table>

Values represent M ± SD.

Fig. 2. Relationship between RBC tocopherol and HDL tocopherol.

Levels of plasma lipids and tocopherol as well as RBC tocopherol were consistent with the values previously reported by Mino et al. (9), and in addition, were normally distributed.

Many studies on the distribution of tocopherol among lipoproteins have yielded conflicting findings as to whether LDL or HDL is the main carrier for delivery of tocopherol to the cells (19–25).

Bjornson et al. (26) and Ogihara et al. (3) have reported that the distribution of tocopherol among lipoproteins is directly related to the total lipid content of the lipoprotein fractions. In view of these findings, it appears reasonable that no age differences were detected with respect to tocopherol distribution among lipoproteins, since no significant age differences in lipoprotein fractions and plasma lipids were found. However, several reports have indicated sex differences with respect to tocopherol distribution in lipoproteins. Lewis et al. (23) and Takahashi et al. (24) suggested that tocopherol distributed in HDL is generally predominant as compared with LDL in adult females. More recently, Behrens et al. (25) reported that in males the tocopherol distribution is predominantly in LDL rather than in HDL, while in females more tocopherol is distributed in HDL. Such sex differences with respect to tocopherol distribution in lipoproteins are attributed to sexual differences in lipoprotein distribution as reported by Behrens et al. (25) and Ogihara et al. (3).

In the present study, no age or sex differences in distribution of tocopherol
among individual lipoprotein fractions was observed in children. The mean of the HDL fraction in our group was slightly larger than that of the LDL fraction, and therefore the tocopherol content in HDL was greater than in LDL, but neither of these differences was statistically significant.

Delivering tocopherol to RBC membranes is believed to be directly related to the tocopherol involved in these lipoproteins. Collision mechanisms between RBC membranes and lipoproteins may play a role in tocopherol transfer, since there are no LDL receptors in RBC membranes. Kayden and Bjornson (2) reported that when individual lipoprotein fractions were incubated with RBCs, the tocopherol present in HDL was preferentially transported to these RBCs. The reason why HDL is thus able to deliver tocopherol to RBCs, is still not clear, but Ogihara et al. (3) suggested that the fact that HDL particles are the smallest among the lipoproteins may account for this, since the chances of contact between RBC membranes and the surface tocopherol in HDL molecules would be greater as compared with larger lipoprotein molecules such as LDL. This may explain the close relationship between RBC tocopherol and HDL tocopherol, which is consistent with the results Ogihara et al. (3) obtained by using ultracentrifugation for lipoprotein separation.

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REFERENCES