Endothelial Function in Healthy 11-Year-Old Children After Dietary Intervention With Onset in Infancy

The Special Turku Coronary Risk Factor Intervention Project for Children (STRIP)

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Background—Early childhood introduction of nutritional habits aimed at atherosclerosis prevention reduces children’s serum total cholesterol concentration, but its effect on vascular endothelial function is unknown.

Methods and Results—Between 1990 and 1992, we randomized healthy 7-month-old infants (n=1062) to intervention (low-saturated-fat diet) and control (unrestricted diet) groups. At the age of 11 years, endothelium-dependent (flow-mediated) and endothelium-independent (nitrate-mediated) vasodilatory responses of the brachial artery were measured with high-resolution ultrasound in 179 intervention and 190 control children. The effect of intervention on endothelial function was significant in boys (P=0.0034) but not in girls (P=0.69). The maximum endothelium-dependent dilation response (mean±SD) was 9.62±3.53% and 8.36±3.85% in intervention boys and control boys and 8.84±4.00% and 8.44±3.60% in intervention girls and control girls, respectively. Intervention had no effect on nitrate-mediated dilation. The difference in endothelial function in boys remained significant after adjustment for current serum total or LDL cholesterol but became nonsignificant after adjustment for mean cholesterol measured under 3 years of age (adjusted means: 9.46% [CI 8.68% to 10.24%] versus 8.54% [CI 7.75% to 9.32%], P=0.11).

Conclusions—A low-saturated-fat diet introduced in infancy and maintained during the first decade of life is associated with enhanced endothelial function in boys. The effect is explained in part by the diet-induced reduction in serum cholesterol concentration. (Circulation. 2005;112:3786-3794.)

Key Words: endothelium ■ pediatrics ■ atherosclerosis ■ cholesterol ■ diet

Hypercholesterolemia is associated with impaired endothelial function and accelerated atherogenesis in children. The relationship between cholesterol and endothelial function is not limited to extreme cholesterol levels, because serum cholesterol concentration also correlates with endothelial function in healthy children and adolescents. We and others have shown that exposure to high serum cholesterol concentration in childhood may induce changes in arteries that contribute to the development of atherosclerosis in adulthood. Consequently, the long-term prevention of atherosclerosis might be most effective when initiated early in life.

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In adults, the reduction of serum cholesterol concentration with diet or drug therapy improves endothelial function and is associated with decreased morbidity and mortality from cardiovascular disease. Enhanced endothelial function may be responsible for part of the benefits of cholesterol lowering on vascular health. The randomized, prospective Special Turku Coronary Risk Factor Intervention Project for children (STRIP) has demonstrated that a fat-modified diet (low in saturated fat) initiated in infancy and combined with repeated individualized dietary and lifestyle counseling lowers serum cholesterol concentration in children without adversely influencing growth or neurological development. We have now examined the effects of the intervention on vascular endothelial function at the age of 11 years. We hypothesized that restricting dietary intake of saturated fat would lead to enhanced endothelium-dependent, flow-mediated dilation of the brachial artery in the intervention children.

Methods

Study Design and Subjects

The study design of the ongoing STRIP study has been published previously. Recruitment took place between March 1990 and June 2000.
At the child’s routine 5-month visit to the child health center, all families were informed of the possibility of participating in the trial. There were 1054 voluntary families with 1062 infants (56.5% of the 1880 eligible infants in the age cohort). The children and their families were randomized to form an intervention group (n = 540) and a control group (n = 522) at the age of 7 months. The intervention group received dietary and lifestyle counseling twice a year given by a team of physicians and dietitians. The families recorded the child’s food consumption for 4 days twice a year. A dietitian examined the food records and gave appropriate individualized dietary instructions. The diet was designed to meet the Nordic Dietary Recommendations. To ensure compliance, parents were given detailed instructions on how to record food and fluid consumption. This was done by including drawings of ordinary foods with weights and common household measures in the instructions to help estimation of food consumption. To ensure compliance at day care, we informed the personnel about the study, emphasized the importance of maintaining the diet, and gave instructions on how to take into consideration the special arrangements with regard to the child’s diet, and gave instructions on how to record food type and consumption. Similarly, at schools, kitchen personnel were informed about the study. Both the parents and the day care personnel were instructed to record the type, brand, and preparation method of all foods used. These records were reviewed by nutritionists for completeness and accuracy at each visit. Individualized counseling was targeted accordingly, and if needed, portion sizes were added to the instructions with, for example, food models and household measures, food descriptions, and food preparation methods provided. The control children received the basic health education given at Finnish well-baby clinics and schools with a minimal amount of nutritional counseling after infancy. During their regular STRIP visits, the control children received no detailed dietary instructions. Based on biannual 4-day food records, the fat intake of the intervention children was constantly ~30% of the energy intake, whereas that of the control children was significantly (P < 0.001) higher, by 2 to 3 calorie percentage units. The intervention children received 2 to 3 calorie percentage units fewer saturated fats and 0.5 to 1.0 calorie percentage units more polyunsaturated fats than the control children. The nutrient intakes of the parents were assessed with 24-hour recall. When children were 5 years of age, both parents of the intervention children consumed less saturated fat (P < 0.001) than control parents. The intervention had no effect on early or later growth of the children.

The study was approved by the Joint Commission on Ethics of the Turku University and the Turku University Central Hospital. Informed consent was obtained from all guardians.

**Physical Examination and Interview**

Weight was measured with an electronic scale to the nearest 0.1 kg (Soehnle S10; Soehnle). Height was measured with the Harpenden stadiometer (Holtain). Blood pressure was measured from the non-dominant arm with a standard sphygmomanometer. The mean of 3 measurements was used in the statistical analyses. Tanner staging was used to classify sexual maturation. Participation in leisure-time physical activity was assessed by asking the frequency of participation in physical activity outside school hours. Exposure to environ-

![Flow diagram of the STRIP trial.](image)
mental tobacco smoke (during the past 3 days) was assessed in an interview. Family history was considered positive if the study subject’s father, mother, grandfather, or grandmother had had a myocardial infarction.

**Measurement of Serum Lipids**

Serum total cholesterol, HDL cholesterol, and triglycerides concentrations were measured with standard methods as described previously. Nonfasting blood samples were drawn when the child was less than 5 years old, and fasting samples were taken thereafter. Cholesterol measurements were performed at ages 7 and 13 months and at 2, 3, 4, 5, 7, 9, 10, and 11 years. Of the study children, 93% had data available on 8 or more cholesterol measurements (mean ± SD = 12 ± 2.3). The Friedewald formula was used to calculate serum LDL cholesterol values. To assess the relation of serum cholesterol measurements at various ages to flow-mediated vasodilation, we calculated 3 mean cholesterol concentration variables: between 7 months and 2 years, between 3 and 5 years, and between 7 and 11 years.

**Brachial Artery Ultrasound Studies**

Recruitment for ultrasound studies began in the middle of the year when the 11-year follow-up visits were ongoing. Children who had not already participated in their 11-year visit were recruited for the study. Thus, the ultrasound group represents a time-restricted cohort. Nonfasting blood samples were drawn when the child was less than 5 years old, and fasting samples were taken thereafter. Cholesterol measurements were performed at ages 7 and 13 months and at 2, 3, 4, 5, 7, 9, 10, and 11 years. Of the study children, 93% had data available on 8 or more cholesterol measurements (mean ± SD = 12 ± 2.3). The Friedewald formula was used to calculate serum LDL cholesterol values. To assess the relation of serum cholesterol measurements at various ages to flow-mediated vasodilation, we calculated 3 mean cholesterol concentration variables: between 7 months and 2 years, between 3 and 5 years, and between 7 and 11 years.

**Statistical Analyses**

The groups were compared with Student’s *t* test or nonparametric Mann-Whitney *U* test as appropriate. Regression coefficients were calculated to study the determinants of flow-mediated vasodilation. ANCOVA was used to calculate adjusted means. We used repeated-measures ANOVA to test whether there were differences between children with and without complete ultrasound data in longitudinal values of serum cholesterol and saturated fat intake, whether there was a sex difference in the effect of the intervention on endothelial function, and whether the magnitude of flow-mediated vasodilatory responses differed between the study groups. A 2-tailed *P* < 0.05 was considered significant. SAS version 8.01 was used for statistical analyses.

**Results**

The baseline characteristics at randomization of all children and those with complete ultrasound data at follow-up are shown in Table 1. Comparison of baseline characteristics between children with and without complete ultrasound data revealed no significant differences (*P* always >0.15). In
addition, those intervention children who had complete ultrasound data did not differ significantly from other intervention children participating in the 11-year study with respect to serum lipoproteins, anthropometry, or blood pressure values (Table 2). Comparison of control children with and without ultrasound data at the age of 11 years yielded similar results (data not shown). Figure 2 shows that the mean values of serum cholesterol concentration and saturated fat intake at each follow-up point was different between intervention and control children but that there were no systematic differences in these 2 variables between children with and without ultrasound data. The results of a dropout analysis (shown in Figure 3) suggested that had been no systematic differences in participants and dropouts with respect to saturated fat intake and serum cholesterol concentration.

The characteristics of the study children at 11 years are shown in Table 3. Intervention children had lower intake of saturated fat and a higher ratio of polyunsaturated to saturated fatty acid than control children (all \( P < 0.001 \)). The boys in the intervention group had 0.23 mmol/L lower serum total cholesterol concentration \(( P = 0.035)\) and 0.26 mmol/L lower LDL concentration \(( P = 0.009)\) than the boys in the control group, whereas these values did not differ between intervention girls and control girls. No significant differences were noted in HDL cholesterol, triglycerides, body size measures, blood pressure, family risk of myocardial infarction, leisure-time physical activity, or exposure to environmental cigarette smoke. At the age of 11 years, puberty was ongoing in 65.7%, and had not started in 34.3% of the children, but none of the children had completed puberty. The distribution of pubertal stages did not differ between intervention and control groups \(( P = 0.64)\).

The results of the ultrasound studies are shown in Table 4. Maximum flow-mediated dilation \(( P = 0.012)\) and the area under the dilation response versus time curve \(( P = 0.004)\) were higher in the intervention boys than in the control boys, Brachial artery vessel size and increase in blood flow after cuff release were similar. The values of intervention and control girls did not differ.

The temporal development of flow-mediated dilation responses measured between 30 and 180 seconds after cuff release followed a similar pattern over time in the study groups, but the magnitude of the response was significantly greater in the intervention boys than in the control boys (group-by-sex interaction \( P = 0.01\); effect of intervention in boys \( P = 0.0034\); effect of intervention in girls \( P = 0.69\); Figure 4).
Significant (interaction terms the interactions between sex and cholesterol were nonsignificant). These relations were similar in boys and girls, because significantly correlated with flow-mediated vasodilation. Mean cholesterol values measured between ages 7 months and 2 years were significantly related to cholesterol values measured between ages 7 and 11 years and between 3 and 5 years were significantly related to cholesterol levels. However, the difference became nonsignificant after adjustments for current total cholesterol and LDL cholesterol levels. Moreover, the difference became nonsignificant after we took into account the mean cholesterol concentration measured after this age. Furthermore, the difference in endothelial function between intervention boys and control boys remained significant after adjustments for current total cholesterol and LDL cholesterol levels. However, the difference became nonsignificant after we took into account the mean cholesterol concentration measured between ages 7 months and 2 years. This suggests that the higher flow-mediated dilation seen in boys in the intervention group is not merely a reflection of recent cholesterol control but reflects the importance of early and long-term cholesterol control in influencing vascular function. These observations, however, need to be interpreted cautiously, because cholesterol values show significant tracking, i.e., a concentration measured at 1 time point reflects the concentration later in life. The mechanisms by which cholesterol interferes with endothelial function are not fully un-
stood but may include reduced availability of nitric oxide through a combination of decreased production, increased inactivation, and abnormal signaling.\textsuperscript{32}

During the trial, the intervention children consistently received 2 to 3 calorie percentage units fewer saturated fats and 0.5 to 1.0 calorie percentage units more polyunsaturated fats than the control children.\textsuperscript{16} With these dietary fat changes, the expected reduction in serum cholesterol concentration would be 0.16 to 0.24 mmol/L according to the Keys’ equation.\textsuperscript{33} In intervention boys, serum cholesterol concen-

\begin{table}
\centering
\caption{Characteristics of 369 Study Children at the Age of 11 Years}
\begin{tabular}{lllll}
\hline
Characteristic & Intervention & Control & \textit{P} & Intervention & Control & \textit{P} \\
\hline
\textit{n} & \textit{n} & \\

\textit{n} & \textit{n} & \\

\textit{n} & \textit{n} & \\

\textit{n} & \textit{n} & \\

\textit{n} & \textit{n} & \\

\hline
Age, mo & 132±2 & 132±1 & 0.38 & 132±1 & 132±1 & 0.38 \\
Height, cm & 148±7 & 148±8 & 0.79 & 148±7 & 146±6 & 0.22 \\
Weight, kg & 40±8 & 40±10 & 0.76 & 39±7 & 38±6 & 0.14 \\
Body mass index, kg/m\textsuperscript{2} & 18.1±2.7 & 18.2±3.2 & 0.68 & 18.0±2.5 & 17.6±2.6 & 0.32 \\
Birth weight, kg & 3.5±0.4 & 3.5±0.5 & 0.55 & 3.7±0.5 & 3.6±0.5 & 0.12 \\
Serum cholesterol, mmol/L & 4.53±0.72 & 4.58±0.80 & 0.62 & 4.37±0.57 & 4.58±0.85 & 0.03 \\
LDL cholesterol, mmol/L & 2.84±0.62 & 2.87±0.72 & 0.70 & 2.64±0.52 & 2.92±0.75 & 0.009 \\
HDL cholesterol, mmol/L & 1.27±0.23 & 1.29±0.26 & 0.41 & 1.34±0.28 & 1.32±0.28 & 0.86 \\
Triglycerides, mmol/L & 0.91±0.47 & 0.87±0.38 & 0.59 & 0.82±0.49 & 0.78±0.33 & 0.53 \\
Blood pressure, mm Hg & & & & & & \\
Systolic & 103±8 & 105±8 & 0.35 & 105±9 & 104±8 & 0.31 \\
Diastolic & 63±6 & 63±5 & 0.89 & 63±6 & 63±5 & 0.93 \\
Saturated fat, % of energy & 11.5±2.9 & 12.6±2.7 & 0.001 & 10.8±2.1 & 13.2±2.9 & <0.0001 \\
P/S ratio & 0.53±0.19 & 0.41±0.13 & <0.0001 & 0.59±0.19 & 0.42±0.14 & <0.0001 \\
Family risk, % & 21.4 & 23.5 & 0.72 & 21.1 & 20.2 & 0.88 \\
Physical activity, % & 34.8 & 32.0 & 0.68 & 33.3 & 34.8 & 0.83 \\
Exposure to tobacco smoke, % & 5.3 & 5.2 & 0.53 & 9.8 & 6.9 & 0.08 \\

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\begin{table}
\centering
\caption{Ultrasound Results of 369 Study Children at the Age of 11 Years}
\begin{tabular}{lllll}
\hline
Characteristic & Intervention & Control & \textit{P} & Intervention & Control & \textit{P} \\
\hline
\textit{n} & \textit{n} & \\

\textit{n} & \textit{n} & \\

\hline
Brachial artery diameter, mm & 2.9±0.3 & 2.9±0.3 & 0.38 & 3.0±0.3 & 3.0±0.3 & 0.91 \\
Increase in blood flow after cuff release, % & 345±155 & 346±145 & 0.99 & 368±155 & 390±157 & 0.35 \\
Nitrate-mediated dilation, % & 10.27±3.58 & 9.91±3.88 & 0.41 & 10.31±3.44 & 10.55±3.47 & 0.66 \\
Area under dilation vs time curve, %×s & 735±495 & 762±484 & 0.53 & 915±490 & 720±503 & 0.004 \\
Adjusted for cholesterol measurements* & & & & & & \\
Current LDL cholesterol, single measure & 734 & 768 & 0.63 & 919 & 731 & 0.01 \\
Age 7 to 11 years, average total cholesterol & 734 & 762 & 0.70 & 909 & 725 & 0.02 \\
Age 3 to 5 years, average total cholesterol & 730 & 761 & 0.66 & 902 & 735 & 0.03 \\
Age 7 months to 2 years, average total cholesterol & 718 & 771 & 0.48 & 893 & 749 & 0.06 \\
Maximum flow-mediated dilation, % & 8.84±4.00 & 8.44±3.60 & 0.47 & 9.62±3.53 & 8.36±3.85 & 0.012 \\
Adjusted for cholesterol measurements* & & & & & & \\
Current LDL cholesterol, single measure & 8.83 & 8.39 & 0.43 & 9.65 & 8.44 & 0.03 \\
Age 7 to 11 years, average total cholesterol & 8.83 & 8.44 & 0.48 & 9.55 & 8.43 & 0.05 \\
Age 3 to 5 years, average total cholesterol & 8.77 & 8.44 & 0.55 & 9.52 & 8.48 & 0.07 \\
Age 7 months to 2 years, average total cholesterol & 8.75 & 8.48 & 0.64 & 9.46 & 8.54 & 0.11 \\

\hline
\end{tabular}
\end{table}
flow-mediated vasodilation (1 percent unit) and 1-unit change in cholesterol (mmol/L).

Ages in All 369 Study Children

and Average Serum Total Cholesterol Measured at Different

TABLE 5. Relationships Between Flow-Mediated Vasodilation

and Average Serum Total Cholesterol Measured at Different Ages in All 369 Study Children

<table>
<thead>
<tr>
<th>Age at Cholesterol Measurement</th>
<th>β±SE</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>7 mo to 2 y</td>
<td>-0.760±0.290</td>
<td>0.009</td>
</tr>
<tr>
<td>3 to 5 y</td>
<td>-0.827±0.288</td>
<td>0.004</td>
</tr>
<tr>
<td>7 to 11 y</td>
<td>-0.496±0.298</td>
<td>0.096</td>
</tr>
<tr>
<td>Current cholesterol*</td>
<td>-0.319±0.263</td>
<td>0.235</td>
</tr>
<tr>
<td>Current LDL cholesterol*</td>
<td>-0.389±0.294</td>
<td>0.187</td>
</tr>
</tbody>
</table>

*Single measurement at 11 years.

The present study had limitations. A substantial number of children were lost to follow-up. The results of a dropout analysis, however, indicated that there were no systematic differences between participants and dropouts with respect to saturated fat intake and serum cholesterol concentration. This suggests that the children remaining in the study are representative of the original sample. The major weakness is that the ultrasound study was performed only in a subgroup of the initial study cohort; however, those children who had complete ultrasound data did not differ significantly from the other children at baseline or at the 11-year study in any of the measured characteristics. Furthermore, children who had complete ultrasound data had similar cholesterol values and similar saturated fat intake as other children participating at the 11-year study in each of the previous study years. These observations suggest that the children with ultrasound data are representative of the other study children.

The magnitude of difference in flow-mediated dilation between intervention and control boys was relatively small. Previous studies have shown much larger differences in endothelial function between children “at risk” and healthy controls; however, all children in the present study were healthy and were recruited from a general population. Our aim was not to improve endothelial dysfunction in a group of high-risk children but to investigate whether a long-term cholesterol-lowering diet would have a beneficial influence on endothelial health in a pediatric population with high adult rates of coronary artery disease. The mean flow-mediated...
dilution values were within the normal range in all children and were comparable for girls and control boys; however, the values for intervention boys were higher, which suggests a better endothelial status than would normally be expected in children consuming a regular Finnish diet. The intervention boys had been exposed to a lower cholesterol concentration throughout their lives, whereas the other groups had been exposed to cholesterol concentrations typical for Finnish children. In all subjects, cholesterol concentration correlated with flow-mediated dilation, which suggests a mechanistic relationship between cholesterol metabolism and endothelial function. Furthermore, the difference between intervention boys and control boys was attenuated when we took into account the cholesterol values measured during their lives. Therefore, the higher flow-mediated vasodilation responses observed in the intervention boys is consistent with the long-term cholesterol control induced by the dietary intervention begun in infancy.

Acknowledgments

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Disclosures

None.

References


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**CLINICAL PERSPECTIVE**

Exposure to high serum cholesterol concentration in childhood may accelerate the development of atherosclerosis. Consequently, the long-term prevention of atherosclerosis might be most effective when initiated early in life. The prospective STRIP trial was launched in 1990 to examine the effects of a low-saturated-fat diet in atherosclerosis risk prevention in children. At the age of 7 months, healthy Finnish children were randomized to low-saturated-fat diet and unrestricted diet groups. During the first decade of the trial, the children in the intervention group received 2 to 3 calorie percentage units less saturated fats than the children in the control group. The serum cholesterol concentration was consistently ~0.2 to 0.3 mmol/L lower in intervention boys than in control boys. Despite similar changes in the diet, no significant cholesterol-lowering effect was observed in girls. Because endothelial function may play a role in the early pathophysiology of atherosclerosis, a noninvasive ultrasound study was performed in these children at the age of 11 years to measure endothelium-dependent flow-mediated vasodilatory responses. Consistent with the long-term cholesterol control induced by the dietary intervention, the intervention was associated with enhanced endothelial function in boys. The STRIP study indicates that it is feasible to reduce saturated-fat intake in children. Estimating the magnitude of risk reduction associated with cholesterol lowering achieved in the STRIP trial will require long-term follow-up of these children, but the associated enhancement of endothelial function in boys suggests potentially advantageous effects on cardiovascular health.
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