Effects of prospective, randomized cholesterol-lowering dietary intervention and apolipoprotein E phenotype on serum lipoprotein(a) concentrations of infants aged 7–24 mo

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ABSTRACT A high serum lipoprotein(a) [Lp(a)] concentration is associated with increased risk of coronary artery disease. Few external factors are able to markedly modify serum Lp(a) concentrations. The aim of this study was to evaluate how serum Lp(a) concentrations of infants between 7 and 24 mo of age change in a cholesterol-lowering dietary intervention, and to assess the influence of apolipoprotein (apo) E phenotypes on serum Lp(a) concentrations. The intervention children (n = 394) had serum cholesterol, non-high-density-lipoprotein cholesterol, and cholesterol corrected for Lp(a)-cholesterol values (P for all < 0.001) lower than those of the control children (n = 390), but median serum Lp(a) concentrations at the age of 24 mo were not different from those of control children. Serum Lp(a) values differed according to the apo E phenotype as the median Lp(a) values increased from E2/E2 to E3/E2, E4/E2, E3/E3, E4/E3, and to E4/E4 (P for the difference = 0.023, Mann-Whitney U test). Our results suggest that apo E phenotype influences serum Lp(a) concentrations noticeably, but the effect of the cholesterol-lowering dietary intervention was not significant in subjects aged 24 mo.

KEY WORDS Lipoprotein(a), children, dietary intervention, apolipoprotein E, prospective trial

INTRODUCTION

A high serum lipoprotein(a) [Lp(a)] concentration is an independent risk factor for cardiovascular and cerebrovascular disease (1–4). An Lp(a) particle consists of a low-density-lipoprotein (LDL) moiety that is covalently linked by a disulfide bridge to apolipoprotein(a) [apo(a)]. Apo(a) is a glycoprotein that closely resembles plasminogen (5). The mechanisms by which high serum Lp(a) concentrations influence the atherosclerotic process and/or fibrinolysis (6) and contribute to coronary artery disease and stroke have remained unresolved.

The serum concentration of Lp(a) is highly genetically determined (7–9). Excessive trans fatty acids or saturated fatty acids in the diet (10–12), exposure to nicotine acid, estrogen, and tamoxifen are the only environmental factors known to influence serum Lp(a) concentration in adults (13–15). We showed earlier that infants who received breast milk as their only milk source at 7 mo of age had lower Lp(a) concentrations than infants who received formula (16). After weaning, serum Lp(a) concentrations increased more in initially breast-fed infants than in initially formula-fed infants (17).

Apo E is a constituent of chylomicrons and very-low-density lipoproteins (VLDLs) and is present in subfractions of high-density lipoproteins (HDLs). Apo E acts as a ligand and mediates endocytosis of the VLDL particles and chylomicrons by the liver (18, 19). It is genetically polymorphic and has three major isoforms (E2, E3, and E4) encoded by three respective alleles (ε2, ε3, and ε4) (20–23). The ε4 allele is associated with high serum concentrations of cholesterol, LDL cholesterol, and apo B, whereas the ε2 allele is associated with low serum total and LDL-cholesterol concentrations (20, 24). However, the results of studies on the association between the different apo E phenotypes and serum Lp(a) concentration are contradictory (25–28). Interestingly, there is a theoretical basis for such an association because there is a structural similarity between Lp(a) and LDL particles, suggesting that catabolism of Lp(a) might be at least partly regulated by the LDL receptors.

In 1990 we launched a prospective randomized trial that aimed to decrease exposure of infants and young children to known coronary artery disease risk factors (29). The trial began with infants aged 7 mo. This study investigated how the cholesterol-lowering dietary intervention influenced serum Lp(a) concentration, how serum Lp(a) and other serum lipids and lipoprotein concentrations correlated, and how the apo E phe...
notype influenced the Lp(a) concentration up to the age of 24 mo.

SUBJECTS AND METHODS

Subjects

The STRIP baby trial (Special Turku coronary Risk factor Intervention Project for babies) comprised 1062 children recruited from the well-baby clinics of the city of Turku (57% of the eligible families) who were all aged 7 mo at the time of recruitment. The study began in March 1990 and recruitment ended in June 1992. The families received information about the project and were randomly assigned into intervention (n = 522) and control (n = 540) groups. At the age of 24 mo, 86.5% of the children continued in the study. The dropout rate was similar in both groups. The most common known reason of dropout was because the family moved to another region. However, many of the parents did not give any explanation for withdrawal from the study.

The Joint Ethics Committee of Turku University and Turku University Central Hospital approved the study. Informed consent was obtained from the parents of the children.

Study design

The intervention group received individualized dietary counseling that aimed to increase the proportion of mono- and polyunsaturated fat and decrease the proportion of saturated fat in the diet. The ideal goal was to modify the ratio of polyunsaturated to monounsaturated to saturated fatty acids to 1:1:1, but to keep energy and fat intakes unchanged. At 24 mo of age, the optimal diet would contain 30% of energy as fat, 15% as protein, and 55% as carbohydrate.

The control group families received the dietary counseling routinely provided in the well-baby clinics in Finland. Such counseling has led to an average diet with 33% of energy as fat, 16% as protein, and 51% as carbohydrate, and with a ratio of polyunsaturated to saturated fatty acids of 0.43 for children aged 1–2 y (30).

Dietary records

Children’s food consumption was recorded using 3–4-d food diaries at 8, 13, and 24 mo of age. The dietitians carefully instructed the parents in food recording. Written instructions with drawings of food proportions were also distributed. The records included at least one weekend day and were evenly distributed throughout the year in the intervention and control families. The timing of the food diaries was occasionally changed due to acute illnesses. The foods were measured with household measures or weighed on domestic scales by the parents and/or daycare providers. The data were entered into a computer by an experienced dietitian. Nutrient compositions of the diets were analyzed by using the MICRO-NUTRICA PC program (Research and Development Unit of the Social Insurance Institution, Turku, Finland). This program calculates the contents of 62 nutrients in over 650 of the most commonly used foods in Finland, including baby foods and infant formulas. The program is continuously updated.

Blood sampling

Nonfasting venous blood samples were collected under cutaneous anesthesia (EMLA, Astra, Södertälje, Sweden) at 7, 13, and 24 mo of age. Serum was separated by low-speed centrifugation (3500 × g) and stored at −25 °C for <1 mo before laboratory analysis. A serum sample was available for Lp(a) determination at the age of 24 mo in 784 children; for 491 children, samples were available at 7 and 13 as well as at 24 mo of age.

Analyses

The Lp(a) concentration was analyzed by using a two-site immunoradiometric assay (Pharmacia, Uppsala, Sweden). The assay is based on a direct sandwich technique in which two monoclonal antibodies directed against separate antigenic determinants on the apo(a) molecule are used (31). The detection limit of the assay is 12 mg/L. The intraassay interassay CVs for the determination were 1.9% (4.4%) at 180 mg/L and 2.3% (4.9%) at 45 mg/L. The serum cholesterol concentration was measured after precipitation of LDLs and VLDLs with dextran sulfate 500000 (33). Intraassay interassay CVs for the cholesterol and HDL-cholesterol assays were 1.5% (2.0%) and 1.2% (1.2%), respectively. Serum cholesteryl ester fatty acids were analyzed with gas-liquid chromatography as described (34). Apo E phenotype was determined by using isoelectric focusing (25). The trans fatty acid contents of 15 vegetable margarine preparations used by the children were measured at the Department of Applied Chemistry and Microbiology of the University of Helsinki. Data on the trans fatty acid content of one preparation were obtained from the manufacturer.

Weights and heights of the children were monitored at each visit. Weight was expressed in absolute and relative values, ie, as percentages of the mean weight-for-height of healthy Finnish children (35). Height was expressed in absolute and as SD values that were based on the height-for-age curves of healthy Finnish children (35). Medical histories of the children (acute and chronic diseases) were recorded at every visit.

Statistical analysis

The Statistical Analysis System (SAS Institute, Cary, NC) and BMDP (BMDP Statistical Software, Los Angeles) software packages were used for data analysis. The distributions of serum Lp(a) concentrations were markedly skewed, and median values were used in statistical analyses. The differences between the groups were tested by using the Mann-Whitney U test. The intragroup differences between consecutive measurements were tested with signed rank tests. The correlations of serum Lp(a) concentration with serum concentrations of other lipoproteins and fatty acids, food diary data, and height and weight were expressed by using Spearman correlation coefficients. In analysis of the contribution of two factors to serum Lp(a) concentrations we used polynomic-regression analysis in which Lp(a) values were log-transformed.

Serum cholesterol and HDL-cholesterol concentrations were expressed as mean ± SD. The serum cholesterol concentration corrected for Lp(a) cholesterol was calculated by subtracting [0.3 × serum Lp(a) (in mg/L) divided by 387] from the serum cholesterol concentration, expressed in mmol/L (36). Serum cholesteryl ester fatty acid contents were expressed as proportions of the sum of all measured cholesteryl ester fatty acids
TABLE 1
Dietary intakes of the intervention and control children at 24 mo of age

<table>
<thead>
<tr>
<th>Dietary intake</th>
<th>Intervention group (n = 326)</th>
<th>Control group (n = 334)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy intake (kcal/d)</td>
<td>4733 ± 828</td>
<td>4828 ± 860</td>
</tr>
<tr>
<td>Protein (% of energy)</td>
<td>17.1 ± 2.4</td>
<td>16.1 ± 2.3</td>
</tr>
<tr>
<td>Carbohydrate (% of energy)</td>
<td>53.2 ± 5.2</td>
<td>51.1 ± 5.3</td>
</tr>
<tr>
<td>Fat (% of energy)</td>
<td>29.6 ± 5.1</td>
<td>32.8 ± 4.8</td>
</tr>
<tr>
<td>PUFA (% of energy)</td>
<td>5.3 ± 1.4</td>
<td>4.5 ± 1.4</td>
</tr>
<tr>
<td>MUF A (% of energy)</td>
<td>10.4 ± 2.3</td>
<td>10.8 ± 2.0</td>
</tr>
<tr>
<td>SAFA (% of energy)</td>
<td>10.8 ± 2.8</td>
<td>14.3 ± 3.0</td>
</tr>
</tbody>
</table>

16:0 (% of energy) | 5.5 ± 1.4 | 7.0 ± 1.4 |
(18:2 (% of fatty acids) | 18.5 ± 3.2 | 21.3 ± 2.6 |
18:2 (% of energy) | 2.3 ± 0.6 | 2.8 ± 0.6 |
(18:2 (% of fatty acids) | 7.8 ± 1.4 | 8.7 ± 1.1 |
18:3 (% of energy) | 4.3 ± 1.2 | 3.6 ± 1.3 |
(18:3 (% of fatty acids) | 14.5 ± 3.7 | 11.0 ± 3.4 |
Trans fatty acids (% of energy) | 0.5 ± 0.5 | 0.4 ± 0.4 |
(Trans fatty acids (% of fatty acids) | 1.8 ± 1.5 | 1.3 ± 1.3 |

1 Mean ± SD.
2 Significantly different from intervention group: *P < 0.001.
3 P < 0.011, 4P = 0.002.

(14:0–22:6). Student’s t test was used in comparisons of the fatty acid concentrations.

TABLE 2
Serum lipoprotein concentrations in the intervention and control groups at 24 mo of age

<table>
<thead>
<tr>
<th>Lipid variables</th>
<th>Intervention group (n = 394)</th>
<th>Control group (n = 390)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol (mmol/L)</td>
<td>4.08 ± 0.69</td>
<td>3.42 ± 0.77</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/L)</td>
<td>0.97 ± 0.19</td>
<td>1.02 ± 0.19</td>
</tr>
<tr>
<td>Non-HDL cholesterol (mmol/L)</td>
<td>3.11 ± 0.65</td>
<td>3.30 ± 0.74</td>
</tr>
<tr>
<td>Cholesterol corrected for Lp(a)-cholesterol (mmol/L)</td>
<td>3.98 ± 0.68</td>
<td>4.21 ± 0.76</td>
</tr>
<tr>
<td>Non-HDL cholesterol corrected for Lp(a) cholesterol (mmol/L)</td>
<td>3.01 ± 0.64</td>
<td>3.19 ± 0.73</td>
</tr>
</tbody>
</table>

RESULTS

Daily energy intakes of the intervention and control children were not different at the age of 24 mo (Table 1). The intervention children received proportionally more energy from protein and carbohydrate and less from fat than the control children (P < 0.001). The fat compositions of the diets also differed; intervention children received more polyunsaturated fatty acids, slightly but significantly less monounsaturated fatty acids, and clearly less saturated fatty acids than control children. The intervention children ingested less palmitic acid (16:0) and stearic acid (18:0) but more linoleic acid (18:2) than the control children.

TABLE 3
Median and mean serum lipoprotein(a) concentrations in children at 7, 13, and 24 mo of age

<table>
<thead>
<tr>
<th>Age</th>
<th>7 mo</th>
<th>13 mo</th>
<th>24 mo</th>
</tr>
</thead>
<tbody>
<tr>
<td>mg/L</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intervention children</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>77.4 ± 115.4 [375]</td>
<td>154.2 ± 232.7 [444]</td>
<td>133.0 ± 199.2 [394]</td>
</tr>
<tr>
<td>Median, range</td>
<td>33.0 (±12–1188)</td>
<td>61.5 (±12–1880)</td>
<td>59.0 (±12–1495)</td>
</tr>
<tr>
<td>Subgroup with complete data</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>85.1 ± 131.9 [254]</td>
<td>162.6 ± 241.6 [254]</td>
<td>143.6 ± 214.8 [254]</td>
</tr>
<tr>
<td>Median, range</td>
<td>33.5 (±12–1188)</td>
<td>62.0 (±12–1880)</td>
<td>62.5 (±12–1495)</td>
</tr>
<tr>
<td>Absolute and relative (%) change in median</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7 to 13 mo</td>
<td>+27.0 (±76.9)</td>
<td>+20.0 (±58.8)</td>
<td>−6.0 (−9.7)</td>
</tr>
<tr>
<td>7 to 24 mo</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13 to 24 mo</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control children</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>91.9 ± 153.4 [372]</td>
<td>156.7 ± 221.4 [413]</td>
<td>132.2 ± 197.3 [390]</td>
</tr>
<tr>
<td>Median, range</td>
<td>35.0 (±12–1147)</td>
<td>72.0 (±12–1565)</td>
<td>52.0 (±12–1430)</td>
</tr>
<tr>
<td>Subgroup with complete data</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>99.0 ± 156.2 [236]</td>
<td>166.0 ± 250.1 [236]</td>
<td>148.6 ± 221.8 [236]</td>
</tr>
<tr>
<td>Median, range</td>
<td>37.0 (±12–1147)</td>
<td>67.5 (±12–1565)</td>
<td>56.0 (±12–1430)</td>
</tr>
<tr>
<td>Absolute and relative (%) change in median</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7 to 13 mo</td>
<td>+23.5 (±54.6)</td>
<td>+14.0 (±44.3)</td>
<td>−3.5 (−7.0)</td>
</tr>
</tbody>
</table>

1 There were no significant differences between groups for any measures, P > 0.05. Significant differences in median lipoprotein(a) concentration for both groups between 7 and 13 mo, between 7 and 24 mo, and between 13 and 24 mo, P < 0.001.
2 Mean ± SD; n in brackets.
To evaluate effects of palmitic, stearic, and trans fatty acid intake on children’s serum Lp(a) concentrations, the children’s intakes were divided into quartiles. There was no difference in median serum Lp(a) concentrations between the quartiles, despite the larger trans fatty acid intake of the intervention children (data not shown). The Lp(a) concentrations of the children who used margarines high or low in trans fatty acids (≥ 10% or < 5% from all fatty acids, respectively) were not different. The intervention children grew according to the Finnish growth curves for healthy children without any delay compared with control children. Serum Lp(a) concentrations and the heights or weights of the children showed no correlation (data not shown). The number of infections recorded was higher in the intervention children during the first 2 y (5.4 ± 4.1 compared with 4.6 ± 3.6 infections, \( P = 0.004 \), possibly due to a reporting bias because the intervention families were seen twice as often as the control families.

At the age of 24 mo the intervention children had lower serum concentrations of cholesterol, non-HDL cholesterol, cholesterol corrected for Lp(a) cholesterol, non-HDL-cholesterol corrected for Lp(a) cholesterol, and HDL cholesterol, than the control children (\( P \) for all < 0.001) (Table 2). However, the ratios of cholesterol to HDL cholesterol were similar in the intervention and control children (4.32 and 4.34, respectively; \( P = 0.74 \)). Serum Lp(a) concentrations correlated in combined intervention and control groups with serum cholesterol (\( r = 0.18, P < 0.001 \)) and non-HDL cholesterol concentrations (\( r = 0.20, P < 0.001 \)), but not with cholesterol corrected for Lp(a) cholesterol (\( r = 0.02, \text{NS} \)) and non-HDL cholesterol corrected for Lp(a) cholesterol values (\( r = 0.03, \text{NS} \)).

Median serum Lp(a) concentrations were not different in the intervention and control infants at the ages of 7, 13, and 24 mo (Table 3). The Lp(a) concentrations increased in both groups between the ages of 7 and 13 mo (\( P < 0.001 \)). Interestingly, median serum Lp(a) concentrations decreased in both the intervention and control groups between 13 and 24 mo of age (absolute changes, both \( P < 0.0001 \); relative change, \( P = 0.002 \) and \( P = 0.013 \), respectively; signed-rank test). The absolute changes in serum Lp(a) concentrations between 7 and 13 mo of age and between 7 and 24 mo of age were not different.

There was a significant overall variance in serum Lp(a) concentrations between children having different apo E phenotypes at the ages of 13 and 24 mo but not at the age of 7 mo (Mann-Whitney \( U \) test) (Table 4 and Figure 1). Children with the highest and lowest serum Lp(a) concentrations belonged to the apo E4/4 and apo E2/2 phenotypes, respectively. At each measurement, the children with the apo E2/2 phenotype had significantly lower Lp(a) concentrations than the children with apo E4/4 (at 7 mo of age, \( P = 0.03 \); at 13 mo of age, \( P = 0.004 \); at 24 mo of age, \( P = 0.006 \), Mann-Whitney \( U \) test). Apo E phenotype was significantly associated with serum cholesterol concentration; subjects with the E2/2 phenotype had the lowest cholesterol concentrations (Figure 1). A similar association was observed between apo E phenotype and serum cholesterol corrected for Lp(a) cholesterol (data not shown). In polynomial-regression analysis, the difference in serum Lp(a) concentration between apo E phenotypes was significant (\( P = 0.009 \) when cholesterol corrected for Lp(a) cholesterol was adjusted for. The association between apo E phenotype and serum Lp(a) concentration persisted when sex or diet group (\( P \) for both = 0.014) was included in the regression model.
DISCUSSION

The serum cholesterol concentration increased with increasing age less markedly in the intervention than in the control group, but the serum Lp(a) concentration increased similarly in both groups of children. A high-cholesterol diet has not been shown to influence serum Lp(a) concentrations although it affects serum cholesterol concentrations (37), suggesting that dietary factors regulating LDL and Lp(a) metabolism are different. Our findings agree with these results.

In our study, serum Lp(a) concentrations correlated with cholesterol and non-HDL-cholesterol concentrations only when cholesterol and non-HDL-cholesterol values were not corrected for the presence of Lp(a) cholesterol. This confirms earlier suggestions of different regulation of serum Lp(a) and LDL-cholesterol concentrations (37). The absence of a correlation between Lp(a) and cholesterol after correction for Lp(a) cholesterol is probably due to the skewness of the Lp(a) distribution, a result of the fact that the contribution of Lp(a) cholesterol varies greatly, from 0.1% to 21.4%, between individuals (38).

In adults, few dietary factors influence the serum Lp(a) concentration. Large amounts of trans fatty acids increase serum Lp(a) concentrations (10, 11). A diet rich in stearic acid leads to a higher serum Lp(a) concentration than does a diet rich in palmitic, myristic (14:0), and lauric (12:0) acids, and polysaturated or monounsaturated fatty acid supplementation decreases serum Lp(a) concentrations (12, 39). The serum Lp(a) concentration is lower in breast-fed 7-mo-old infants than in their formula-fed peers (16). Because serum Lp(a) concentrations increase markedly after weaning (17), we expected that other dietary factors might also influence serum Lp(a) concentrations in early childhood. However, in this study serum Lp(a) concentrations and changes in serum Lp(a) between 7 and 24 mo of age were similar in the intervention and control children despite the 3.5% (of energy) lower intake of saturated fat by the intervention group at 24 mo of age. Serum Lp(a) values and nutrient intakes also correlated poorly and the effect of trans fatty acid intake on the serum Lp(a) concentration was minimal. However, the amount of trans fatty acids in the diet was only 5–10% of that used in some studies in adults (10, 11). Therefore, we are unable to exclude the possibility that the high trans fatty acid content in the diet may affect serum Lp(a) concentration in early childhood.

Data on the influence of apo E phenotypes on the serum Lp(a) concentration are controversial. In Finnish children aged 3–18 y serum Lp(a) values were not associated with the apo E phenotype (25). The age distribution of this study population was broad, including pubertal children. The children in our study were all of the same age (7, 13, or 24 mo). Our results suggest that the median Lp(a) values increased from E2/2 to E3/2, E4/2, E3/3, E4/3, and to E4/4. In accordance with our results, two other studies showed that the serum Lp(a) concentration depends on apo E phenotype (26, 40). Others have also found that subjects with the apo E2 phenotype have the lowest serum Lp(a) concentrations (28, 40).

At first glance, our results may look partly controversial: serum Lp(a) concentrations failed to correlate with serum cholesterol corrected for Lp(a) cholesterol, but there was an association between serum Lp(a) concentration and the apo E phenotype, and between concentrations of cholesterol or cholesterol corrected for Lp(a) cholesterol and the apo E phenotype. The formation of Lp(a) includes synthesis of apo(a) in the liver followed by assembly of apo(a) with apo B (41, 42). Thus, different factors may regulate formation of LDL, its cholesterol content, and Lp(a), but the catabolism of LDL and Lp(a) may share common characteristics. In fact, there is evidence that Lp(a) is partly catabolized through the LDL-receptor pathway (26, 43). A likely explanation for the similar association of serum cholesterol and Lp(a) concentrations with the apo E phenotype is the apo E phenotype–dependent affinity of the apo E molecules toward the LDL receptor. The reduced affinity of apo E2 for the LDL receptor results in reduced delivery of remnant and intermediate-density-lipoprotein cholesterol to the liver, thereby enhancing hepatic LDL receptor expression (44). In turn, this may account for the reduced serum concentrations of cholesterol and Lp(a) observed in our subjects with the E2/2 phenotype.

In conclusion, this study shows that a dietary intervention aimed at decreased intake of saturated fat and cholesterol fails to influence the serum Lp(a) concentration in early childhood. The association of serum Lp(a) concentration and serum cholesterol concentration with apo E phenotype suggests that catabolism of Lp(a) and LDL particles may at least partly occur via the same pathway.
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