Apoprotein E phenotype determines serum cholesterol in infants during both high-cholesterol breast feeding and low-cholesterol formula feeding

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Abstract Our objective was to establish the role of the apoprotein (apo) E phenotype in determining serum cholesterol levels in infants fed exclusively on high-fat, high-cholesterol human milk and in those fed a low-cholesterol, high-unsaturated fat formula. The total and lipoprotein cholesterol, apoB, and triglyceride concentrations in serum were quantified and related to the apoE phenotype in 151 infants at birth and at 2, 6, 9, and 12 months of age. Forty-four had the E3/4 or 4/4 phenotype (E4 group), 94 had the E3/3 phenotype (E3 group), and 13 had the E2/3 or 2/4 phenotype (E2 group). In cord blood, cholesterol concentrations tended to be higher in the E4 than in the E2 group. With exclusive breast-feeding, the concentrations rose significantly faster and higher in the E4 group than in the E3 group or, especially, the E2 group. The values (mmol/L, mean ± SEM) were 1.6 ± 0.15, 1.5 ± 0.05, 1.4 ± 0.1 (P = n.s.) at birth; 4.2 ± 0.1, 3.8 ± 0.08, 3.4 ± 0.2 (P < 0.001) at 2 months; 4.4 ± 0.15, 3.9 ± 0.1, 3.4 ± 0.15 (P < 0.001) at 4 months; 4.3 ± 0.17, 4.0 ± 0.13, 3.7 ± 0.26 (P < 0.001) at 6 months; 4.8 ± 0.28, 4.4 ± 0.11, 3.8 ± 0.05 (P < 0.001) at 9 months; and 4.7 ± 0.11, 4.4 ± 0.08, 4.1 ± 0.19 (P < 0.001) at 12 months, for the E4, E3, and E2 groups, respectively. Increases in LDL cholesterol and LDL apoB behaved similarly. The total triglyceride, and total HDL, HDL2, and HDL3 cholesterol concentrations did not depend on the apoE phenotype. Among infants fed high-fat, high-cholesterol human milk, the total and LDL cholesterol concentrations and the LDL apoB concentration of those with the apoE phenotype 4/4 or 3/4 rose faster and to higher levels than in other infants. Among formula-fed infants, receiving a low-cholesterol, high-unsaturated fat diet, the differences between the apoE groups were smaller. — Kallio, M. J. T., L. Salmenperä, M. A. Siimes, J. Perheentupa, H. Gylling, and T. A. Miettinen. Apoprotein E phenotype determines serum cholesterol in infants during both high-cholesterol breast feeding and low-cholesterol formula feeding. J. Lipid Res. 1997. 38: 759–764.

Supplementary key words child * nutrition * cholesterol * LDL cholesterol * apoprotein E * human milk * breast feeding

Apolipoprotein (apo)E is involved in cholesterol homeostasis, and its various phenotypes exert different influences on the serum level, synthesis, and elimination of cholesterol and removal of chylomicron remnants, and even on the risk of coronary artery disease (1–12). ApoE is a constituent of triglyceride-rich lipoproteins, chylomicrons, and VLDL and its remnants, and is also present in small amounts in HDL. The main source of apoE is the liver, but it is also synthesized by several peripheral tissues (13). ApoE is an arginine-rich glycoprotein which, like apoB, serves as a ligand for the low-density lipoprotein (LDL) receptor and the LDL receptor-related protein, the postulated chylomicron-remnant receptor (13–15). Hence, triglyceride-rich lipoproteins are rapidly taken up by the liver.

There are three circulating alleles of apoE, namely e4, e3, and e2, that differ from each other by one unit of net charge due to single base-pair substitution at two codons in the fourth exon of the apoE gene on the long arm of chromosome 19 (16–18). As a consequence, six phenotypes of apoE can be detected in plasma: apoE2/2, E3/2, E3/3, E4/2, E4/3, and E4/4, which differ by an amino acid substitution at one or both of two sites (residues 112 and 158) (19, 20). ApoE4 differs from apoE3 due to an arginine-for-cysteine substitution at residue 112, while apoE2 differs from apoE3 due to a cysteine-for-arginine substitution at residue 158. Because of the similarity of their physiologic effects, these are commonly grouped as the E2 group (4/2, 3/2 and 2/2), the E3 group (3/3) and the E4 group (4/4 and 4/3).

The apoE phenotypes have several clinical implications; the E2 group is associated with low concentra-

Abbreviations: apo, apoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein; HDL2, high density lipoprotein 2; HDL3, high density lipoprotein 3; SEM, standard error of the mean; g, gram; kJ, kilojoule; kcal, kilocalorie; mL, milliliter.

†To whom correspondence should be addressed.
tions and the E4 group with high concentrations of serum total and LDL cholesterol (1, 2, 4, 5, 16, 21). In addition, the E4 group, as compared with the E2 group, has a higher efficiency of cholesterol absorption, and lower rates of cholesterol synthesis and removal of LDL apoB (3, 11, 22). Because of the different binding affinities of apoE4 and apoE2 to the LDL receptor and the LDL-related protein receptor (20), rates of postprandial clearance of remnant lipoproteins are low in subjects with the apoE 2/2 phenotype, and high in those with the apoE 4/4 phenotype (6). Subjects of the E4 group, in contrast to subjects of the E2 groups, are usually, but not consistently, responders to modifications of dietary cholesterol and fat content (8, 23–26).

Dietary fat and cholesterol strongly influence serum total cholesterol levels in infancy. Breast-fed infants develop higher total cholesterol concentrations than formula-fed infants during the first months of life, but the difference gradually diminishes over the first year (27, 28). As compared to infant formulas, human milk is rich in cholesterol and saturated fatty acids but poor in unsaturated fatty acids (29, 30), which explains its hypocholesterolemic effect (31, 32). Exclusively breast-fed infants receive 15–20 mg/kg cholesterol daily, while infants on formula-feeding receive only 2–5 mg/kg. Thus, healthy infants on prolonged exclusive breast-feeding offer a unique physiologic dietary model for assessing the effects of a high-cholesterol, high-saturated fat diet on serum lipid levels in infancy. The aim of the present study was to follow exclusively breast-fed infants and infants on a formula diet over the first year of life and compare the effects of the apoE phenotype on the serum lipids in these groups.

MATERIALS AND METHODS

Subjects

This investigation is part of a nutritional follow-up study of 200 parents and their infants (33). They were recruited 2–3 days after delivery with these criteria: a healthy and nonsmoking mother with uncomplicated pregnancy and delivery, and a full-term singleton infant with appropriate weight for gestational age, a 1-min Apgar score ≥8, and no evidence of disease by the age of 3 days. The mothers were encouraged to breast-feed exclusively as long as possible. The apoE phenotypes were determined in 151 of these infants. The total serum cholesterol concentration was measured at birth from umbilical vein blood, and from scalp or antecubital vein blood at the ages of 2 (n = 147), 6 (n = 144), 9 (n = 140), and 12 months (n = 147).

The exclusively breast-fed infant group consists of the infants that were fed exclusively from the breast without any supplementary formula or solid foods; their number was 129 at 2, 97 at 6, 31 at 9, and 6 at 12 months of age. The infants leaving this group were weaned to a formula and/or gradually to solid foods. The infant formula used was Tutteli® (Valio, Helsinki, Finland); it contained fat, 1.2 g/100 kJ (5.2/100 kcal); cholesterol, 1.4 mg/100 kJ (6.0 mg/100 kcal). Of its fatty acids, 56% were saturated, 27.7% monounsaturated, and 16.3% polyunsaturated. Expressed as percent of total fatty acid content, the proportions for fatty acids were: 14/0 = 7.8%, 16/0 = 22.5%, 18/0 = 11.0%, 18/1 = 25.3%, and 18/2 = 12.6%. VLDL, LDL, HDL2, and HDL3 cholesterol, triglyceride, and apoB concentrations were determined repeatedly in a subgroup of 30 infants at 2, 6, 9, and 12 months of age. In this subgroup, 19 infants were fed exclusively with breast milk and solid foods up to the age of 9 months.

Methods

Serum total cholesterol (34) and triglyceride levels (35) were determined with an AutoAnalyzer. For lipoprotein cholesterol measurement, an enzymatic method was used (36). VLDL was separated by ultracentrifugation at a density <1.006 g/mL. Cholesterol and apoB were quantified from the infranatant (apoB with commercial kits, M-Partigen; Behringwerke AG, Marburg, Germany) followed by measurement of HDL cholesterol after precipitation of apoB-containing particles with heparin-manganese (37). The difference between the infranatant and HDL cholesterol gave the LDL cholesterol level. After precipitation of apoB-containing lipoproteins with heparin-manganese, HDL2 cholesterol was precipitated by the addition of dextran sulfate (37), and HDL3 cholesterol was determined from the resulting supernatant. HDL3 cholesterol was calculated as the difference between total HDL cholesterol (heparin-manganese supernatant) and HDL3 cholesterol (dextran sulfate supernatant).

ApoE phenotyping was performed by isoelectric focusing (38). For data analysis, apoE 4/4 (n = 4) and 4/3 (n = 40) phenotypes were combined as the E4 group (n = 44), and apoE 4/2 (n = 2), and 3/2 (n = 11) were combined as the E2 group (n = 13), while homozygotes for the E3 allele were denoted as the E3 group (n = 94).

Significances were tested with analysis of variance for repeated measurements and with the two-sided Student’s t test.

The study was approved by the Ethical Committee of the Children’s Hospital, and is in accordance with the Helsinki Declaration.
ence between the apoE4 and E2 groups was also evident 2 months (Fig. 1). The values of the E3 group were always between the values of the E2 and E4 groups. The differences increased linearly between the ages of 2 and 12 months (Fig. 1). Table 1 presents the total cholesterol concentrations assessed in the exclusively breast-fed infants (Table 2).

Table 1: Serum levels of total cholesterol (n = 151), and VLDL, LDL, total HDL, HDL2, and HDL3 cholesterol (n = 30) during the first year of life, in all infants together and separated into exclusively breast-fed and formula-fed groups

<table>
<thead>
<tr>
<th>Age (months)</th>
<th>Total Chol</th>
<th>VLDL</th>
<th>LDL</th>
<th>Total HDL</th>
<th>HDL2</th>
<th>HDL3</th>
</tr>
</thead>
<tbody>
<tr>
<td>All infants together</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>3.7 ± 0.06</td>
<td>0.21 ± 0.03</td>
<td>2.4 ± 0.1</td>
<td>1.1 ± 0.06</td>
<td>0.69 ± 0.07</td>
<td>0.45 ± 0.02</td>
</tr>
<tr>
<td>6</td>
<td>5.9 ± 0.07</td>
<td>0.26 ± 0.03</td>
<td>2.8 ± 0.2</td>
<td>0.9 ± 0.04</td>
<td>0.47 ± 0.04</td>
<td>0.47 ± 0.01</td>
</tr>
<tr>
<td>9</td>
<td>4.3 ± 0.07</td>
<td>0.21 ± 0.02</td>
<td>2.8 ± 0.1</td>
<td>1.0 ± 0.03</td>
<td>0.49 ± 0.03</td>
<td>0.50 ± 0.01</td>
</tr>
<tr>
<td>12</td>
<td>4.5 ± 0.06</td>
<td>0.15 ± 0.02</td>
<td>2.9 ± 0.1</td>
<td>1.0 ± 0.03</td>
<td>0.46 ± 0.04</td>
<td>0.51 ± 0.02</td>
</tr>
<tr>
<td>Breast-fed infants</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>3.8 ± 0.06</td>
<td>0.13 ± 0.02</td>
<td>2.5 ± 0.1</td>
<td>1.2 ± 0.06</td>
<td>0.72 ± 0.06</td>
<td>0.47 ± 0.02</td>
</tr>
<tr>
<td>6</td>
<td>4.0 ± 0.09</td>
<td>0.23 ± 0.03</td>
<td>2.9 ± 0.2</td>
<td>1.0 ± 0.04</td>
<td>0.49 ± 0.03</td>
<td>0.48 ± 0.02</td>
</tr>
<tr>
<td>9</td>
<td>4.5 ± 0.12</td>
<td>0.23 ± 0.07</td>
<td>2.7 ± 0.3</td>
<td>1.1 ± 0.07</td>
<td>0.56 ± 0.05</td>
<td>0.50 ± 0.07</td>
</tr>
<tr>
<td>12</td>
<td>4.6 ± 0.10</td>
<td>0.11 ± 0.02</td>
<td>3.0 ± 0.2</td>
<td>1.0 ± 0.07</td>
<td>0.50 ± 0.05</td>
<td>0.53 ± 0.04</td>
</tr>
<tr>
<td>Formula-fed infants</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>2.9 ± 0.1</td>
<td>0.17 ± 0.07</td>
<td>1.6 ± 0.1</td>
<td>1.0 ± 0.14</td>
<td>0.55 ± 0.11</td>
<td>0.49 ± 0.05</td>
</tr>
<tr>
<td>6</td>
<td>3.5 ± 0.08</td>
<td>0.26 ± 0.06</td>
<td>2.2 ± 0.2</td>
<td>0.9 ± 0.05</td>
<td>0.45 ± 0.03</td>
<td>0.50 ± 0.02</td>
</tr>
<tr>
<td>9</td>
<td>4.1 ± 0.09</td>
<td>0.22 ± 0.03</td>
<td>2.5 ± 0.1</td>
<td>0.9 ± 0.07</td>
<td>0.43 ± 0.06</td>
<td>0.52 ± 0.02</td>
</tr>
<tr>
<td>12</td>
<td>4.4 ± 0.06</td>
<td>0.17 ± 0.03</td>
<td>2.8 ± 0.1</td>
<td>0.9 ± 0.06</td>
<td>0.42 ± 0.04</td>
<td>0.50 ± 0.03</td>
</tr>
</tbody>
</table>

Values are means ± SEM.

RESULTS

The serum total and LDL cholesterol concentrations increased gradually throughout the first year of life (Table 1, Table 2, and Table 3), while the concentrations of the other lipoproteins remained rather stable. At birth, the mean (±SEM) total cholesterol was 1.4 ± 0.1 mmol/L in the E2 group, 1.5 ± 0.05 mmol/L in the E3 group, and 1.6 ± 0.01 in the E4 group (n.s.). It rose, significantly faster and higher in the exclusively breast-fed infants of the E4 group than in the others. Thus, in the exclusively breast-fed infants, the total cholesterol concentrations were higher in the E4 group than in the E2 group at all time points after birth (Fig. 1). The values increased linearly between the ages of 2 and 12 months (Fig. 1). The values of the E3 group were always between the values of the E2 and E4 groups. The difference between the apoE4 and E2 groups was also evident when increments in serum cholesterol from birth were assessed in the exclusively breast-fed infants (Table 2).

Table 2: Increments in serum cholesterol at different ages from birth in exclusively breast-fed and formula-fed infants grouped by apoE phenotype

<table>
<thead>
<tr>
<th>Age (months)</th>
<th>4/4 and 4/3</th>
<th>3/3</th>
<th>2/3 and 2/4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Breast</td>
<td>Formula</td>
<td>Breast</td>
</tr>
<tr>
<td></td>
<td>mmol/L</td>
<td></td>
<td>mmol/L</td>
</tr>
<tr>
<td>2</td>
<td>2.6 ± 0.16</td>
<td>1.4 ± 0.5</td>
<td>2.3 ± 0.1</td>
</tr>
<tr>
<td>6</td>
<td>2.6 ± 0.21</td>
<td>2.0 ± 0.4</td>
<td>2.5 ± 0.14</td>
</tr>
<tr>
<td>9</td>
<td>3.5 ± 0.29</td>
<td>2.5 ± 0.39</td>
<td>2.8 ± 0.14</td>
</tr>
<tr>
<td>12</td>
<td>3.6 ± 0.32</td>
<td>3.0 ± 0.22</td>
<td>2.9 ± 0.16</td>
</tr>
</tbody>
</table>

Values are means ± SEM.

The LDL cholesterol concentrations of the three groups of infants differed similarly after the age of 2 months. The E4 group had the highest and the E2 group the lowest values (Table 3). The LDL apoB level showed similar differences at 9 and 12 months (Table 3). The total triglyceride, and total HDL, HDL2, and HDL3-cholesterol concentrations were not related to the apoE phenotype.

In the formula-fed infants, the apoE phenotype also strongly influenced the cholesterol levels (Fig. 2). Their cholesterol levels increased by 6 months, as in the exclusively breast-fed infants, although less. At the age of 6 months, the E4 group had the highest cholesterol concentration (3.6 ± 0.18 mmol/L) compared with the E3 group (3.5 ± 0.12 mmol/L) and the E2 group (2.8 ± 0.10 mmol/L) (P < 0.01). The differences between the groups were greatest at the age of 9 months (Fig. 2).

Obtaining a clear picture was hampered by the small sizes of the age groups. Hence, we completed the picture for the age of 6 months, estimating the missing

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values for each infant on each diet by extrapolating forward and backward from his or her true values according to the regression observed for each diet and E group. In the apoE4 group the high-cholesterol, high-saturated fat diet of breast feeding increased total cholesterol significantly more than the formula diet Fig. 3. The difference between the apoE3 and apoE2 groups was also clear.

DISCUSSION

The present data show clearly for the first time that the apoE phenotype strongly influences the serum total and LDL cholesterol levels during the first year of life in both exclusively breast-fed infants and in infants weaned to a diet of formula and solids. During exclusive breast feeding, the serum total cholesterol concentration was highest in the E4 group and lowest in the E2 group. The LDL cholesterol and apoB levels appeared to follow the same pattern. In the weaned infants, the
influence of the apoE phenotype was also clear. For each apoE group, the absolute values were up to 1 mmol/L lower than in the exclusively breast-fed infants, especially at the age of 2-9 months. In the apoE4 group this difference tended to persist even at the age of 12 months. The marked difference in the composition of the two diets, i.e., the high-cholesterol, high-saturated fatty acid intake from human milk versus the low-cholesterol, high-unsaturated fatty acid intake from the diet of formula and solids is responsible for the differences in cholesterol level among the groups of infants with different apoE phenotypes. During the first 6 months of life, the weaned infants of the E4 group were similar in cholesterol levels to the exclusively breast-fed infants of the E2 group.

The main reason for the higher cholesterol response in the exclusively breast-fed infants is presumably down-regulation of the LDL apoB receptor activity by the large intake of cholesterol and saturated fat. It is well known that, in general, such a diet results in enhanced cholesterol absorption and decreased LDL apoB uptake through reduced LDL apoB receptor activity. Thus, the relative increase in serum cholesterol during the first 6 months of life was clearly faster and higher in the exclusively breast-fed infants than in the formula-fed infants, irrespective of the apoE phenotype.

The increment in serum cholesterol in the exclusively breast-fed infants above the level of the formula-fed infants was higher in the E4 group than in the E2 group (Fig. 3). The difference between the two diets was significant mainly in the E4 group. Why then did the E4 group respond more strongly to exclusive breast feeding and, to some extent, to the formula diet than the apoE2 group? Saturated fatty acids may down-regulate LDL apoB receptor activity more sensitively in the E4 group than in the E2 group; in adults such mechanisms appear significant. Also, removal of remnants (of both chyomicrons and VLDL) by the liver may have been faster in the E4 group (6), resulting in a higher accumulation of LDL cholesterol. On a high-cholesterol diet, such as breast feeding, LDL removal might be effectively inhibited by down-regulation of the LDL apoB receptor. Furthermore, intestinal cholesterol absorption might be more efficient in the E4 subjects than in the apoE2 individuals (3). Adults of the E4 group, as compared with the E2 group, have more efficient intestinal absorption, and slower elimination and synthesis of cholesterol associated with slower removal of LDL apoB (12, 22). In addition, adults of the E2 group are usually nonresponders to cholesterol feeding because, in comparison to the E4 group, they have more efficient compensatory mechanisms for regulating cholesterol metabolism so that serum cholesterol remains low. In infants consuming mother’s milk, which is rich in cholesterol (15–20 mg/kg/day) and saturated fat, efficient cholesterol absorption may explain the high serum cholesterol levels, especially in the E4 group. The apoE-related regulation of the serum cholesterol level was less clearly expressed in the weaned infants than in the exclusively breast-fed infants. Similarly, the apoE-related regulation of the serum cholesterol level is usually less strongly expressed during a cholesterol-lowering diet than during the high-saturated fat, high-cholesterol Western diet.

Healthy infants on prolonged exclusive breast feeding offer a unique physiologic dietary model for assessing the effects of a high-cholesterol, high-saturated fat diet on serum lipid levels. Our results confirm earlier findings that the relation of apoE polymorphism to serum lipoprotein concentrations noted in adults can be seen already in children (39, 40). The apoE4/4 phenotype together with a diet high in cholesterol and saturated fat may be a significant inducer of hypercholesterolemia in this subpopulation. Because of the greater sensitivity of E4 individuals to dietary cholesterol and saturated fat, dietary intervention in this group may be more effective in reducing serum total cholesterol and LDL-cholesterol concentrations, and further in reducing the risk of coronary heart disease in these high-risk individuals in future. However, dietary intervention may be postponed to some extent, because infants do need the increased caloric density provided in human milk and also other nutrients contained in high-fat breast milk.

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