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)

Olli T. Raitakari, MD, PhD; Tapani Rönnemaa, MD, PhD; Mikko J. Järvisalo, MD, PhD; Tuuli Kaitosaari, MD; Iina Volanen, MD; Katariina Kallio, MD; Hanna Lagström, PhD; Eero Jokinen, MD, PhD; Harri Niinikoski, MD, PhD; Jorma S.A. Viikari, MD, PhD; Olli Simell, MD, PhD

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[1] and accelerated atherogenesis in children.[2,3] 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.[4] 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.[5–7] Consequently, the long-term prevention of atherosclerosis might be most effective when initiated early in life.[8]

Clinical Perspective p 3794

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.[9] Enhanced endothelial function may be responsible for part of the benefits of cholesterol lowering on vascular health.[10] 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[11–13] without adversely influencing growth or neurological development.[14] 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.[12] Recruitment took place between March 1990 and June

Received April 6, 2005; de novo received August 16, 2005; revision received September 27, 2005; accepted October 7, 2005.

From the Departments of Clinical Physiology (O.T.R.), Medicine (T.R., J.S.A.V.), Research Centre of Applied and Preventive Cardiovascular Medicine (M.J.J, T.K, I.V., K.K., H.L.), and Pediatrics (H.N., O.S.), University of Turku, Turku, Finland, and Hospital for Children and Adolescents (E.J.), University of Helsinki, Helsinki, Finland.

Correspondence to Olli T. Raitakari, MD, PhD, Department of Clinical Physiology, University of Turku, PO Box 52, FIN-20521 Turku, Finland. E-mail olli.raitakari@utu.fi

Circulation is available at http://www.circulationaha.org DOI: 10.1161/CIRCULATIONAHA.105.583195

1992. 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.[15] 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, 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.[16] 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.[16] The nutrient intakes of the parents were assessed with 24-hour recall.[17] 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[18] or later[19] 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 nondominant arm with a standard sphygmomanometer. The mean of 3 measurements was used in the statistical analyses. Tanner staging was used to classify sexual maturation.[20] Participation in leisure-time physical activity was assessed by asking the frequency of participation in physical activity outside school hours. Exposure to environ-

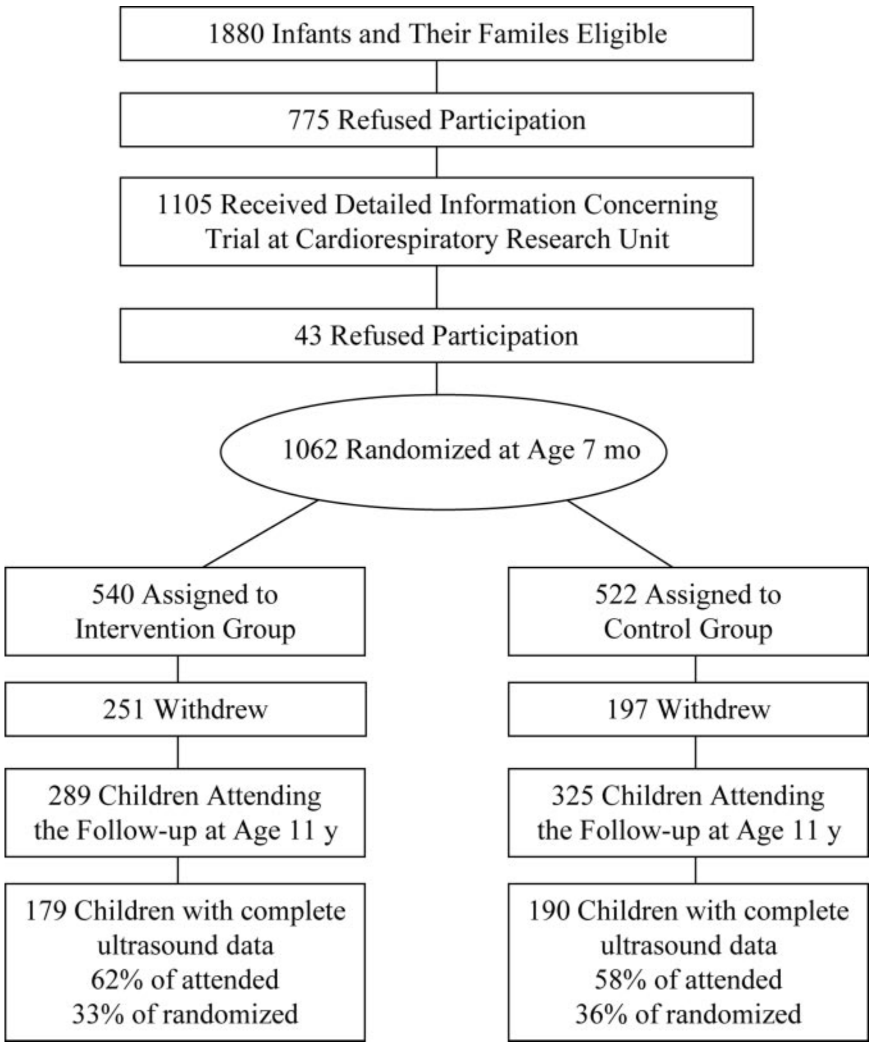

Figure 1. Flow diagram of the STRIP trial.

TABLE 1. Characteristics of Study Children at Randomization

	Intervention Children		Control Children	
	Boys	Girls	Boys	Girls
All children, n	284	256	266	256
Weight, kg	8.8±0.9	8.3±0.9	8.8±1.0	8.2±0.9
Height, cm	71.2±2.2	69.5±2.2	71.2±2.3	69.4±2.3
Body mass index, kg/m^2	17.4±1.4	17.1±1.5	17.4±1.5	16.9±1.5
Birth weight, kg	3.63±0.51	3.50±0.49	3.63±0.56	3.46±0.53
Birth height, cm	51.1±2.2	50.2±2.0	51.0±2.5	49.8±2.3
Serum cholesterol, mmol/L	3.94±0.75	4.32±0.86	3.97±0.64	4.23±0.82
Children with ultrasound data, n	90	89	92	98
Weight, kg	8.9±1.0	8.3±0.8	8.8±0.8	8.1±1.0
Height, cm	71.2±2.4	69.5±2.2	71.1±2.1	69.2±2.7
Body mass index, kg/m^2	17.6±1.4	17.2±1.2	17.4±1.3	16.8±1.4
Birth weight, kg	3.74±0.5	3.49±0.43	3.62±0.54	3.45±0.53
Birth height, cm	51.5±2.0	50.1±2.0	51.0±2.4	49.7±2.4
Serum cholesterol, mmol/L	3.96±0.77	4.23±0.78	4.09±0.55	4.35±0.92

Plus-minus values are mean±SD. P always >0.15 in comparisons between intervention and control groups.

Body mass index was calculated as weight in kilograms divided by the square of the height in meters.

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.[12,21] 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 9.2±1.0 measurements per child). The Friedewald formula was used to calculate serum LDL cholesterol values.[22] To assess the relation of serum cholesterol measured 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 begun 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. The reasons given for nonparticipation for the ultrasound study were child's unwillingness to participate (50%, n=42), lack of time (25%, n=21), fear of the study (20%, n=17), and transportation problems (5%, n=4). Of the 1062 children randomized, 614 children attended the 11-year follow-up study. Complete ultrasound data were available on 369 children, with similar rates in the intervention and control groups (Figure 1).

All ultrasound studies were performed with an Acuson Sequoia 512 mainframe (Acuson) with a 13.0-MHz linear-array transducer. To minimize external stimuli, all studies were performed in silence in a temperature-controlled clinical research laboratory. Left brachial artery diameter was measured from B-mode ultrasound images at rest, during reactive hyperemia, and after administration of sublingual nitroglycerin as described previously.[4] Briefly, a resting scan was performed and arterial flow velocity measured with a Doppler signal. Increased flow was then induced by inflation of a blood pressure cuff placed around the forearm to a pressure of 250 mm Hg for 4.5 minutes, followed by release. A second continuous scan was recorded between 30 and 180 seconds after cuff deflation, including a repeated flow-velocity measurement during the first 15 seconds after cuff release. All ultrasound scans were analyzed by the same reader, who was blinded to the subject's details. Vessel diameter was measured offline at a fixed position with ultrasonic calipers at end diastole, incident with the R wave on a continuously recorded ECG. We measured dilation from baseline at 10-second intervals between 30 and 180 seconds. In addition, we assessed the total dilatation response. This was defined as the area under the dilation response versus time curve between 30 and 180 seconds after hyperemia. The nitrate-mediated endothelium-independent dilation response was tested by administration of a 250-μg sublingual dose of nitroglycerin. Maximum arterial diameter 5 minutes after nitrate administration was used to calculate nitrate-mediated dilation. In our laboratory, the interobserver variation (coefficient of variation) of flow-mediated dilation measurements was 8.6%, and the between-study coefficient of variation was 9.3%.[23]

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

TABLE 2. Comparison of Intervention Children With and Without Complete Ultrasound Data at the Age of 11 Years

	With Ultrasound Data (n=179)	Without Ultrasound Data (n=110)	P
Height, cm	148±7	147±7	0.26
Weight, kg	40±8	39±8	0.23
Body mass index, kg/m^2	18.0±2.6	17.7±2.8	0.34
Birth weight, kg	3.6±0.4	3.6±0.4	0.56
Serum cholesterol, mmol/L	4.43±0.65	4.43±0.80	0.92
LDL cholesterol, mmol/L	2.75±0.58	2.76±0.54	0.88
HDL cholesterol, mmol/L	1.30±0.26	1.34±0.30	0.23
Triglycerides, mmol/L	0.86±0.49	0.73±0.29	0.07
Blood pressure, mm Hg			
Systolic	107±10	107±10	0.54
Diastolic	58±6	58±6	0.81
Saturated fat, % of energy	11.2±2.5	11.1±2.5	0.86
P/S ratio	0.56±0.20	0.56±0.17	0.99
Family risk, %	21.2	25.5	0.41
Physical activity, %*	34.1	33.7	0.94
Exposure to tobacco smoke, %†	7.6	6.7	0.60

P/S ratio indicates polyunsaturated to saturated fatty acid ratio.

Plus-minus values are mean±SD. Body mass index was calculated as weight in kilograms divided by the square of the height in meters.

*Engaging in leisure-time physical activity ≥3 times per week.

†Within the previous 3 days.

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

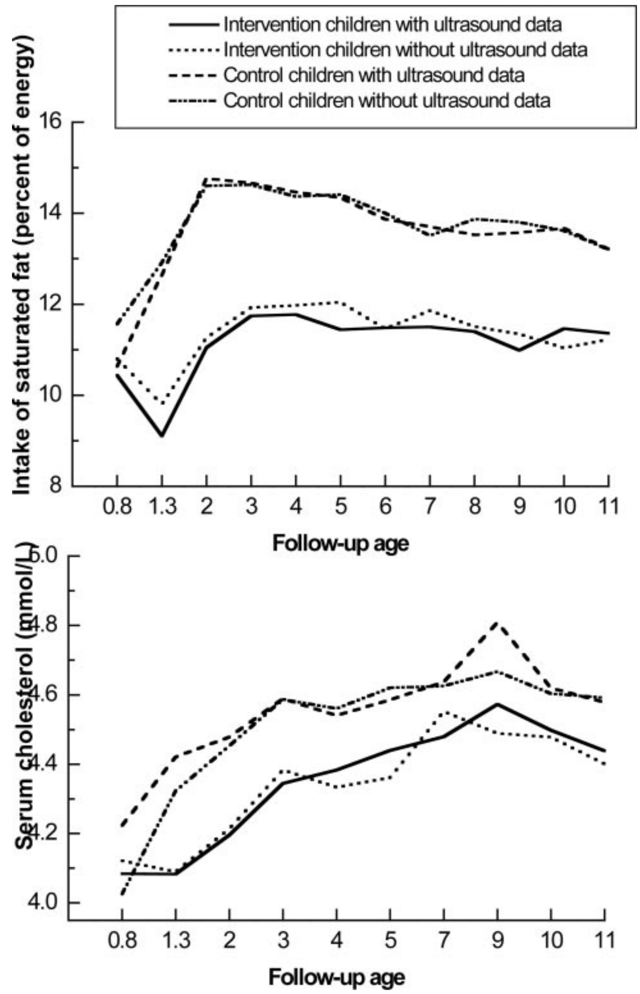

Figure 2. Serum saturated fat intake values (top) and total cholesterol values (bottom) are shown at each study point in children with and without complete data on ultrasound at the 11-year visit. Intervention children had consistently lower saturated fat intake ($P<0.001$) and mean cholesterol concentration ($P<0.001$) in repeated-measures of analysis from age 13 months than control children. However, intervention and control children with ultrasound data had similar cholesterol ($P=0.11$) and saturated fat intake ($P=0.13$) values as other intervention and control children.

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).

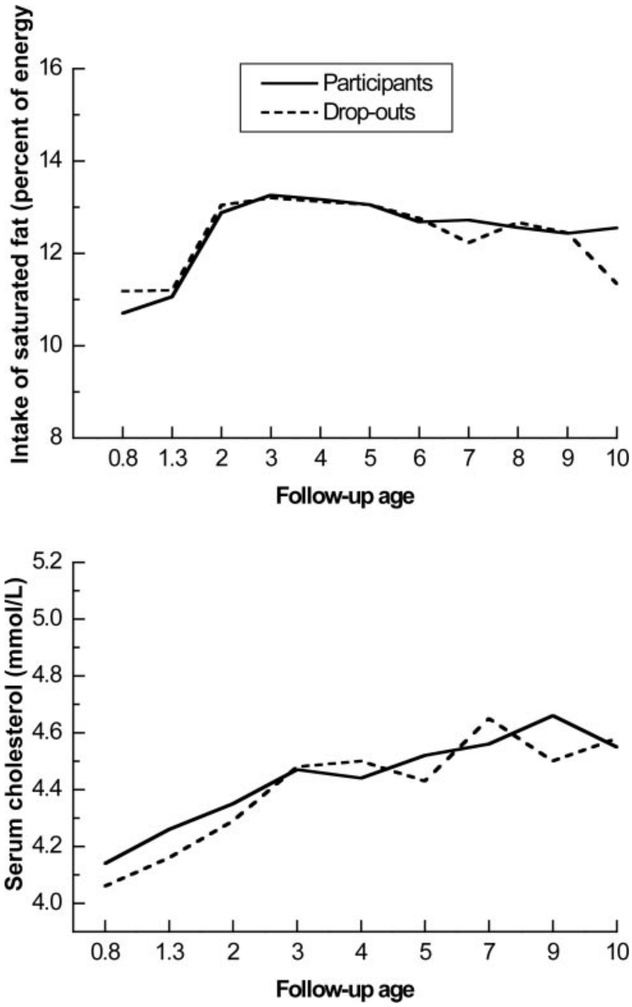

Figure 3. Saturated fat intake values (top) and total cholesterol values (bottom) are shown at each study point in participants and in children who were lost to follow-up after the particular study point. The values of cholesterol and saturated fat intake were similar in each study year (P always >0.05 in multiple pairwise comparison at each follow-up study point for both cholesterol and saturated fat intake).

The relations between maximum flow-mediated dilation and mean serum cholesterol concentrations measured at various ages in all subjects are shown in Table 5. Mean cholesterol values measured between ages 7 months and 2 years and between 3 and 5 years were significantly related to flow-mediated dilation. Mean cholesterol values measured between 7 and 11 years and current cholesterol and current LDL cholesterol (single measurements at 11 years) were not significantly correlated with flow-mediated vasodilation (Table 3). These relations were similar in boys and girls, because the interactions between sex and cholesterol were nonsignificant (interaction terms P always >0.4).

The difference in endothelial function between the intervention and control boys remained significant after adjustments for current LDL cholesterol and mean total cholesterol measured between ages 7 and 11 years (Table 2). Similarly, the difference remained significant after adjustment for body mass index. The body mass index adjusted means were 9.60% versus 8.43% ($P=0.036$) for maximal flow-mediated dilation and 921%×s versus 727%×s ($P=0.014$) for area under dilation versus time curve, in intervention and control boys, respectively. After the adjustment for mean total cholesterol measured between 7 months and 2 years, the difference in maximum flow-mediated dilation became statistically nonsignificant ($P=0.11$; Table 2).

Discussion

Alterations in endothelial function have been documented in children with hypercholesterolemia or diabetes.[1,24,25] This supports the concept that endothelial dysfunction plays a role in the early pathophysiology of atherosclerosis. Endothelial function may be enhanced by various therapies.[26] Interventions that have been applied successfully in high-risk children have included the use of antioxidants[27], lipid-lowering with statins,[28] and exercise training.[29] The results of previous dietary interventions have been inconsistent. Woo et al[30] studied the effect of a hypocaloric low-fat diet on flow-mediated vasodilation in obese children. The intervention decreased serum cholesterol level and improved endothelial function in the short term (at 6 weeks). The effect of a low-saturated-fat diet on endothelial function has been studied by Engler and coworkers.[27] They examined the effects of the National Cholesterol Education Program Step II diet on endothelium-dependent vasodilation in children with familial hypercholesterolemia or familial combined hyperlipidemia. The diet induced a significant reduction in LDL cholesterol concentration but failed to improve endothelial function during the 6-month trial. de Jongh et al[31] evaluated the effect of plant sterols on cholesterol and vascular function in children with familial hypercholesterolemia. Daily intake of plant sterols for 4 weeks decreased total cholesterol and LDL cholesterol but did not improve flow-mediated vasodilation. Compared with these studies, the present study differed markedly in length and target population. The present study cohort was recruited from the general population and included a relatively large number of healthy children who had been exposed to life-long cholesterol-lowering dietary intervention immediately after weaning.

In the present study, the mean serum cholesterol concentration measured during the life course correlated with flow-mediated dilation. Interestingly, cholesterol concentration measured before the age of 5 years was a stronger correlate than 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, ie, 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 under-

TABLE 3. Characteristics of 369 Study Children at the Age of 11 Years

Characteristic	Girls			Boys		
	Intervention (n=89)	Control (n=98)	*P*	Intervention (n=90)	Control (n=92)	*P*
Age, mo	132±2	132±1		132±1	132±1	
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^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	12.8±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

P/S ratio indicates polyunsaturated to fatty acids ratio.

Plus-minus values are mean±SD. Body mass index was calculated as weight in kilograms divided by the square of the height in meters.

*Engaging in leisure-time physical activity ≥3 times per week.

†Within the previous 3 days.

stood but may include reduced availability of nitric oxide through a combination of decreased production, increased inactivation, and abnormal signaling.[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.[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.[33] In intervention boys, serum cholesterol concen-

TABLE 4. Ultrasound Results of 369 Study Children at the Age of 11 Years

	Girls			Boys		
	Intervention (n=89)	Control (n=98)	*P*	Intervention (n=90)	Control (n=92)	*P*
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

Plus-minus values are mean±SD.

*Values shown are mean values of markers of endothelial function adjusted for current LDL cholesterol value and average total cholesterol measurement values at various ages.

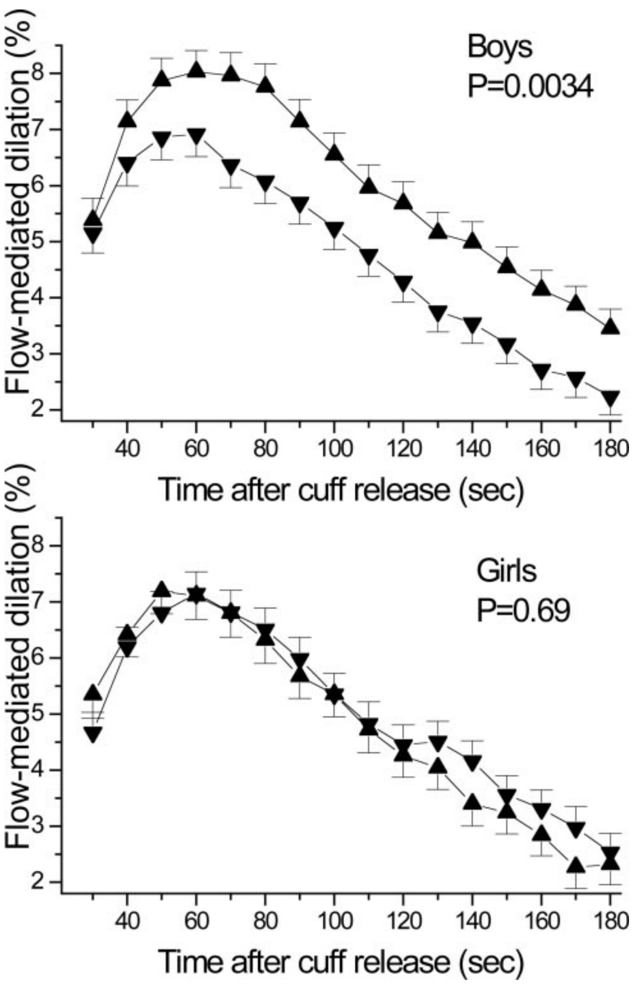

Figure 4. Flow-mediated vasodilation responses in the intervention (▲) and control children (▼) at each measurement point between 30 and 180 seconds after cuff release (top, boys; bottom, girls). There was a significant sex difference in the effect of the intervention on flow-mediated vasodilation (interaction term P=0.01). The magnitude of the response was greater in intervention boys than in control boys (effect of intervention P=0.0034) but similar in intervention girls compared with control girls (effect of intervention P=0.69). Error bars show SEM.

tration has been consistently ≈0.2 to 0.3 mmol/L or 5% to 8% lower than in control boys, ie, the intervention effect has been slightly greater than predicted. A meta-analysis by Law et al[34] indicated that the benefits of cholesterol reduction are greater in younger subjects: a 10% reduction in cholesterol concentration produces a reduction in ischemic heart disease of 50% at age 40 and 20% at age 70 years. 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.

TABLE 5. Relationships Between Flow-Mediated Vasodilation and Average Serum Total Cholesterol Measured at Different Ages in All 369 Study Children

Age at Cholesterol Measurement	$\beta\pm$SE	P
7 mo to 2 y	−0.760±0.290	0.009
3 to 5 y	−0.827±0.288	0.004
7 to 11 y	−0.496±0.298	0.096
Current cholesterol*	−0.319±0.263	0.235
Current LDL cholesterol*	−0.389±0.294	0.187

β-Values are regression coefficients for a 1-unit change in flow-mediated vasodilation (1 percent unit) and 1-unit change in cholesterol (mmol/L).

*Single measurement at 11 years.

The intervention influenced endothelial function in boys but not in girls. This is consistent with the lack of intervention effect on serum cholesterol concentration in girls.[12,13] Despite similar intervention-control group differences in the composition of the diet throughout the years,[16] the follow-up has consistently shown significant cholesterol-lowering effects only in boys. Some[35–38] but not all[39] earlier studies in adults have suggested that men show greater changes than women in LDL cholesterol concentration in response to changes in dietary intake of saturated fatty acids. The mechanism for the possible higher resistance to diet-induced LDL lowering in females is unknown but may relate to differences in sex hormone levels. Estrogens regulate serum LDL cholesterol concentration by affecting the number of hepatic LDL receptors.[40] Similarly, changes in dietary saturated fat intake influence serum LDL cholesterol concentration by inducing changes in the number of LDL receptors.[41] Therefore, a potential for interaction in lipoprotein metabolism exists between fat intake and estrogens. Differences in estrogen levels between boys and girls are many-fold and become evident before puberty.[42] Thus, it is plausible that the inefficient impact of intervention in girls may be due to gender differences in serum sex hormone concentrations.

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.[43] The mean flow-mediated dilation values were within the normal range in all children[4] 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

This study was financially supported by the Academy of Finland (grants 34316, 11053, 73582, and 64/2003); City of Turku; the Finnish Foundation for Pediatric Research; the Finnish Foundation of Cardiovascular Research; the Finnish Cultural Foundation; government grants for Turku University Hospital; Juho Vainio Foundation; the Mannerheim League for Child Welfare; Raisio Group Research Foundation; the Social Insurance Institution of Finland; the Turku University Foundation; the Van den Bergh Foods Company; the Yrjö Jahnsson Foundation; and the Sigrid Juselius Foundation.

Disclosures

None.

References

1. Celermajer DS, Sorensen KE, Gooch VM, Spiegelhalter DJ, Miller OI, Sullivan ID, Lloyd JK, Deanfield JE. Noninvasive detection of endothelial dysfunction in children and adults at risk of atherosclerosis. *Lancet*. 1992;340:1111–1115.
2. Tonstad S, Joakimsen O, Stensland-Bugge E, Leren TP, Ose L, Russell D, Bonaa KH. Risk factors related to carotid intima-media thickness and plaque in children with familial hypercholesterolemia and control subjects. *Arterioscler Thromb Vasc Biol*. 1996;16:984–991.
3. Järvisalo MJ, Jartti L, Näntö-Salonen K, Irjala K, Rönnemaa T, Hartiala JJ, Celermajer DS, Raitakari OT. Increased aortic intima-media thickness: a marker of preclinical atherosclerosis in high-risk children. *Circulation*. 2001;104:2943–2947.
4. Järvisalo MJ, Rönnemaa T, Volanen I, Kaitosaari T, Kallio K, Hartiala JJ, Irjala K, Viikari JS, Simell O, Raitakari OT. Brachial artery dilatation responses in healthy children and adolescents. *Am J Physiol Heart Circ Physiol*. 2002;282:H87–H92.
5. Davis PH, Dawson JD, Riley WA, Lauer RM. Carotid intimal-medial thickness is related to cardiovascular risk factors measured from childhood through middle age: the Muscatine Study. *Circulation*. 2001;104:2815–2819.
6. Raitakari OT, Juonala M, Kähönen M, Taittonen L, Laitinen T, Mäki-Torkko N, Järvisalo MJ, Uhari M, Jokinen E, Rönnemaa T, Åkerblom HK, Viikari JSA. Cardiovascular risk factors in childhood and carotid artery intima-media thickness in adulthood: the Cardiovascular Risk in Young Finns Study. *JAMA*. 2003;290:2277–2283.
7. Li SX, Chen W, Srinivasan SR, Bond MG, Tang R, Urbina EM, Berenson GS. Childhood cardiovascular risk factors and carotid vascular changes in adulthood: the Bogalusa Heart Study. *JAMA*. 2003;290:2271–2276.
8. McGill HC Jr, McMahan CA. Starting earlier to prevent heart disease. *JAMA*. 2003;290:2320–2322.
9. Schaefer EJ. Lipoproteins, nutrition, and heart disease. *Am J Clin Nutr*. 2002;75:191–212.
10. Cohen JD, Drury JH, Ostdiek J, Finn J, Babu BR, Flaker G, Belew K, Donohue T, Labovitz A. Benefits of lipid lowering on vascular reactivity in patients with coronary artery disease and average cholesterol levels: a mechanism for reducing clinical events? *Am Heart J*. 2000;139:734–738.
11. Lapinleimu H, Viikari J, Jokinen E, Salo P, Routi T, Leino A, Rönnemaa T, Seppänen R, Välimäki I, Simell O. Prospective randomised trial in 1062 infants of diet low in saturated fat and cholesterol. *Lancet*. 1995;345:471–476.
12. Niinikoski H, Viikari J, Rönnemaa T, Lapinleimu H, Jokinen E, Salo P, Seppänen R, Leino A, Tuominen J, Välimäki I, Simell O. Prospective randomized trial of low-saturated-fat, low-cholesterol diet during the first 3 years of life: the STRIP baby project. *Circulation*. 1996;94:1386–1393.
13. Kaitosaari T, Rönnemaa T, Raitakari O, Talvia S, Kallio K, Volanen I, Leino A, Jokinen E, Välimäki I, Viikari J, Simell O. Effect of 7-year infancy-onset dietary intervention on serum lipoproteins and lipoprotein subclasses in healthy children in the prospective, randomized special Turku coronary risk factor intervention project for children (STRIP) study. *Circulation*. 2003;108:672–677.
14. Rask-Nissilä L, Jokinen E, Terho P, Tammi A, Lapinleimu H, Rönnemaa T, Viikari J, Seppänen R, Korhonen T, Tuominen J, Välimäki I, Simell O. Neurological development of 5-year-old children receiving a low-saturated fat, low-cholesterol diet since infancy: a randomized controlled trial. *JAMA*. 2000;284:993–1000.
15. Nordic nutrition recommendations. *Scand J Nutr*. 1996;40:161–165.
16. Talvia S, Lagström H, Räsänen M, Salminen M, Räsänen L, Salo P, Viikari J, Rönnemaa T, Jokinen E, Vahlberg T, Simell O. A randomized intervention since infancy to reduce intake of saturated fat: calorie (energy) and nutrient intakes up to the age of 10 years in the Special Turku Coronary Risk Factor Intervention Project. *Arch Pediatr Adolesc Med*. 2004;158:41–47.
17. Lagström H, Seppänen R, Jokinen E, Rönnemaa T, Salminen M, Tuominen J, Viikari J, Simell O. Nutrient intakes and cholesterol values of the parents in a prospective randomized child-targeted coronary heart disease risk factor intervention trial: the STRIP project. *Eur J Clin Nutr*. 1999;53:654–661.
18. Niinikoski H, Viikari J, Rönnemaa T, Helenius H, Jokinen E, Lapinleimu H, Routi T, Lagström H, Seppänen R, Välimäki I, Simell O. Regulation of growth of 7- to 36-month-old children by energy and fat intake in the prospective, randomized STRIP baby trial. *Pediatrics*. 1997;100:810–816.
19. Simell O, Niinikoski H, Rönnemaa T, Lapinleimu H, Routi T, Lagström H, Salo P, Jokinen E, Viikari J. Special Turku coronary risk factor intervention project for babies (STRIP). *Am J Clin Nutr*. 2000;72:1316S–1331S.
20. Tanner J. *Growth in Adolescence*. 2nd ed. Oxford, UK: Blackwell Scientific Publications; 1962.
21. Salo P, Viikari J, Rask-Nissilä L, Hamäläinen M, Rönnemaa T, Seppänen R, Simell O. Effect of low-saturated fat, low-cholesterol dietary intervention on fatty acid compositions in serum lipid fractions in 5-year-old children: the STRIP project. *Eur J Clin Nutr*. 1999;53:927–932.
22. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem*. 1972;18:499–502.
23. Järvisalo MJ, Jartti L, Karvonen MK, Pesonen U, Koulu M, Marniemi J, Hammar N, Kaprio J, Paakkunainen U, Simell O, Raitakari OT, Ronnemaa T. Enhanced endothelium-dependent vasodilation in subjects with Proline7 substitution in the signal peptide of neuropeptide Y. *Atherosclerosis*. 2003;167:319–326.
24. Singh TP, Groehn H, Kazmers A. Vascular function and carotid intimal-medial thickness in children with insulin-dependent diabetes mellitus. *J Am Coll Cardiol*. 2003;41:661–665.
25. Järvisalo MJ, Raitakari M, Toikka JO, Putto-Laurila A, Rontu R, Laine S, Lehtimäki T, Rönnemaa T, Viikari J, Raitakari OT. Endothelial dysfunction and increased arterial intima-media thickness in children with type 1 diabetes. *Circulation*. 2004;109:1750–1755.
26. Raitakari OT, Celermajer DS. Testing for endothelial dysfunction. *Ann Med*. 2000;32:293–304.
27. Engler MM, Engler MB, Malloy MJ, Chiu EY, Schloetter MC, Paul SM, Stuehlinger M, Lin KY, Cooke JP, Morrow JD, Ridker PM, Rifai N, Miller E, Witztum JL, Mietus-Snyder M. Antioxidant vitamins C and E improve endothelial function in children with hyperlipidemia: Endothelial Assessment of Risk from Lipids in Youth (EARLY) Trial. *Circulation*. 2003;108:1059–1063.
28. de Jongh S, Lilien MR, op't Roodt J, Stroes ES, Bakker HD, Kastelein JJ. Early statin therapy restores endothelial function in children with familial hypercholesterolemia. *J Am Coll Cardiol*. 2002;40:2117–2121.

29. Watts K, Beye P, Siafarikas A, O'Driscoll G, Jones TW, Davis EA, Green DJ. Effects of exercise training on vascular function in obese children. *J Pediatr*. 2004;144:620–625.
30. Woo KS, Chook P, Yu CW, Sung RY, Qiao M, Leung SS, Lam CW, Metreweli C, Celermajer DS. Effects of diet and exercise on obesity-related vascular dysfunction in children. *Circulation*. 2004;109:1981–1986.
31. de Jongh S, Vissers MN, Rol P, Bakker HD, Kastelein JJ, Stroes ES. Plant sterols lower LDL cholesterol without improving endothelial function in prepubertal children with familial hypercholesterolaemia. *J Inherit Metab Dis*. 2003;26:343–351.
32. Vogel RA. Cholesterol lowering and endothelial function. *Am J Med*. 1999;107:479–487.
33. Keys A, Anderson JT, Grande F. Serum cholesterol response to changes in diet, I: iodine value of dietary fat versus 2S-P. *Metabolism*. 1965;14:747.
34. Law MR, Wald NJ, Thompson SG. By how much and how quickly does reduction in serum cholesterol concentration lower risk of ischaemic heart disease? *BMJ*. 1994;308:367–372.
35. Barnard RJ. Effects of life-style modification on serum lipids. *Arch Intern Med*. 1991;151:1389–1394.
36. Savolainen MJ, Rantala M, Kervinen K, Jarvi L, Suvanto K, Rantala T, Kesäniemi YA. Magnitude of dietary effects on plasma cholesterol concentration: role of sex and apolipoprotein E phenotype. *Atherosclerosis*. 1991;86:145–152.
37. Clifton PM, Noakes M, Nestel PJ. Gender and diet interactions with simvastatin treatment. *Atherosclerosis*. 1994;110:25–33.
38. Li Z, Otvos JD, Lamon-Fava S, Carrasco WV, Lichtenstein AH, McNamara JR, Ordovas JM, Schaefer EJ. Men and women differ in lipoprotein response to dietary saturated fat and cholesterol restriction. *J Nutr*. 2003;133:3428–3433.
39. Walden CE, Retzlaff BM, Buck BL, McCann BS, Knopp RH. Lipoprotein lipid response to the National Cholesterol Education Program step II diet by hypercholesterolemic and combined hyperlipidemic women and men. *Arterioscler Thromb Vasc Biol*. 1997;17:375–382.
40. Kovanen PT, Brown MS, Goldstein JL. Increased binding of low density lipoprotein to liver membranes from rats treated with 17 alpha-ethinyl estradiol. *J Biol Chem*. 1979;254:11367–11373.
41. Mustad VA, Etherton TD, Cooper AD, Mastro AM, Pearson TA, Jonnalagadda SS, Kris-Etherton PM. Reducing saturated fat intake is associated with increased levels of LDL receptors on mononuclear cells in healthy men and women. *J Lipid Res*. 1997;38:459–468.
42. Klein KO, Baron J, Colli MJ, McDonnell DP, Cutler GB Jr. Estrogen levels in childhood determined by an ultrasensitive recombinant cell bioassay. *J Clin Invest*. 1994;94:2475–2480.
43. Tunstall-Pedoe H, Kuulasmaa K, Amouyel P, Arveiler D, Rajakangas AM, Pajak A. Myocardial infarction and coronary deaths in the World Health Organization MONICA Project: registration procedures, event rates, and case-fatality rates in 38 populations from 21 countries in four continents. *Circulation*. 1994;90:583–612.

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.

Circulation
JOURNAL OF THE AMERICAN HEART ASSOCIATION

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)

Olli T. Raitakari, Tapani Rönnemaa, Mikko J. Järvisalo, Tuuli Kaitosaari, Iina Volanen, Katariina Kallio, Hanna Lagström, Eero Jokinen, Harri Niinikoski, Jorma S.A. Viikari and Olli Simell

Circulation. 2005;112:3786-3794; originally published online December 5, 2005;
doi: 10.1161/CIRCULATIONAHA.105.583195
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231

Print ISSN: 0009-7322. Online ISSN: 1524-4539