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Influence of maternal hypercholesterolaemia during pregnancy on progression of early atherosclerotic lesions in childhood: Fate of Early Lesions in Children (FELIC) study

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Summary

Background Children generally have low cholesterol and no clinical manifestations of atherosclerosis, but fatty-streak formation begins in fetuses and is greatly increased by maternal hypercholesterolaemia during pregnancy. In the FELIC study we assessed the evolution of such lesions during childhood.

Methods Computer-assisted imaging was used to measure the area of the largest individual lesion and the cumulative lesion area per section in serial cross-sections through the entire aortic arch and abdominal aorta of 156 normocholesterolaemic children aged 1–13 years, who died of trauma and other causes. Children were classified by whether their mother had been normocholesterolaemic (n=97) or hypercholesterolaemic (n=59) during pregnancy. Atherosclerosis was correlated with 13 established or potential risk factors.

Findings The largest fatty streaks in the aortic arch of children younger than 3 years of hypercholesterolaemic mothers were 64% smaller than those previously found in corresponding fetuses ($p<0.0001$), which suggests that fetal fatty streaks may regress after birth. In the two groups, lesion size in the aortic arch and abdominal aorta increased linearly with age ($r=0.87$ – 0.98). However, lesions progressed strikingly faster in children of hypercholesterolaemic mothers than in those of normocholesterolaemic mothers ($p<0.0001$). Conventional risk factors for atherosclerosis in children or mothers correlated with lesion size, but did not account for the faster progression of atherogenesis in normocholesterolaemic children of hypercholesterolaemic mothers.

Interpretation Our results suggest that maternal hypercholesterolaemia during pregnancy induces changes in the fetal aorta that determine the long-term susceptibility of children to fatty-streak formation and subsequent atherosclerosis. If so, cholesterol-lowering interventions in hypercholesterolaemic mothers during pregnancy may decrease atherogenesis in children.

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See Commentary page ???

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Introduction

Hypercholesterolaemia is associated with an increased incidence of atherosclerosis and its common clinical sequelae, coronary heart disease, and ischaemic stroke.^{1,2} Clinical trials with cholesterol-lowering drugs have substantially decreased cardiovascular morbidity and mortality,^{3–6} and the mechanisms by which hypercholesterolaemia and lipoprotein oxidation induce and promote atherogenesis are increasingly understood.^{7–11} Current guidelines for the prevention of atherosclerosis therefore emphasise detection of hypercholesterolaemia and lowering of cholesterol.¹²

In the absence of genetic defects, such as familial hypercholesterolaemia, children generally have low cholesterol concentrations and do not develop clinically important atherosclerosis. However, some atherosclerotic lesions have been seen in young adults and, occasionally, children.^{13,14} We have shown previously that the earliest lesions of atherosclerosis, fatty streaks, are already formed in fetal arteries and that maternal hypercholesterolaemia during pregnancy greatly increases their number and size.^{15,16} Even the earliest fetal fatty streaks contained native and oxidised LDL, as well as macrophage-derived foam cells, and analysis of lesion composition showed that LDL oxidation contributed to the initiation of the atherogenic process. Oxidation increases atherogenicity of LDL^{9–11} and has substantial immunological consequences.¹⁷ We have also seen that fetal cholesterol concentrations were highest in earlier pregnancy and decreased with increasing fetal age. Since fatty-streak formation was related to hypercholesterolaemia, this finding suggested that fetal lesions would regress in infancy, when cholesterol concentrations are low.

We designed the Fate of Early Lesions in Children (FELIC) study to investigate whether such regression occurs and whether the events in the arterial wall during pregnancy influence the extent of lesion formation during childhood.

Patients and methods

Patients

We studied 156 children, aged 1–13 years, who died of acute trauma, cancer, and cerebral aneurysms. Children were allocated to groups according to whether their mothers had been normocholesterolaemic (n=97) or hypercholesterolaemic (n=59) during pregnancy. Mothers were asked to provide all medical records and we obtained current lipid profiles. To be classified as hypercholesterolaemic according to the joined recommendations of the Task Force of the European Society of Cardiology, European Atherosclerosis Society, and European Society of Hypertension,¹⁸ and the American Heart Association/National Heart, Lung, and Blood Institute¹⁹ (total plasma cholesterol concentrations 4.65–5.17 mmol/L, dependent on age), mothers had to have been hypercholesterolaemic during pregnancy (based on three to four measurements; data obtained from clinical

Characteristic	Normocholesterolaemic mothers		Hypercholesterolaemic mothers		p*
	n	Value	n	Value	
Demography					
Sex (m/f)	49/48	..	28/31	..	0.71
Median (range) age (years)		5.9 (1.5–13.4)	..	5.4 (1.4–12.9)	0.42
Cause of death					
Trauma†	62	..	42	..	0.65
Cancer	23	..	11	..	
Cerebral aneurysm	12	..	6	..	
Clinical					
Total plasma cholesterol (mmol/L)‡	97	3.12 (0.36)	59	2.96 (0.25)	0.004
Total plasma cholesterol (mmol/L)§	68	4.24 (0.49)	38	4.29 (0.6)	0.72
LDL cholesterol (mmol/L)§	68	2.52 (0.47)	38	2.52 (0.55)	0.92
VLDL cholesterol (mmol/L)§	68	0.70 (0.06)	38	0.68 (0.05)	0.08
HDL cholesterol (mmol/L)§	68	1.09 (0.07)	38	1.09 (0.01)	0.88
Plasma triglycerides (mmol/L)§	68	1.71 (0.21)	38	1.65 (0.25)	0.23
Birthweight (kg)	73	3.19 (0.49)	41	3.15 (0.45)	0.64
Coronary heart disease (%)	80	37.5	44	20.5	0.051
Type 1 diabetes (%)	78	19.2	45	17.8	0.84
Hypertension (%)	78	9.0	44	20.5	0.07

Data are mean (SD) except where shown otherwise. n for clinical parameters shows number of patients for whom data could be obtained.

*Pearson's χ^2 or independent Student's *t* test. †Acute death n=23 normocholesterolaemic, n=11 hypercholesterolaemic; in hospital n=39 normocholesterolaemic, n=31 hypercholesterolaemic. ‡Measured in intensive care or at time of death, by the same laboratory. §Previously measured at different times by different laboratories (taken from medical records).

Table 1: Characteristics of children

records) and at the time of the interview (shortly after the death of their child). Conversely, mothers were classified as normocholesterolaemic when normal cholesterol concentrations were documented on medical records and they were normocholesterolaemic at the time of the study.

Mothers who were temporarily hypercholesterolaemic during pregnancy but who had normal cholesterol concentrations at the time of the interview or before pregnancy and those with intermittent hypercholesterolaemia were excluded. In our previous study, we had shown that temporary hypercholesterolaemia during pregnancy also significantly increased formation of fetal fatty streaks.¹⁵ We did not include such mothers in the FELIC study for several reasons. First, classification would have depended entirely on cholesterol measured during pregnancy by different laboratories. By the use of a stringent definition of maternal hypercholesterolaemia that included measurements by our central laboratory, we decreased this source of error. Second, because we do not know at what time during fetal development the aorta is most susceptible to pathogenic effects of maternal hypercholesterolaemia, inclusion of mothers with temporary hypercholesterolaemia would not be meaningful without taking into account the time of onset. Finally, we judged that comparison of two well-defined and clearly separated groups would increase the likelihood of detecting a potential impact of fetal fatty-streak formation on atherogenesis during childhood.

Total plasma cholesterol concentrations were measured in mothers and children by an automated enzymatic procedure and kit (Boehringer Mannheim, Mannheim, Germany). Because lipoprotein concentrations in children measured in intensive care or after death may not reflect normal concentrations, total,

Mothers	Normocholesterolaemic mothers (n=97)	Hypercholesterolaemic mothers (n=59)	p
Median (range) age at time of birth (years)	28.3 (18.2–36.0)	27.7 (19.9–32.9)	0.23
Mean (SD) plasma cholesterol during pregnancy (mmol/L)*	3.85 (1.61)	9.41 (2.16)	0.0001
Mean (SD) plasma cholesterol after pregnancy (mmol/L)†	4.08 (1.12)	7.15 (1.14)	0.0001
Primigravida (%)	30	27	0.71
Lactation and breastfeeding (%)	39	44	0.55
History of coronary heart disease (%)	37	56	0.02
Smoking (%)	45	48	0.80
Type 1 diabetes (%)	11	20	0.12
Hypertension (%)	40	36	0.57

*Average of three to four measurements throughout pregnancy (taken from medical records). †Single measurement after death of the child by central laboratory; normocholesterolaemic n=54, hypercholesterolaemic n=43.

Table 2: Characteristics of mothers during pregnancy

LDL, VLDL, and HDL cholesterol, as well as triglyceride concentrations were obtained from medical records.

We took detailed medical histories of mothers and children to find out whether potential risk factors for atherosclerosis were present. Maternal risk factors during pregnancy included: history of coronary heart disease (clinically manifest coronary heart disease among first-degree relatives and maternal and paternal grandparents); hypertension (systolic pressure >100 mm Hg plus age, higher than 95th percentile of systolic and diastolic values adjusted for age and sex, or continuing antihypertensive treatment); type 1 diabetes mellitus; habitual smoking; and maternal age at birth. In children, risk factors were birthweight, hypertension, and type 1 diabetes. Family history of coronary heart disease in children (symptomatic coronary heart disease among siblings, parents, or grandparents) was assessed separately from that of the mothers to include paternal history of coronary heart disease. Diet was not included among risk factors because an accurate retrospective determination of all potential dietary factors during pregnancy would have been difficult. However, diet-related questions during our interviews showed that participants followed a homogeneous Mediterranean diet previously described in detail.²⁰

Plasma triglycerides were similar in the two groups (mean 2.01 [SD 0.31] and 1.85 [0.29] vs 1.77 [0.32] and 1.80 mmol/L [0.34] before and after pregnancy, respectively), which also suggested a similar influence of diet in the two groups.

In mothers of the hypercholesterolaemic group, the mean total cholesterol concentration during pregnancy was 9.41 mmol/L (2.16). However, pregnancy is known to lead to substantial increases in total cholesterol^{21–23} and values after pregnancy were lower than those during pregnancy (7.15 mmol/L [1.14]; table 2). Maternal total cholesterol concentrations before pregnancy obtained from medical records were similar to those after pregnancy (7.41 mmol/L [1.37], $p<0.05$). The characteristics of children and mothers are shown in tables 1 and 2.

The study was approved by the human ethics committee of Federico II University, Naples, and we obtained informed consent from all participating parents.

Tissue preparation and immunocytochemistry

Aortas were obtained during necropsy within 3.5–6.0 h of death. After dissection and thorough washing with cold, sterile, phosphate-buffered saline containing 2 mmol/L edetic acid, the aorta was separated into 46–56 segments (5–8 mm long), dependent on the total aortic length. Segments too large to be sectioned as an intact aortic ring were subdivided longitudinally. Segments were immersed in OTC medium, flash-frozen in liquid nitrogen, and from each block containing a segment of the

Figure 1: Microphotographs of oil red O stained aortic sections from children

A: early fatty streak without substantial intimal thickening in abdominal aorta of child aged 14.2 years of normocholesterolaemic mother (final magnification $\times 93$). B: shoulder area of transitional lesion in the abdominal aorta of child aged 12.2 years from a hypercholesterolaemic mother (magnification $\times 37$). C: advanced lesion in same aorta (magnification $\times 30$).

arch (15–18 blocks) and abdominal aorta (16–21 blocks), 36 consecutive cryosections were prepared at $7\ \mu\text{m}$ thickness. The rest of each block was also sectioned and ten additional equidistant sections were used for morphometry. On average, 759 sections of each aortic arch and 851 sections of each abdominal aorta were analysed. Sections for morphometry were stained with oil red O and counterstained with hematoxylin.

Computer-assisted imaging was used to measure the surface area of the single largest lesion and the cumulative lesion area in each section by an investigator masked to study group.^{15,16} The aortic diameter was calculated from the outer vascular circumference measured for each aortic segment. Although this cross-sectional approach is labour-intensive, the measurement of lesion areas is accurate even for very early lesions that are difficult to assess in Sudan-stained en-face preparations traditionally used,^{13,14} and, more importantly, allowed us

to compare our results with those previously obtained by the same method in human fetuses to assess lesion regression after birth.¹⁵

18 serial sections from the arch and abdominal aorta were fixed in buffered 10% formalin and used for immunocytochemistry. Sections were immunostained with: MDA2 and NA59, monoclonal antibodies to malondialdehyde (MDA)-lysine and 4-hydroxynonenal (4-HNE)-lysine epitopes, which are oxidation-specific epitopes that occur on oxidised LDL and other adducts between peroxidation products and proteins;²⁴ NP1533975 (Boehringer Mannheim), a mouse monoclonal antibody (IgG1) to human apolipoprotein B; and HAM-56 (Axcel Accurate, Westbury, NY, USA), a monoclonal antibody to human monocytes and macrophages.²⁵ All antibodies were used at a dilution of 1:500. Epitopes recognised by the primary antibody were detected by an avidin-biotin-peroxidase method.¹⁵

Statistical analysis

Descriptive results for continuous variables are reported as mean (SD) and for categorical data as percentages. Demographic variables and clinical characteristics of children and of mothers were compared between the two groups. Comparison of means for continuous variables were done with independent Student's *t* test. We compared proportions for categorical data with Pearson's χ^2 test. Observed significance levels (*p* values) are presented for all hypothesis tests.

We aimed to identify prognostic indicators of lesion formation. Four variables of atherosclerosis were assessed in aortic cross-sections: area of the greatest lesion in the aortic arch and abdominal aorta, and cumulative lesion area in the arch and abdominal aorta. These four measures are not independent *a priori*, and were highly correlated ($r=0.86$ – 0.97 , $p<0.0001$). Therefore, we used principal-components analysis to combine information from these measures. The logarithm of the measures was used, and the first principal component accounted for more than 95% of the variation of the data (eigenvalue 3.8). We therefore used this first principal component as a composite measure of atherosclerosis and designated it as the dependent outcome variable in subsequent analyses.

To explore the impact of risk factors of mothers and children on the size of atherosclerotic lesions, the data analytical strategy was to first identify candidates with potential as significant risk factors of atherosclerosis and then to find out which combination of these factors provided a significant estimate of

Figure 2: Presence of native and oxidised LDL, monocytes, and macrophages in aortic intima of children

Data for children aged 1–4 years ($n=88$) and those aged 8–13 years ($n=68$) shown separately, since younger children had mainly fatty streaks, whereas older children also had transitional and more advanced lesions. All values were higher for children from hypercholesterolaemic than for children from normocholesterolaemic mothers ($p<0.0001$).

Figure 3: Lesion sizes in aortic arch and abdominal aorta of children
Slopes of regression lines in all panels significantly different ($p < 0.0001$).

lesion formation. From the list of selected potential risk factors, we entered separately each continuous variable (child's age, birthweight, total plasma cholesterol at the time of death, and maternal age at birth) into a univariate linear regression model. For this analysis, data were pooled from children of normocholesterolaemic and hypercholesterolaemic mothers and from the mothers themselves. We tested each dichotomous variable (risk group, sex, history of coronary heart disease [mother and child], type 1 diabetes [mother and child], hypertension [mother and child], and maternal smoking status) for significance by independent Student's *t* test. We entered into a stepwise multiple linear regression all variables with a *p* value less than 0.20 in the univariate analysis to lessen the chance of omitting a variable that, although independently weak, might, in combination, be influential. The criteria to be entered into the stepwise model was an enter value of 0.25 and an elimination value of 0.10. From the resultant model, we tested individual covariates with Bonferroni-adjusted criteria for a significance of 0.05. Finally, with the resulting variables, obvious candidates for interaction were tested for significance by the same procedures. An observed significance level () of 0.05 was taken to be significant. We did all analyses on JMP 3.2.1 software.

Results

In all children, lipid-rich lesions were found in the aortic arch and abdominal aorta. Lesions in younger children consisted mostly of fatty streaks with only minimal intimal thickening, whereas in children older than 10 years, especially those from hypercholesterolaemic mothers, transitional lesions and even some classical

atheromas were seen (figure 1), consistent with previous reports.¹²⁻¹⁴ Intimal lipid accumulations in the aorta of younger children visible only under the microscope would commonly not be taken to be atherosclerotic lesions. We nevertheless use the terms early atherosclerotic lesion and atherogenesis, because lesion sizes were measured without differentiating between stages and because we believe that fatty streaks represent the initial stage of the disease, irrespective of whether they later progress to a more advanced stage or regress.

Immunocytochemistry showed macrophages and oxidised LDL (defined as the presence of apolipoprotein B and oxidation-specific epitopes^{15,16,24}) in the vascular intima, even in early fatty streaks of younger children from normocholesterolaemic mothers. Significantly fewer children of normocholesterolaemic mothers than children of hypercholesterolaemic mothers had positive immunostaining in at least one lesion (figure 2), which reflects that children of normocholesterolaemic mothers had noticeably fewer lesions. In children younger than 4 years of hypercholesterolaemic mothers, 45-65% of sections contained macrophages and oxidised LDL. By contrast with children from normocholesterolaemic mothers, the proportion of sections that contained macrophages and oxidised LDL did not increase with age, even though lesions in older children were larger. However, since each section containing at least one immunostained lesion was classified as positive, increases

Figure 4: Correlation between age of children and plasma cholesterol concentrations at or shortly before death

in the area or number of lesions in each section were not reflected.

In the two groups, the size of lesions in the arch and abdominal aorta progressively increased with age ($p < 0.0001$, figure 3). This increase was linear ($r = 0.87-0.98$, $p < 0.0001$), which suggests that, overall, lesion formation was a continuous process. Contrary to expectation, the rate of lesion progression, indicated by the slope of the regression lines, was strikingly greater in children of hypercholesterolaemic mothers than in children of normocholesterolaemic mothers ($p < 0.0001$). This finding was true irrespective of whether the lesion size was measured as the area of the largest lesion per section or as the cumulative lesion area per section. The values of these two variables were greater for the abdominal aorta than for the aortic arch, which is consistent with the higher prevalence of lesions in the abdominal aorta in adults.

The difference in the rate of lesion progression was not due to hypercholesterolaemia of the children. All children had plasma cholesterol concentrations in the normal range²⁶ and concentrations were actually slightly higher in children from normocholesterolaemic mothers (table 1, figure 4). Plasma cholesterol concentrations of children obtained from medical records (ie, determined by different laboratories and at different time points) were higher in absolute terms than those measured under uniform conditions at the time of death (table 1), but were not significantly different in the two groups. LDL, VLDL, and HDL cholesterol concentrations, as well as triglyceride concentrations were similar in the two groups (table 1).

Waste could have affected lipoprotein metabolism and atherogenesis in cancer patients and, therefore, we also did a subgroup analysis. In children of normocholesterolaemic mothers, plasma lipoprotein concentrations and atherosclerosis did not differ significantly between those who did or did not have cancer, whereas among children from hypercholesterolaemic mothers, cancer patients were

Continuous variables	R ²	r	Coefficient	SE	p
Age of child	0.417	0.65	0.32	0.03	0.0001
Birthweight	0.045	0.21	-0.87	0.38	0.05
Total plasma cholesterol of child	0.045	0.21	0.03	0.01	0.01
Maternal age at birth	0.026	0.16	0.09	0.04	0.05

R²=coefficient of determination; r=correlation coefficient; coefficient=regression (β) coefficient; SE=SE of estimate.

Table 3: Significance of continuous variables in estimating cumulative measure of atherosclerosis in univariate analysis

Dichotomous variables	R ²	√MSE	p
Group	0.494	1.39	0.0001
Sex of child	0.001	1.96	0.74
Coronary heart disease in child	0.009	1.94	0.30
Type 1 diabetes in child	0.009	1.95	0.30
Hypertension in child	0.065	1.88	0.005
Maternal coronary heart disease	0.029	1.93	0.03
Maternal smoking	0.021	1.94	0.07
Maternal hypertension	0.006	1.95	0.33
Maternal type 1 diabetes	0.019	1.94	0.08

√MSE=root of mean square error.

Table 4: Significance of dichotomous variables in estimating cumulative measure of atherosclerosis in univariate analysis

younger and had smaller aortic diameters and lesion sizes than children who died of other causes (data not shown). In addition, the percentage deviation of weight from the expected weight was about 10% (range 5–16) in all children with cancer. However, this difference did not affect the correlation between age and atherosclerosis or the rate of progression of atherosclerosis in the normocholesterolaemic or the hypercholesterolaemic groups. Analysis of only children who did not have cancer yielded regression lines, regression coefficients, and p values similar to those in figure 3.

In univariate analysis for risk factors, age of the child, total plasma cholesterol, maternal age at birth, cholesterolaemia group, hypertension in the child, and maternal history of coronary heart disease correlated positively and birthweight correlated negatively with cumulative lesion size (tables 3 and 4). Risk factors that were significant in the univariate analysis were used to build regression models to find out which of these variables remained correlated with lesion size when combined with group and age of the child (the two factors that had the strongest influence in the univariate analysis). The initial model is shown in table 5. After application of the Bonferroni-adjusted criteria for covariate significance, only group, age, and birthweight remained significantly associated with the composite measure of atherosclerosis (model II). Low birthweight has previously been linked to hypertension and increased atherosclerosis.^{27–29} To further explore the influence of the birthweight, we added a group by birthweight interaction

Model	Covariate	Model	R ²	Coefficient	SE
Model I					
Group	<0.0001				
Age	<0.0001				
Birthweight	<0.0001	<0.0001	0.98	..	..
Mother's age at birth	0.32				
Maternal coronary heart disease	0.30				
Model II					
Group	<0.0001				
Age	<0.0001	<0.0001	0.98	..	..
Birthweight	<0.0001				
Model III					
Group	<0.0001				
Age	<0.0001				
Birthweight	<0.0001	<0.0001	0.98	..	..
Group×age	0.83				
Group×birthweight	0.03				
Model IV					
Group	<0.0001			1.07	0.20
Age	<0.0001	<0.0001	0.98	0.34	0.01
Birthweight	<0.0001			-0.28	0.06
Group×birthweight	0.03			0.14	0.06

R²=Coefficient of determination; ×=interaction; Coefficient=regression (β) coefficient.

Table 5: Models of multiple linear-regression analysis assessing role of group, age of child, birthweight, maternal age, and coronary heart disease history on atherosclerosis of child

Figure 5: Comparison of lesion sizes in abdominal aorta between fetuses and children younger than 3 years

Data previously reported for 28 normocholesterolaemic mothers and 39 hypercholesterolaemic mothers;¹⁵ 18 fetuses added to increase numbers. Lesions reported as average area of single largest lesion per section in all sections from aortic arch or abdominal aorta.

*Significant decrease ($p<0.0001$). †Significant increase ($p<0.0001$).

term (as well as a group by age interaction term), into the model (model III, table 5). In the final regression model (model IV), the combination of group, age of the child, birthweight, and group by birthweight term significantly estimated atherosclerosis. However, a separate analysis of birthweight in each group showed a significant negative correlation with lesion sizes only in children of normocholesterolaemic mothers ($r=-0.34$, $p<0.003$), but not in children of hypercholesterolaemic mothers ($r=0.05$, $p>0.75$).

We previously reported that fetal plasma cholesterol concentrations decrease with decreasing fetal age.¹⁵ We therefore formed the hypothesis that fetal fatty streaks would regress towards the end of the pregnancy or after birth. To test this hypothesis, we compared lesion sizes in children younger than 3 years with those previously determined by the same method in fetuses. We chose age 3 years as the cut-off age so that sufficient numbers of children were included in the comparison. Because the average diameter of the aorta was larger in children than in fetuses (11.2 [1.4] vs 2.4 mm [0.7] for the abdominal aorta, $p<0.0001$), we compared the area of the largest lesion per section rather than the cumulative lesion area. This variable can be assumed to be largely independent of the vascular calibre, because the absolute size of these lesions was small and they occupied only a small part of the luminal circumference in fetuses and younger children.

The area of the largest lesion per section in the aortic arch of young children was significantly smaller than that of fetuses ($p<0.0001$), especially in the hypercholesterolaemic group, in which the largest lesion was 64% smaller than that in fetuses (figure 5). By contrast, in the abdominal aorta, the area of the largest lesion in children was 24% bigger than that in fetuses. Corresponding differences in the normocholesterolaemic group were also significant, but were smaller in absolute terms. Fatty streaks induced by maternal hypercholesterolaemia during pregnancy^{30,31} do, therefore, regress to some extent during the late stages of pregnancy or shortly after birth. Even the apparent increase of lesion size in the abdominal aorta is consistent with this conclusion. Given the linear correlation between the size of lesions and the age of children in figure 3, it is apparent that striking lesion growth occurred from age 1

year to 3 years and that a lower cut-off age would have yielded smaller lesion sizes. When we analysed only data for children up to age 2.5 years, the average area of the largest lesion per section in children from hypercholesterolaemic mothers was almost identical to that of fetuses (0.73 [0.13] vs 0.75 mm² [0.13], $p>0.05$).

Discussion

Little is known about the progression of fatty streaks in human beings. It is assumed that fatty streaks can progress to more advanced atherosclerotic lesions because they occur at the same anatomical sites and because transitional stages have been seen.³² Conversely, the assumption that fatty streaks may regress rests on observations in animal studies and angiographic studies which show that some advanced human lesions may partially regress as a result of hypolipidaemic intervention.³³ The FELIC study showed that human fatty streaks formed during fetal development can regress. However, the most important result was that changes induced by or associated with maternal hypercholesterolaemia during pregnancy or formation of fatty streaks that do not fully regress predetermine the rate of progression of atherosclerosis throughout childhood. This finding may have implications for understanding of early pathophysiological events in atherogenesis and its prevention.

One potential explanation for the faster progression of atherosclerosis in children of hypercholesterolaemic mothers could be the prevalence of one or several atherogenic genes. Increased susceptibility to atherosclerosis has been linked to particular genes in mice,^{34,35} and the same may be true for human beings. Hypercholesterolaemic mothers had almost twice the rate of type 1 diabetes and history of coronary heart disease compared with normocholesterolaemic mothers, and the mere fact that they were hypercholesterolaemic may reflect a different gene pool. The normal plasma cholesterol and HDL concentrations found in children of hypercholesterolaemic mothers rule out gross defects in lipoprotein receptors (eg, familial hypercholesterolaemia), but differences in apolipoprotein isoforms (eg, apolipoprotein E), point mutations in apolipoproteins, or apolipoprotein receptors, or differences in genes not involved in lipoprotein metabolism (detectable only by advanced genetic analysis)^{36,37} may have influenced long-term lesion formation. However, since there was virtually no overlap in the extent of atherosclerosis in children of normocholesterolaemic and hypercholesterolaemic mothers, it is unlikely that the differences were attributable to a small number of recessive or dominant genes contributed by the mother. These genes would be expected to segregate in their children, leading to overlapping distribution of the extent of atherosclerosis.

A second mechanism could be the induction of a constitutional state of overexpression of "atherogenes" in the fetal vascular wall by maternal hypercholesterolaemia or the resulting fatty-streak formation. We propose that such genes exist and that they are activated directly by hypercholesterolaemia or by processes that take place during the increased formation of fatty streaks resulting from maternal hypercholesterolaemia during pregnancy. Expression of such genes could be promoted by LDL peroxidation that occurs in atherosclerotic lesions.^{9,38} Oxidation-related processes are known to influence

regulation of NFkB-dependent genes³⁹ and regulation of other nuclear receptor pathways,⁴⁰ and could also affect cell cycle or apoptosis. Such atherogenes could specifically interact with growth-factors expressed by vascular cells, analogous to the interaction between oncogenes and growth factors in cancer.⁴¹ Identification of potential atherogenes could be attempted by comparing gene expression and transcription in fetal arteries from normocholesterolaemic and hypercholesterolaemic mothers, or in genetically homogeneous animal models. Similarly, gene regulation could be compared between lesion-prone and resistant areas of arteries, or between intracranial and extracranial arteries.¹⁶

The mechanisms by which maternal hypercholesterolaemia modulates expression of specific genes in the fetus remain to be established. We have previously reported that in fetuses at less than 26 weeks' gestation, maternal and fetal plasma cholesterol concentrations correlate strongly, whereas in older fetuses there is no correlation.¹⁵ The influence of maternal hypercholesterolaemia may therefore be strongest during the early development of fetal arteries. To date, it has been presumed that fetal cholesterol requirements are met by de-novo synthesis rather than by use of maternal or placental cholesterol.⁴² In addition to the possibility that during early fetal development the placenta may be permeable to non-esterified fatty acids, oxidised fatty acids, or native lipoproteins, the hypercholesterolaemic status of the mother may promote an increase of oxygen radical production and peroxidative compounds.^{43,44} These reactive metabolites may permeate the placenta and induce peroxidative chain-reactions in the fetal bloodstream, which could affect several cell-cell signalling processes and gene expression and transcription in the arterial wall.^{9,39,40} Antioxidant intervention studies during pregnancy may help to clarify whether lesion progression in children is dependent on oxidation-related processes.⁴⁵

Although none of the traditional risk factors could independently explain the more rapid progression of atherogenesis in children of hypercholesterolaemic mothers, the scatter in figure 3 was noticeably wider in older children than in younger ones. At older ages, therefore, the impact of traditional risk factors or socioeconomic differences may increase.^{46,47}

Our results also suggest that the long-term benefits of lipid-lowering therapy in hypercholesterolaemic mothers during pregnancy should be investigated. If it can be confirmed that a faster rate of progression persists throughout adulthood, even in the presence of hypercholesterolaemia and other risk factors of atherosclerosis, lipid-lowering interventions in hypercholesterolaemic mothers during pregnancy may offer long-term benefits to their children. The obvious approach would be dietary intervention. A 20% decrease in cholesterol concentrations has been achieved in pregnant women by dietary intervention.³⁰ Statins, the most effective lipid-lowering drugs currently available, are contraindicated during pregnancy, but a cautious, strictly controlled treatment with bile-acid sequestrants or newly developed drugs that are safe during pregnancy is conceivable.

Contributors

C Napoli and W Palinski jointly designed the study and wrote the paper. W Palinski was the senior investigator and was responsible for data analysis. F P D'Armiento and C Napoli jointly directed and coordinated

clinical activities and participated in data collection and interpretation. R Deutsch did the statistical analysis and wrote the description of the statistical methods. C K Glass, J L Witztum, and F P D'Armiento contributed to the interpretation of results. All investigators reviewed the paper.

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