Maternal hypercholesterolemia during pregnancy influences the later development of atherosclerosis: clinical and pathogenic implications

Hypercholesterolaemia plays a major causal role in atherogenesis and reduction of blood cholesterol is therefore a primary therapeutic target. Although great progress has been made in identifying pathogenic mechanisms of atherosclerosis, in particular with regard to the role of apolipoproteins and scavenger receptors, low density lipoprotein (LDL) oxidation, adhesion molecules, growth factors, interleukins, nitric oxide, and intracellular signalling in arterial cells\[1–7\], little is known about genes or other factors that determine the basic susceptibility to atherosclerosis in humans. Recent studies provided new insights by showing that the atherogenic process begins in fetal arteries and that the fetal onset of atherogenesis profoundly influences the rate of its progression throughout childhood and adolescence. The potential clinical implications of these findings will be discussed here.

Until recently, atherogenesis was thought to begin during late childhood, even though fatty streaks had occasionally been observed in younger children\[8,9\]. A single fatty streak had also been reported in a human fetus with familial hypercholesterolaemia\[10\], but elevated maternal cholesterol levels during pregnancy were not considered to be a risk factor of atherosclerosis, because animal studies had indicated that the placenta is impermeable to large, cholesterol-carrying apolipoproteins\[11\], and because in the absence of heritable defects of the lipoprotein metabolism, cholesterol levels in term-newborns are not correlated with maternal cholesterol levels. However, a systematic morphometric analysis of cross-sections through the entire aorta of premature human fetuses (fetal age 6·2 ± 1·3 months) demonstrated that the formation of fatty streaks, the precursors of more advanced atherosclerotic lesions, is prevalent in all fetal aortas, and that their number and size is markedly increased in fetuses whose mothers were hypercholesterolaeamic during pregnancy\[12\]. Fetal lesions contained elements typical of early atherosclerotic lesions, such as native and oxidized LDL and macrophages, and reached intimal medial ratios of approximately 1. Lesion distribution in fetal aortas reflected that of more advanced atherosclerosis in adults, i.e. was most extensive in the abdominal aorta, followed by the aortic arch, whereas only minimal intimal lipid accumulation was seen in the thoracic aorta. Although absolute lesion areas were greatest in fetuses from mothers who were permanently hypercholesterolaeamic (i.e. before, during, and after pregnancy), fetuses from mothers who were only temporarily hypercholesterolaeamic during pregnancy also showed marked increases, compared to fetuses from normocholesterolaeamic mothers, indicating that maternal and/or fetal hypercholesterolaeemia contributed to enhanced lesion formation. Fetal plasma cholesterol levels showed a striking inverse correlation with fetal age and correlated with maternal cholesterol levels up to the 6th month of fetal age, whereas there was no correlation beyond 6 months\[12\]. This suggests that during the earlier stages of pregnancy, maternal hypercholesterolaeemia leads to fetal hypercholesterolaeemia, which in turn may give rise to increased LDL oxidation and lesion formation.

LDL oxidation is prevalent in atherosclerotic lesions\[13\] and may enhance atherogenesis by a number of mechanisms\[3\], including the recruitment of circulating monocytes\[12\]. The assumption that LDL oxidation is a contributor to fatty streak formation in fetal arteries was also supported by a later study comparing lesion formation in intra- and extracranial fetal arteries\[14\]. In this study, the middle cerebral and basilar arteries of fetuses contained significantly smaller lesions than the aorta and common carotid arteries, even when differences in vascular calibre were corrected for. This is consistent with the observation that cerebral atherosclerosis in adults occurs later and is less extensive than atherosclerosis in coronary or carotid arteries. However, when lesions in the same artery were compared between fetuses from normo- and hypercholesterolaeamic mothers, maternal hypercholesterolaeemia was associated with a marked increase in lesion size.
only in extracranial, but not in intracranial arteries. Determinations of the arterial activities of manganese superoxide dismutase, catalase, and glutathion peroxidase indicated that overall, intracranial arteries of human fetuses were better protected against oxidation than extracranial arteries. Although other factors such as anatomical particularities of brain arteries or exposure to less pressure could have contributed to the difference, these results support the assumption that better protection against free-radical mediated oxidation may contribute to the greater resistance of intracranial arteries to hypercholesterolaemia-induced atherogenesis. This is also consistent with the previous observation that exposure to mildly oxidized LDL impaired vasodilation of carotid, but not intracranial arteries.[15]

The above studies demonstrated that fatty streaks are prevalent in premature human aortas and that they are greatly enhanced in fetuses from hypercholesterolaemic mothers. A recent study also confirmed in other human fetal arteries that intimal thickening and smooth muscle cell proliferation begins prior to birth[16]. However, the relevance of fetal fatty streaks for atherosclerosis later in life remained uncertain. Given that fetal cholesterol levels decreased sharply with increasing fetal age[12], it was likely that fetal fatty streaks would regress towards the end of a normal pregnancy or during normocholesterolaemic infancy.

To ascertain whether fetal fatty streaks indeed regress and or whether they influence atherogenesis during childhood and adolescence, we designed the ‘Fate of Early Lesions in Children’ (FELIC) study, the results of which were published in a recent edition of the Lancet[17]. In this study, 156 normocholesterolaemic children, aged 1–13 years, who died of trauma and other causes were divided into two groups, based on whether their mother had been normocholesterolaemic or hypercholesterolaemic during pregnancy. Atherosclerosis was determined by computer-assisted image analysis of cross-sections through the entire aortic arch and abdominal aorta. The impact of 13 established or potential risk factors of atherosclerosis in both children and their mothers on the extent of atherosclerosis was then determined by univariate and multivariate analysis.

When lesion sizes in fetuses were compared to those in children younger than 3 years, the average area of the largest fatty streak in each cross-section through the aortic arch of children from hypercholesterolaemic mothers was 64% smaller than that of corresponding fetuses. In contrast, in the abdominal aorta, lesions of children were greater than those of fetuses. This indicates that fetal fatty streaks do regress during infancy, but that regression is neither complete nor equal in all areas of the aorta.

In both groups of children, atherosclerosis in the aortic arch and abdominal aorta increased linearly with increasing age. The most remarkable result of the FELIC study, however, was that lesions progressed much faster in children of hypercholesterolaemic mothers than in children of normocholesterolaemic mothers, even though both groups of children had normal total plasma cholesterol levels and lipid profiles. None of the 13 risk factors assessed could account for the much faster progression of atherogenesis in children of hypercholesterolaemic mothers. In univariate analysis of pooled data, many of the ‘traditional’ risk factors correlated with atherosclerosis, e.g. hypertension of the child and maternal coronary heart disease history. The birthweight showed a significant inverse correlation. However, multiple regression analysis indicated that of all risk factors, only the birthweight remained significantly correlated with atherosclerosis when examined together with the age and group of children (defined by the maternal cholesterol level during pregnancy), the two parameters that correlated best in the univariate analysis. Low birthweight has previously been linked to hypertension and increased atherosclerosis[18,19], but a separate analysis of birthweight in each group showed that the correlation was significant only in children of normocholesterolaemic mothers.

These findings have profound implications for our understanding of the pathogenesis of atherosclerosis. Although genetic differences in the mothers are bound to exist and are likely to contribute to the different susceptibility of children to the disease, several reasons speak against a predominantly genetic explanation of the different rate of progression. These include the absence of abnormalities in the lipoprotein profiles that could have indicated genetic defects of apoproteins or their receptors. More importantly, combinations of several different genes should have resulted in a broad scatter of lesion sizes in children, rather than in lesion sizes falling on either one of two very distinct regression lines (see Fig. 3 in[17]). We therefore hypothesized that maternal/fetal hypercholesterolaemia induces constitutive changes in gene expression in arterial cells and that these changes are associated with a greater susceptibility to the disease later in life. A detailed discussion of the scientific implications of this hypothesis and the approaches necessary to validate it is provided elsewhere[20]. The following will focus mainly on the clinical implications.

The most important therapeutic implication of the FELIC study is the possibility that lipid-lowering therapy of hypercholesterolaemic mothers during
pregnancy may offer persistent benefits to their children. Clearly, any recommendation to treat mothers is dependent on fulfillment of two conditions. The first is that our observations in human subjects are confirmed in genetically more homogeneous animal models. This will rule out a major contribution of genetic differences and support a causal role of hypercholesterolaemia during pregnancy. The second condition is that the increased susceptibility to atherosclerosis noted in children persists into adulthood, even in the presence of conventional risk factors of atherosclerosis, and that it is associated with increased morbidity and mortality from atherosclerosis-related causes, such as myocardial infarction and ischaemic stroke. Studies testing this are in progress.

Statins, the most effective lipid-lowering drugs available, are currently contraindicated during pregnancy, but a strictly controlled treatment with bile acid sequestrants or new drugs that are safe during pregnancy is conceivable. Dietary intervention in pregnant women, the most obvious way of intervening, has been reported to lower cholesterol levels by up to 20%[21].

Theoretically, plasma-exchange and LDL-apheresis are also conceivable in extreme cases of hypercholesterolaemia. This has been shown to increase the longevity of patients with homozygous familial hypercholesterolaemia. Frequent plasma exchange also prevented extreme serum cholesterol elevations that occur in pregnant homozygous familial hypercholesterolaemia patients and was associated with a significant improvement in uteroplacental circulation[22]. In four such pregnancies the clinical course was uneventful, ending in normal deliveries at full-term, but the third pregnancy of a patient with severe aortic stenosis had to be terminated due to the development of hypotension and syncope during plasma exchange. Thus, long-term plasma exchange during pregnancy seems feasible, but close monitoring for haemodynamic complications will be necessary. Obviously, the determination of the optimal frequency of plasma exchange therapy will require a more detailed analysis of the maternal cholesterol levels associated with increased fetal atherogenesis. In our studies, maternal cholesterol data represented the mean of several determinations throughout pregnancy. It is well known that even in normal pregnancies, elevated cholesterol levels are regularly seen in the last trimester[23], but the increase in cholesterol during pregnancy appeared to be far greater in our ‘hypercholesterolaemic’ group than in ‘normocholesterolaemic’ mothers (Napoli and Palinski, unpublished data). Although one can be fairly certain that no significant increase in fetal fatty streak formation occurs below an average maternal cholesterol level of 220 mg. dl⁻¹, the threshold level associated with increased risk clearly needs to be better defined. Independent of the type of cholesterol lowering intervention chosen, potential adverse effects of excessive cholesterol lowering will have to be carefully monitored. Finally, if studies in animal models provide experimental evidence for the causative role of oxidation, administration of antioxidants during pregnancy may be considered, such as vitamin E[24]. In this context, it is of interest that LDL apheresis also reduces the susceptibility of LDL to oxidation[25].

If it can be established that the pathogenetic events occurring during fetal development not only significantly enhance atherosclerosis in children, but also increase the incidence of chronic heart disease in adults, maternal hypercholesterolaemia during pregnancy should also be added to the list of risk factors determining the need for monitoring and preventive therapy[26]. Given the major role of hypercholesterolaemia in atherogenesis and the success of lipid-lowering trials in reducing clinical events, current clinical guidelines place great emphasis on detection of hypercholesterolaemia[27]. However, such screening would not detect increased risk associated with maternal hypercholesterolaemia, as all children in the FELIC study had normal lipid profiles.

Until more insights on the pathogenetic mechanisms associated with hypercholesterolaemia during pregnancy have been gained, an early and intense lipid-lowering intervention may be the best option for children combining several risk factors. As indicated by a recent meta-analysis of studies on the development of coronary artery disease in children and adolescents[28], an average reduction of LDL-cholesterol by 25% can be obtained with HMG-CoA reductase inhibitors (lovastatin, pravastatin, or simvastatin) in combination with a lipid-lowering diet. Statins are generally well-tolerated in children and adolescents, even though transient, asymptomatic elevations in creatinine phosphokinase and hepatic transaminase have been reported in a small percentage of the children. Current data also do not indicate adverse effects on growth and sexual development in male adolescents, but formal evaluations have not been performed in females. Nevertheless, larger and more prolonged studies will be necessary before statins are routinely prescribed to children.

In the case of high-risk children, follow-up may also have to include earlier than usual non-invasive diagnosis of atherosclerosis. This may be difficult for the type of lesions prevailing in children, but recent advances in non-invasive methods offer some promise. Potential approaches include measurement of coronary flow velocity in the distal portion of the left coronary artery and assessment of plaque burden using ultrasound imaging.

Eur Heart J, Vol. 22, issue 1, January 2001
antior descending coronary artery by transthoracic Doppler echocardiography\textsuperscript{29}, determination of the degree of stenosis in the proximal left anterior descending coronary artery by transoesophageal Doppler\textsuperscript{30}, or measurement of coronary flow reserve in the left anterior descending coronary artery by contrast-enhanced transthoracic second harmonic echo Doppler\textsuperscript{31}. Another non-invasive diagnostic method may be electron beam computerized tomography, which measures coronary atherosclerosis by estimating calcification and allows three-dimensional renderings of the coronary arteries, veins, and other cardiac structures to be constructed from transaxial tomograms\textsuperscript{32}. Several clinical studies comparing electron beam computerized tomography coronary angiography to conventional cine-coronary angiography showed that coronary calcium scores correlated strongly with severity of atherosclerotic lesions determined by conventional angiography\textsuperscript{33,34}. Currently, electron beam computerized tomography is a reasonably robust technique for the visualization and assessment of the left main and left anterior descending coronary artery, but substantially underestimates the overall extent of coronary plaque, and electron beam computerized tomography-detected calcium may not be present in minimal diffuse coronary plaques of children. At the moment, a relatively high proportion of the right and circumflex coronary angiograms are non-interpretable, but improvements in image acquisition and post-processing techniques are expected to improve the diagnostic accuracy of the technique\textsuperscript{35}. Valuable information on the usefulness of electron beam computerized tomography for the detection of coronary artery disease among young, asymptomatic subjects is expected to be provided by the Prospective Army Coronary Calcium (PACC) study of the US Army Cardiovascular Screening Program, started in October 1998\textsuperscript{36}. Magnetic resonance imaging is another very promising approach to assess lesion severity in the proximal and left anterior descending coronary artery\textsuperscript{37} or carotid artery\textsuperscript{38}. Finally, ultrasonography of carotid arteries provides less information on lesion severity than electron beam computerized tomography, but some studies showed a moderate correlation between carotid intimal–medial thickness measured by ultrasound and coronary atherosclerosis\textsuperscript{39,40}. Another important clinical goal is to study whether early atherogenesis in children and young adults affects cardiac function. This could be done by echocardiography, which permits a comprehensive assessment of resting regional and global left ventricular function, the presence and extent of inducible myocardial ischaemia, as well as the identification of myocardial viability\textsuperscript{41}. In some patients, however, a suboptimal transthoracic echocardiogram may limit the usefulness of this technique. Recently, transoesophageal echocardiography in combination with dobutamine stress has been used for the evaluation of patients with coronary artery disease\textsuperscript{42}. This technique is semi-invasive, more time-consuming, and requires a greater degree of expertise. Colour kinesis is a new echocardiographic technique based on acoustic quantification\textsuperscript{43}. Colour kinesis might improve our ability to distinguish contraction abnormalities from normal hypokinesis, something that has always been difficult in clinical echocardiography. Segmental analysis of colour kinetic images also allows objective detection of dobutamine-induced regional wall motion abnormalities. Moreover, cardiovascular magnetic resonance imaging allows non-invasive visualization of the heart with a high spatial and temporal resolution\textsuperscript{43}. Gradient echo cardiovascular magnetic resonance images permit an exact and reproducible determination of global and regional left ventricular function, and identical pharmacological stress protocols (i.e. dobutamine stress echocardiography), can be implemented for cardiovascular magnetic resonance imaging. PET measures residual oxidative metabolism by \textsuperscript{11}C-labelled acetate PET and it is more accurate than \textsuperscript{18}F-fluorodeoxyglucose PET, which reflects both anaerobic and oxidative metabolism\textsuperscript{44}. Because fatty acids are metabolized only aerobically, they are excellent candidates for the clinical assessment of myocardial viability. Derivatives of fatty acids that are not metabolized but accumulate in the myocyte are attractive for myocardial imaging. Examples are \textsuperscript{123}I-beta-methyl-p-iodophenyl pentadecanoic acid and 15-(o-\textsuperscript{123}I-phenyl)-pentadecanoic acid\textsuperscript{45}. These tracers can be detected by planar scintigraphy and SPECT, which are more economical and widely available than PET. In addition, \textsuperscript{511} keV collimators have been developed recently, making the detection of positron emitters by planar scintigraphy and SPECT feasible.

This paper is dedicated to the memory of Gaetano Salvatore (Naples, Italy). We thank F. P. D’Armiento, J. L. Witztum, C. K. Glass, L. J. Ignarro, G. Palumbo, G. Ambrosio, M. Chiariele, R. Deutsch, F. P. Mancini, F. de Nigris, A. Cali, A. Bianchi, and M. Mancini for many valuable discussion. We regret that for reasons of brevity many relevant original papers could not be quoted. This work was supported by NHLBI grant HL56989, MURST 96.40% and IS.NIH grant 5698099.

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