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Significance and Need of Screening for Hyperlipidemia in Childhood*

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Both hyperchylomicronemia, which may cause pancreatic disease, and familial hypercholesterolemia, which leads to premature coronary sclerosis, have clinical relevance in childhood. However, these forms of dyslipoproteinemia are rare with an incidence rate of $< 1\%$ taken together. The question whether other more frequent forms of hyperlipoproteinemia of adulthood already have clinical significance in early years of life cannot be answered today with certainty. Support in favor of this idea is derived from epidemiologic studies in adults (Kannel et al. 1971; Chapman and Massey 1964; Stamler et al. 1960; Keys et al. 1963), which have now clearly established that the increase of certain lipid and lipoprotein fractions of plasma must be considered as a major risk factor predicting morbid events related to atherosclerotic disease.

Although the causes have not yet been established, few diseases have been given more attention by clinicians and medical scientists than atherosclerosis since this term was coined almost 150 years ago by Lobstein. In the meantime the etiology of atherosclerosis and the search for effective means of prevention and treatment have become questions not only of clinical significance but of high priority in health policy. Therefore, this disease needs to be considered in future health screening programs. There can be no doubt that the pathogenesis of atherosclerosis is of a multifactorial nature. Although pathologists pointed out more than 50 years ago the need to study young persons for early development of atherosclerosis, little work has been accomplished since then to determine plasma lipids and lipoproteins in the early years of life and to elucidate the question of atherogenesis in childhood and its possible relationship to disorders of lipid metabolism.

Before evaluating the need for screening for hyperlipoproteinemia in childhood, some major points of the possible relationship between plasma lipids or lipoproteins and atherogenesis should be briefly recalled. The discovery of Windaus that atherosclerotic alterations in the arteries mainly contain cholesterol and its esters was the first indication of a relationship between cholesterol and atherosclerosis. Today, 65 years later, we know that this cholesterol is mainly derived from the plasma pool of the circulating soluble plasma lipoproteins. Proliferation of arterial smooth muscle cells to "foam cells" is a prior condition for the deposition of cholesterol in the arterial wall and thus for the develop-

* This contribution is dedicated to Wilhelm Doerr with best wishes for his 65th birthday.

ment of the atherosclerotic lesion. Therefore, the endothelium as the natural barrier between arterial smooth muscle cells located in the media and the constituents of plasma have central importance in atherogenesis. The integrity of the endothelium may be altered by established risk factors, i.e., hypertension, endotoxines, carbon monoxide, etc., making contact of the smooth muscle cells with plasma lipoproteins possible. The first phase of lipid deposition into the intima, the so-called fatty streaks, are regarded as reversible and may be manifest in childhood at the age of 10 years and even earlier. It is subject to dispute, however, whether such early lesions are precursors of fibrous plaques, which may further develop to become complicated lesions with all clinical consequences.

What is the significance of plasma lipids in atherogenesis? With the original lipid theory an attempt was made to ascribe a "disease value" to individual lipid classes. This has, however, proved to be too inaccurate in many respects. The measurement of plasma triglycerides or cholesterol alone gives no information about the form of lipoproteins in which these lipids are present in plasma. Consequently, such measurements cannot reveal the metabolic defects that lead to raised plasma lipids and to the development of premature atherosclerosis. Genetic and biochemical research in this field has clearly shown that the pathogenesis of atherosclerosis can only be understood and successfully dealt with if our analysis is concentrated on defined lipoprotein units and their metabolic regulation.

More than 35 years ago, Gofman et al. (1954) first pointed out that it is important to evaluate the metabolic and structural properties of the complex particles in which cholesterol and triglycerides are transported in plasma if one wishes to correlate plasma lipid concentrations with the development of atherosclerotic vascular disease. The rapid development of chemical, physicochemical, and biochemical techniques have greatly promoted the characterization of the various lipoproteins and in particular their apoproteins. This new information constituted a promising basis for a molecular approach in this research field.

Goldstein and Brown (1975) were pioneers in the use of fibroblast cultures for the study of lipoprotein metabolism. Today a clear relation between the cholesterol-transporting lipoproteins and most body tissues seems to be established and mediated by the protein moiety of the lipoproteins. Goldstein and Brown were able to demonstrate in human tissue cultures that cellular cholesterol synthesis is crucially regulated by high affinity receptors for apolipoprotein-B (Apo-B). In the meantime information has accumulated that apolipoprotein-E may not only interact with the same cell surface receptor but even with higher activity. According to our present knowledge, Apo-B-carrying lipoproteins [primarily very low-density lipoproteins (VLDL) and low-density lipoproteins (LDL)] are bound through the high affinity receptor via the protein moiety and taken up into the cell in the form of endocytotic vesicles. The apoprotein moiety is hydrolized by lysosomal enzymes to amino acids. The cholesterol esters that are now released from the lipoproteins are cleaved by an acid lysosomal cholesterol esterase, an enzyme that may play a dominant role in atherogenesis. The newly formed free cholesterol can now reach the endoplasmatic reticulum where it inhibits hydroxymethylglutaryl (HMG)-CoA-reductase activity and thus depresses cellular cholesterol synthesis. The activity of the high affinity receptors itself is regulated via a feedback mechanism. In depletion of cellular

cholesterol, there is an increase in Apo-B-receptor formation at the cell surface, while accumulation of cholesterol in the cell leads to a diminution of receptors. While Apo-B is primarily responsible for the influx of cholesterol into the cells, the α- or high-density lipoproteins (HDL), characterized primarily by Apo-AI and Apo-AII, seem to exert an opposite effect, i.e., they may promote removal of cellular cholesterol (Stein et al. 1975). More than that, high concentration of HDL may cause an inhibition of Apo-B receptor: Apo-B interaction of smooth muscle cells and by this inhibit the lipoprotein-B-mediated influx of plasma cholesterol into the cells.

Although most mammalian cells possess the enzymatic activity necessary for formation of cholesterol, there are differences between tissues with respect to the regulatory kinetics of this synthesis and only liver tissue can degrade or excrete cholesterol. In this regard it is relevant to note that the binding and uptake of LDL via high affinity receptors on smooth muscle cells are only qualitatively comparable to that of fibroblasts. In smooth muscle cells the negative feedback, leading to a reduction of receptors when LDL uptake is high, proceeds very slowly and high LDL levels may enhance non-Apo-B-receptor-mediated uptake. This seems to be an argument and good explanation for the almost selective proliferation and lipid storage of smooth muscle cells in familial type II hyperlipoproteinemia, although intercellular enzymatic dysfunctions play an additional role. For familial hypercholesterolemia, Goldstein and Brown have shown that homozygous patients do not have active receptors on their cell membranes or the receptors present are defective. These subjects cannot bind low-density lipoproteins, and thus these can only be degraded at very slow rates, resulting in a prolonged biologic half-life of the particles. Furthermore, little or no cholesterol is transferred across the cell membrane to regulate cellular cholesterol synthesis, which proceeds on without any regulation. At very high concentrations of LDL, cellular cholesterol synthesis in the heterozygous subjects is controlled, but at LDL levels twice that of normals.

In relation to our topic it was most interesting to learn that this genetic disease can already be diagnosed with certainty using cultured amionic fluid cells (Brown et al. 1978). Amniotic cells from an affected fetus in a family with type II hyperlipoproteinemia showed no binding of LDL and thus no degradation of low-density lipoproteins. In comparison, control amniotic cells behaved quite similar to fibroblasts in culture. This genetic defect, which causes the most severe forms of atherosclerosis, demonstrates that atherogenesis, and in particular coronary artery disease, may already occur in early childhood (Horn and Schwartzkopf 1975) and that severe dyslipoproteinemia alone may be followed by early development of atherosclerosis. Literature reports of young children suffering from coronary infarction due to familial type II hyperlipoproteinemia are not rare. On the other hand, it is well known that deposition of lipids in the arterial wall in childhood takes place primarily in the form of fatty streaks, alterations that are to our knowledge reversible without any particular treatment.

A major problem that emerges is how to recognize an early onset of atherosclerosis. If we want to elucidate the early natural history of atherosclerosis, it is evident that we must also start to study in children the risk factors established for adults. Interpretation of the outcome of such studies is more difficult in children because of the limitation in the ability to observe the severity or progressiveness of the disease. Since hyperlipoproteinemia is a multifactorial and multigenetic condition, the interpretation of screening

procedures is also very difficult in newborns. It has been reported that hypercholesterolemia may or may not occur following prenatal exposure to steroids for prevention of the respiratory distress syndrome. This suggests that normal medical procedures may cause false positive findings in newborn screening (C.R. Scriver 1978, personal communication). However, in spite of such difficulties, we must realize that the time has come to attack these problems before seriously considering prevention of risk factors at a young age in an attempt to reduce atherosclerosis and coronary artery disease in the general population.

As screening is defined as the "presumptive identification of unrecognized disease by the application of tests that can be applied rapidly," to our mind screening for hyperlipidemia cannot yet be recommended for childhood.

Since it is clear that prevention is better than cure and that atherosclerosis develops slowly, it is urgent to start large, well-defined, and well-controlled epidemiologic surveys in children to find an answer to our question of whether screening for hyperlipoproteinemia in childhood is indicated or not. The outcome of such studies should permit elucidation of the prevalence and incidence of hyperlipoproteinemia in childhood and its eventual relevance for atherogenesis in adulthood.

Assuming that the outcome of such studies would indicate predictive biochemical and clinical parameters, we would need additional information as to whether early treatment of the identified patients would be of any benefit to them. Only if both questions can be answered positively should screening for hyperlipoproteinemia be recommended in childhood. For the time being, it seems to be justified only to identify the genetic marker causing hyper-β-lipoproteinemia by the use of cell culture systems. Any plasma cholesterol level above 250 mg% at any age must be considered to be suspicious for this disease and must be followed up; the same is true for a familial history of an early manifestation of atherosclerosis or cardiovascular disease.

Although a large number of studies in which plasma lipids or plasma lipoproteins were measured in childhood are available, we are still far away from being able to provide exact data for the "normal range" of this age group or even to indicate a risk level.

Tables 1–5 summarize some of the work in this field. The data presented here clearly indicate that low levels of both cholesterol and triglycerides at birth increase rapidly during the first weeks of life and may during the first years of life reach values found in adults. It was suggested by G.M. Komrower (1978, personal communication) that for this reason screening should not be done earlier than at the nursery school age (at 4 years) and primarily in children from families at risk.

Table 1. Total plasma cholesterol concentration at birth (mg/dl)

	Country	n	X	SD
Glueck et al. (1971)	U.S.A.	1800	64	18
Barnes et al. (1972)	Australia	747	75	19
Goldstein et al. (1974)	U.S.A.	2000	82	20
Greten et al. (1974)	G.F.R.	1323	60	20
Mishkel (1974)	Canada	2937	70	17
Carlson and Hardell (1977)	Sweden	2817	70	17

Table 2. β-Cholesterol concentration at birth (mg/dl)

	Country	n	X	SD
Glueck et al. (1971)	U.S.A.	596	37	21
Kwiterovich et al. (1973)	U.S.A.	36	31	6
Greten et al. (1974)	G.F.R.	1323	35	12
Mishkel (1974)	Canada	240	35	11

Table 3. Total plasma triglycerides at birth (mg/dl)

	Country	n	X	SD
Barnes et al. (1972)	Australia	747	52	–
Goldstein et al. (1974)	U.S.A.	2000	42	25
Mishkel (1974)	Canada	1805	40	19
Carlson and Hardell (1977)	Sweden	1817	41	14

Table 4. Total plasma cholesterol concentration in childhood (mg/dl)

	Country	Age	n	X	SD
Ballester et al. (1965)	Switzerland	4 wk	11	135	19
Darmady et al. (1972)	U.K.	4 mo	265	184	36
Greten et al. (1974)	G.F.R.	1 yr	64	174	37
Friedman and Goldberg (1976)	U.S.A.	3 yr	420	154	14
Golubjatnikov et al. (1972)	U.S.A.	5–9 yr	328	187	31
Golubjatnikov et al. (1972)	Mexico	5–9 yr	209	100	28
Andersen and Clausen (1972)	Denmark	5–10 yr	27	177	41
Golubjatnikov et al. (1972)	U.S.A.	10–14 yr	328	185	40
Golubjatnikov et al. (1972)	Mexico	10–14 yr	209	100	24
Clarke et al. (1970)	U.S.A.	15 yr	121	165	35

Table 5. Total plasma triglyceride concentration in childhood (mg/dl)

	Country	Age	n	X	SD
Frerichs (1976)	U.S.A.	5 y	287	66	25
Frerichs (1976)	U.S.A.	4 y	359	75	40
Berenson et al. (1974)	U.S.A.	15–16 yr	32	87	45
Dyerberg and Hjörne (1973)	Denmark	16–20 yr	54	87	30

To get some preliminary ideas about plasma lipid and lipoprotein fluctuations during the first 12 years of life, we have in collaboration with the Childrens Hospital and the Institute of Statistics of the University of Heidelberg determined concentrations of plasma cholesterol, plasma triglycerides, and plasma lipoprotein fractions in various age groups (cord blood, 4 weeks, ½ year, 1 year, 2 years, 6 years, and 12 years; see Figs. 1 and 2).

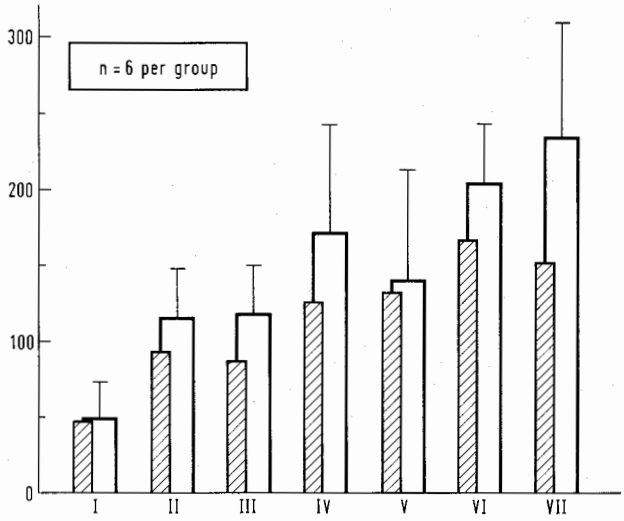

Fig. 1. Total plasma cholesterol and cholesterol in the lipoprotein density fraction d > 1.006 both in mg/dl in various age groups. *I*, cord blood; *II*, 4 weeks; *III*, 0.5 years; *IV*, 1 year; *V*, 2 years; *VI*, 6 years; *VII*, 12 years

The statistical examination showed an age-dependent increase for both cholesterol and triglycerides as well as for α- and β-lipoproteins. However, the data also indicate a high degree of variation within these age groups. In accordance with other studies, plasma cholesterol in children may be in the range of an adult level already after the age of 2. Determination of the lipoprotein fractions revealed a relatively high concentration of α-lipoproteins in cord blood, while already after 4 weeks of life the β-lipoproteins drastically increase. The biologically important ratio of β:α-lipoproteins is high during the first weeks of life and decreases toward the age of 12. The question raised by C. Bachmann (personal communication 1978) of whether HDL cholesterol has a better predictive value for atherosclerosis even in childhood cannot be answered at present but certainly needs consideration in future prospective studies.

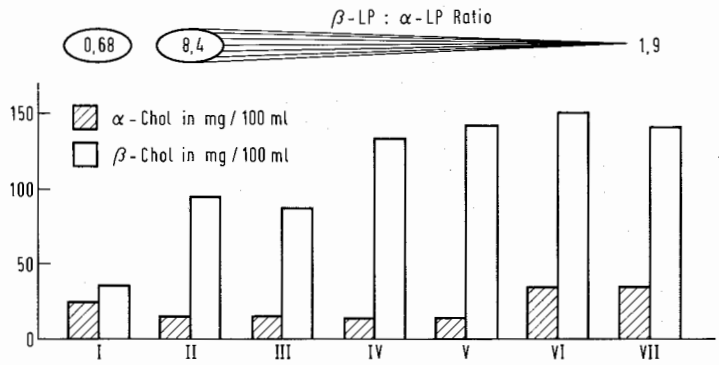

Fig. 2. α-Cholesterol and β-cholesterol in mg/dl and β-LP:α-LP ratio in various age groups. *I*, cord blood; *II*, 4 weeks; *III*, 0.5 years; *IV*, 1 year; *V*, 2 years; *VI*, 6 years; *VII*, 12 years

The possibility of measurement of Apo-B in the filter paper dried blood spot as indicated by R. Guthrie (1978, personal communication) is an interesting approach and requires further consideration. The well-known technical problems, however, in the measurement of apolipoproteins by immunologic means also holds for this procedure and may limit its value.

As for screening for hyperlipoproteinemia in adulthood, the method used in future studies in children must fulfill basic requirements for screening procedures in clinical chemistry. Besides accuracy and precision of the method, the diagnostic power of the parameter determined needs to be well established. Besides measurements of total plasma cholesterol and total plasma triglycerides, enzymatic determination and quantification of two major cholesterol-transporting plasma lipoprotein fractions seem to be justified at the present and can now easily be achieved by the use of quantitative plasma lipoprotein electrophoresis (Wieland and Seidel 1978; Seidel 1979).

Further development of sequential precipitation techniques to fractionate the plasma lipoprotein spectrum may also provide meaningful tools in the future.

Because of our present knowledge about the possible antagonistic function of the two major lipoprotein fractions in atherogenesis, measurement of only one of the two fractions alone (HDL or LDL) provides only limited information and therefore should not be recommended for this purpose.

In summary, we should like to emphasize that our understanding of the pathogenesis of atherosclerosis has increased in recent years through the outcome of experimental studies at the cellular level and also through clinical trials. These studies have clearly demonstrated the important role of the plasma lipoproteins in atherogenesis. The question of whether atherosclerosis starts early in life cannot be answered yet, with the exception of the familial type II hyperlipoproteinemia. General screening for dyslipoproteinemia in childhood, therefore, cannot be recommended at present. However, it is urgent to start large epidemiologic studies, performing measurements on defined populations, to elucidate the prevalence and incidence of dyslipoproteinemia in childhood and to compare these data with the natural history of atherosclerosis. To do this it will be important that pediatricians, pathologists, and clinical chemists colaborate and that the clinical chemistry profile not only aids in measuring lipids but also in following individual plasma lipoprotein concentrations (Castelli et al. 1977).

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Addendum

T.P. Foley, Jr.

In agreement with this report and because atherosclerotic vascular disease has a proformed impact on morbidity, mortality, and delivery of health care services in the United States, I think that it is most important that research studies as described be developed to determine if screening is feasible at all and therapeutic intervention beneficial in favorably alterating the course of this disease. Certainly we would agree that routine "service" non-research screening should not be instituted at this time.